Mosquitoes: The Urban Pests

Public Health Entomology

(3rd Edition)

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1.1. Introduction

Mosquitoes have been causing a nuisance since the ancient times and are known as the human predators. Since, they are associated with human diseases so they are said to be "vectors". In the holy book Quran, we can get reference about the mosquitoes: "The death of the Pharos King by a little mosquito; which entered through the nose and reached his brain and started biting, the cause of his death." In 1878, the first arthropod to be formally incriminated as the intermediate host of parasites was, the **mosquitoes** (Foster and Walker, 2009). Mosquitoes are vectors of arthropod borne diseases e.g. filariasis, malaria, yellow fever, encephalitis and dengue fever. Due to which they have attained much attention in research during the past century. These sicknesses are more prevalent in the tropics and sub-tropics. Early death and chronic debilitations caused by these arthropods vectors pressurize the public health amenities and reduce the human fitness, there by spreading economic and financial burden.

The bites of these minute annoying insects are quite discomforting; not only for the poor but for the richer persons as well. Mosquitoes are correlated with the slums and the dark shady moist areas but they are also present in the highly industrialized areas of the temperate zone. Because they are tiny flying arthropods (in which the female species is the blood sucking vector); they can fly off far distances and also can be transported via vehicles, shipping parcels, barrels, tires and crops etc. Mostly mosquitoes disperse by flight and every important mosquito species has its own flight range and pattern (Table 01), e.g. *Aedes aegypti* has a flight range of about 10-800m (Tripis and Hausermann, 1988). For long distance movement of mosquitoes trade and movement has played a major role e.g. Cargo and tires. *Aedes albopictus* was introduced in the USA by tyre trade; as desiccated mosquito eggs were present inside the tires (Knudson, 1995). In Pakistan Aedes arrival is linked to the import of tires from Thailand, Malaysia, Vietnam and Singapore etc. This human mediated dispersal of *Aedes* also favored dispersal of dengue virus from city to city in Pakistan. During Eid-ul-Fitar patients infected were carrying DENV (dengue virus) in their blood moved to KPK (Khaberpakhtunkhwa) and then dengue cases were also reported from KPK.

Not only humans are the bare hosts of these tiny predators but the domestic and wild life animals are also its hosts. Rather, if, management is not taken against these nuisance spreading arthropods, the result would be loss of productivity and even death. Mosquitoes are widely distributed in all over the world except Polar Regions. Mosquitoes flourished and acclimatized wide range of biosphere such as, arctic tundra, forests, mountains tops, grasslands, deserts, brine swamps, and marine tidal zone. As mosquitos cause nuisance everywhere they spread, whether due to their biting habits or as disease spreading vectors. Mosquitoes belong to Phylum Arthropoda order Diptera (two winged) and family: Culicidae. There are two important sub-families in the mosquito family (Culicidae): Anopheline and Culicinae. All over the world there are 43 mosquito genera are known to present, but in this book, we would discuss only the most common genera in Pakistan: *Aedes, Anopheles* and *Culex, Culiceta* and *Mansonia* (formally "**urbanized mosquito's**" belong to these genera, which live in urban setting). Among this *Anopheles* spp. are vectors of malaria in man, *Culex* spp. vector of malaria in animals especially in birds and *Aedes* spp. vector of dengue, yellow fever and filarial diseases.

In Pakistan, two *Aedes* species are responsible for the spread of dengue fever i.e. *Aedes aegypti* and *Aedes albopictus*. But the main cause of dengue fever is the indoor breeder *Aedes aegypti* that is primary vector of yellow fever virus and of four serotypes of dengue virus (Herrena *et al.*, 2006) and dengue hemorrhagic fever. Mosquito's life cycle is very short approximately completed in two weeks with little variations among different genera. They undergo metamorphosis showing four stages in their life i.e. egg, larvae, pupa and fully-grown adult. Male and female are present but only female bits animals and human because; blood meal is necessary for the female mosquito to nourish its eggs.

Female mosquitoes of *Aedes* act as a vector of dengue virus in rural and urban areas. Female mosquitoes are also highly anthropohilic, means it prefers breeding places close to humans i.e. mostly indoors (Arunachalam *et al.*, 2008). Mosquitoes prefer to live in dark damp areas and some species are diurnal while others are nocturnal. Mosquitoes are flying insects and can fly too far of distances as well. The body is divided into three distinct parts: head thorax and abdomen. Head contains the mouth parts which are modified for piercing and sucking in the adults. *Anopheles*, *Aedes* and *Culex* are major source of vector borne diseases like malaria, dengue, dengue hemorrhagic fever and filariasis.

In 2011, 2012 and 2013 dengue epidemic caused a great havoc on the people of the province Punjab, Pakistan; the spread of DENV (Dengue virus) via its vectors *Aedes aegypti* and *Aedes albopictus* (the vectors may be affected with any of the four serotypes of the dengue virus i.e. DENV-I, DENV-II, DENV-III, DENV-IV in the transmission cycle). The main reason for this is the subtropical climate of Pakistan is suitable for its breeding. In Pakistan dengue fever dates back to the early 1980's, when DENV-II was detected in the blood samples taken from the people of Rawalpindi, Pakistan. Human activities have always been favoring some kind of imbalance in the ecosystem and the mosquito species have been benefited in man-made forests. Many mosquito species have been benefited via man-made alterations in the ecosystem and some of them have become domesticated e.g. *Aedes aegypti* (common yellow fever mosquito). Due to their enormous significance mosquitoes have also been a subject of interest for the researchers and always seem highlighted in various journal, articles and books.

2.1. Taxonomy

Classification	
Kingdom:	**Animalia**
Phylum:	**Arthropoda**
Class:	**Insecta**
Order:	**Diptera**
Suborder:	**Nematocera**
Infraorder:	**Culicomorpha**
Superfamily:	**Culicoidea**
Family:	**Culicidae (Meigen, 1818)**

The family **Culicidae**, derived from *Culex* (*Latin*, means gnat) is one of the main genera of Nematocera, the infraorder Culicomorpha. It comprises two superfamilies that include all the piercing/sucking nematocerans, both are predators and blood sucking biters (Foster and Walker, 2009). The overview of nematoceran phylogeny and classification is provided in Wood and Borkent (1989). Culicidae includes approximately 3,500 species (Lawniczak *et al*., 2010) and these numbers are lay open to to constant alteration as new species are been exposed with the progress of molecular biology and gene sequencing. The family Culicidae classified in to two subfamilies and 43 genera. The two major subfamilies in which the mosquitoes are divided comprise the: Anophelinae and Culicinae. The Anophelinae is thought to be the most primitive group.

Among the 43 genera, 40 belong to the subfamily Culicinae. The most important species are the *Anopheles gambiae, Culex pipiens complexes* and *Aedes* subgenus *Stegomyia*. The

***Anopheles gambiae* complex** species are anthropophilic, endophilic and endophagic i.e. prefer to bite humans and other life stock, hence they are consider as important vectors. The ***Culex pipiens* complex** is a medically important group which include most important taxa of the southern house mosquito e.g. *Cx. quinquefasciatus* (*fatigans*) and the northern house mosquito temperate species *Cx. pipiens*. They act as vector of disease causing agents, such as St. Louis encephalitis virus, West Nile virus and filariasis. The ***Aedes* subgenus *Stegomyia*,** contains the brightly marked species known of great medical importance; *Aedes aegypti*, the common yellow fever mosquito and *Aedes albopictus*, the Asian tiger. The yellow fever mosquito, *Aedes aegypti* is distributed worldwide in the tropics and the subtropics. It is known to spread dengue and yellow fever virus.

3.1. Life Cycle

The life cycle of mosquito is holometabolous i.e. egg, larvae, pupa and adult. They are found in aquatic as well as terrestrial habitat. The larvae and pupae stages develop in a aquatic habitats, while adult occupies terrestrial habitat. However, few species of mosquitoes acclimatized to breeding in phytotelmata (*sing.* Phytotelma) are hazardous disease vectors e.g. *Anopheles* and *Aedes* species. In nature, they may breed in a resonating tree trunk to a curl leaf filled with water. In nature, they may breed in a resonating tree trunk to a curl leaf filled with water. They also breed in man-made artificial environment, such as the water filled plastic container, plant pot, empty bottle or discarded tire. Such species with diverse breeding habits are well-associated to pick up disease causing agents from infected person and pass them to healthy person. Female mosquito laid eggs in aquatic habitat which develop in to larva and pupa. All these stages are aquatic and last for 5 to 14 days, which in turn depends on the species type and microclimatic conditions and sessional temperature. Mosquitoes living in cold regions go in to dormant stage and arrested their developmental and growth stages for some part of the year. As enough warm and suitable environment temperature achieved they break their diapause stage and carry on their development. For instance, *Wyeomyia* larvae typically get frozen into solid lumps of ice during winter so, arrest their development and only complete their development in spring. During dry period *Aedes* egg's undergoes diapause and remain unharmed throughout this period, they hatched as they are covered with water. The eggs of some species of *Aedes* remain unharmed in diapause if they dry out, and hatch later when they are covered by water. At first, the eggs are white in color, but within hours the chorion tans and become darker. In tropics, subtropics, and summers of some temperate regions, eggs usually complete embryonic development within two to three days after being laid, but may take up to a week or more in cool climates. Soon after embryonation larvae hatch from eggs in water, including most tribes of Culicinae and all Anophelinae.

The members of the some genera such as *Ochlerotatus*, *Aedes* and other Aedini usually develop in temporary water. These mosquitos lay eggs on solid substrates out of water, and the larvae within them stay inactive until flooded with water. The *Anopheles* and *Culex* eggs laid above the water surface and once they submerge they do not hatch into larvae or they cannot even withstand desiccation, rather, *Aedes* eggs are laid above the water surface and if submerged, will hatch into 1^{st} instar; also in dry season they undergoes diapause and hatch later when submerged with water. The eggs hatch after 24-48 hours (if proper water level) into 1^{st} instar larvae depending upon the species. If mosquito larvae are well nourished and the water level is maintained they will molt into 2^{nd}, 3^{rd} and finally 4^{th} instar after each 24hours interval.

Larvae develop from eggs, which undergoes radical changes and attain pupae form. Depending on the water conditions and type of species most mosquito larvae either spend most of their life at the water surface or at the water bottom, move to the surface for light and air only occasionally, or not at all. In the presence of adequate food and temperature (26-28 ˚C), the entire larval phase of *Aedes aegypti*, may last as few as five to six days. The larval stage undergoes four instars before pupate, first three instars stages lasts about a day each while fourth instar slightly longer and takes about three days. In male sexes these periods are slightly shorter as compared to females, so the male mosquitos pupate about one day earlier than females. As pupa mature they come toward the surface and fully-grown mosquito emerges from the mature pupa as it floats at the water surface. The pupa spends nearly all its time at the water surface. As time pass it has molted within the pupal cuticle and form a pharate adult. The time taken by the pupal stage depends upon the water temperature. In warm water the entire pupa stage typically lasts about two days in both sexes while at lower temperature pupal period takes much time and lasts longer.

Males tend to emerge earlier than females, because of their shorter larval growth periods. At emergence time, the pupa come to the water surface and remains stationary remains stationary, and the abdomen become straightens over 12 to 15 min. Pupa ingest air through cuticle, split the cephalothorax and adult emerges from the pupa cuticle and stand on the water surface. The whole process completed only in few minutes. As adult emerge they make a short flight only for few minutes but not for long time. Until the cuticle become fully sclerotized. Store glycogen and lipids, carried over from larval reserves; afford adequate energy for a few days of survival. They have potential adult life spans ranging from as short as a week to as long as several months depending on availability of food, weather conditions and gender. Some species can lie dormant in winter season as adults in diapause.

Univoltine mosquito species complete only one generation per year. This occurs either if the developmental time is slow in relation to the season favorable for development or if the life cycle includes an obligate form of diapause, a compulsory phase of arrested development. **Bivoltine** and **multivoltine** species can complete two or more generations, respectively, during each breeding season, but the number actually completed may depend on temperature, available larval habitats, or available hosts. Mosquitoes pass through the winter or dry season as eggs, larvae or adults, depending on the species and the climate. In cold climates, overwintering takes place in a state of diapause.

The larvae of the mosquitoes are motile. The *Anopheline* larvae lie horizontal below the water surface, supported by small notched organs of the thorax and clusters of float hairs along the abdomen. They have no prominent siphon, while, *Culicinae sp*. larvae hang vertically downwards with their siphon below the water surface. Mosquito larvae feed on suspended particles in the water as well as microorganisms. However, there are a few species that capture and eat other species. The 4th instar larvae finally molts in to pupa, comma shaped and motile as well. The pupa which are about to molt into adults are more darker in color than those which are not yet ready; while the male pupae will hatch earlier than the female pupae. The pupae will also hatch in about 2-4 days. The T-shaped mark present on the pupa breaks open and releases the adult mosquito. After 24 hours, the adult mosquito dries out its wings and grooms its head appendages before flying away. Now it is ready for its flight and to take its meal. Accordingly, this is a critical stage in the survival of mosquitoes. If there is too much wind or wave action, the emerging adult will fall over, becoming trapped on the water surface to die. The duration of egg to adult depends on ambient temperature and varies strongly among different species.

3.2. Ecology and Behavior

Both male and female mosquitoes obtain their energy from basic metabolic mechanism and plants nectar. However females require blood as well for the development of egg. Female mosquito bites the vertebrates and humans for obtaining the blood. For sucking the blood they have specialized mouth part called as proboscis. Breeding sites are different for different species. However it is categorized in to three major types i.e. **floodwater breeders**, **permanent water breeders and artificial container/tree hole breeders**. *Anopheles* and various *Culex* mosquito species select permanent water bodies, such as swamps, ponds, lakes and ditches that do not usually dry up.

Floodwater mosquitoes lay eggs on the ground in low areas subject to repeated flooding. During heavy rains, water collecting in these low areas covers the eggs, which hatch from within minutes to a few hours.

Mosquito species that prefer the flood water mosquitoes as breeding sites usually lay eggs in the area which are subjected to repeated flooding. Eggs remain dormant during dry season but during heavy rain, rain water collected in the areas and cover the eggs, as egg get water they become metabolically active and start development. Anopheles mosquito usually prefers the floodwater habitat for egg lying. Similarly (*Ae. aegypti*), Asian tiger mosquitoes (*Ae. albopictus*) species usually prefer the artificial containers and tree holes for egg lying. Some mosquito species lay eggs on the wall of the empty container, as these container fill with water, eggs submerge in water and start development. In the same way some species lay egg directly on the water surface. Anopheles female usually laid egg on the surface of water body during night time. Their eggs are elongated cigar-shape about 1mm long. At suitable environmental conditions eggs usually hatched within 72 hours. *Aedes* and *Ochlerotatus* mosquitoes lay their eggs on moist ground around the edge of the water or on the inside walls of artificial containers just above the water line. The eggs of *Aedes* and *Ochlerotatus* mosquitoes can resist desiccated conditions for long periods of time. This feature has allowed *Aedes/Ochlerotatus* mosquitoes to use temporary water bodies for breeding, such as artificial containers, periodically flooded salt marshes, fields and tree holes. *Ochlerotatus taeniorhynchus* and *Oc. sollicitans* are salt marsh mosquitoes they breed in salt marsh pools flooded by tides or rain unbearable. Their flight range is between 5 and 10 miles, but they can travel 40 miles or more.

Eggs are laid on the soil and hatch once the rice field is irrigated. *Culex* mosquitoes lay batches of eggs attached together to form little floating rafts. On close inspection of a suitable breeding site, these egg rafts can often be seen floating on the water's surface. Where breeding conditions are favorable, *Culex* mosquitoes also occur in enormous numbers. Several *Culex* species are notorious for their aggravating high-pitched hum when flying about the ears, but *Aedes* species being totally opposite; fly very silently and that's why it's quite difficult to catch or to kill them. In tropical areas, mosquito breeding may continue year-round, but in temperate climates, many species undergo a diapause in which the adults enter a dormant state similar to hibernation. In preparation for this, females become reluctant to feed, cease ovarian development and develop fat body. In addition, they may seek a protected place to pass the approaching winter. Some species, instead of passing the winter as hibernating adults, produce dormant eggs or have larvae that can survive the harsh effects of winter.

Mosquitoes vary in their biting patterns. Most species are nocturnal in activity, biting mainly in the early evening for example *Culex Sp.* However, some species especially *Ae. aegypti* and *Ae. albopictus*, bite in broad day light (although there may be a peak of biting early and late in the day). Others, such as salt marsh species and many members of the genus *Psorophora* ordinarily bite during the night but will attack if disturbed during the day (such as walking through high grass harboring resting adults). Most *Anopheles* mosquitoes are crepuscular (active at dusk or dawn) or nocturnal (active at night). Those anopheline species that prefer a variety of vertebrate hosts are known as **zoophilic**, while those species that distinctly prefer humans are known as **anthropophilic**. Some feed indoors (endophagic), while others feed outdoors (exophagic). After feeding, some blood mosquitoes prefer to rest indoors (endophilic), while others prefer to rest outdoors (exophilic), though this can differ regionally based on local vector ecotype and vector chromosomal makeup, as well as housing type and local microclimatic conditions. Biting by nocturnal, endophagic *Anopheles* mosquitoes can be markedly reduced through the use of insecticide-treated bed nets or through improved housing construction to prevent mosquito entry (e.g. window screens). Endophilic mosquitoes are readily controlled by indoor spraying of residual insecticides. In contrast, exophagic/exophilic vectors are best controlled through source reduction (destruction of the breeding sites).

3.2.1. *Aedes aegypti*

(Yellow Fever Mosquito)

Host preference: Humans

Larval habitat: Artificial containers

Flight range: ≈ 500 ft.

Biting activity: Daytime

Overwinter stage: Egg

Broods per year: Multiple

Diagnostic description:

- Palpi short
- Silvery lyre-shaped stripes on scutum
- Pointed abdomen
- Broad white basal bands on hind tarsi
- Hindtarsomere 5 entirely white
- Clypeus with white scales

Disease: Yellow Fever, Dengue and Chikungunya

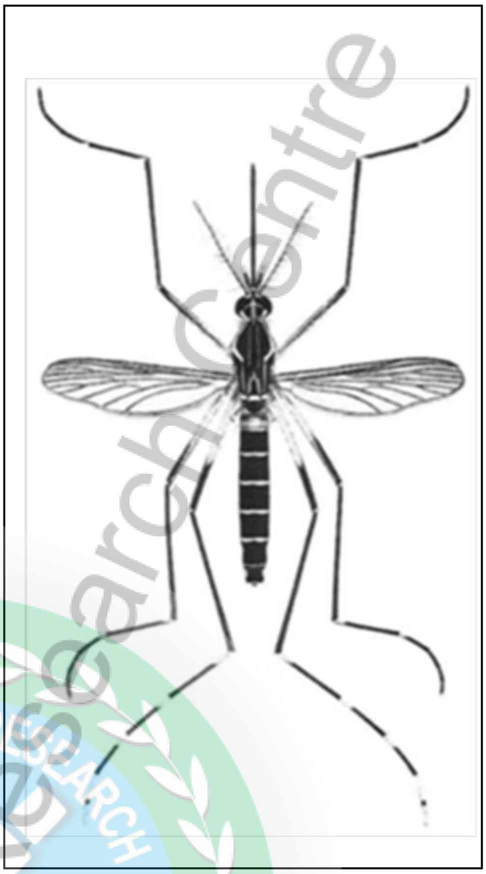


Figure.3.2.1. *Aedes aegypti* (Adult female)

3.2.2. *Aedes albopictus*

(Asian Tiger Mosquito)

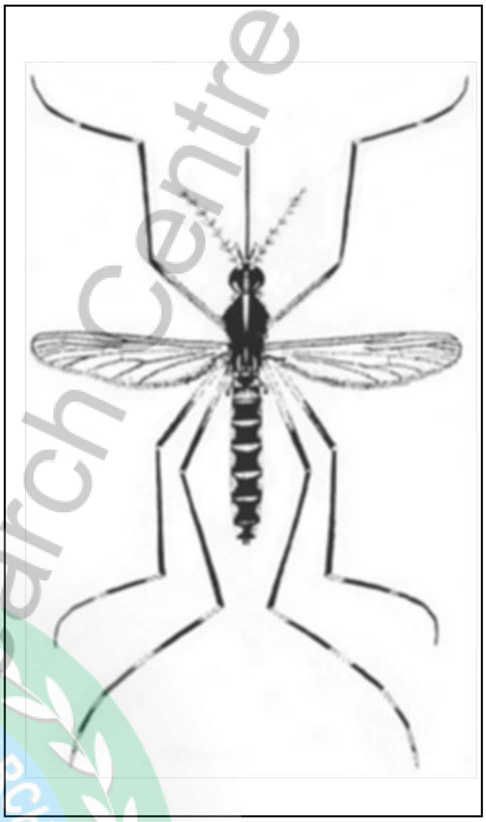


Figure.3.2.2. *Aedes albopictus*

Host preference: Humans, Mammals and Birds

Larval habitat: Artificial containers, tree holes

Flight range: ≈ 500 ft.

Biting activity: Daytime

Overwinter stage: Egg

Broods per year: Multiple

Diagnostic description:

• Palpi short

• Scutum black with narrow silver stripe

• Abdomen pointed

• Proboscis entirely dark (female)

• Wings entirely dark

• Hindtarsi with broad basal white bands

• Hindtarsomere 5 entirely white

• Clypeus without scales

Disease: Believed to be involved in transmission of LaCrosse encephalitis in urban areas; vector of Dengue, Chikungunya, and West Nile Virus.

3.2.3. *Culex quinquefasciatus*

(Southern House Mosquito)

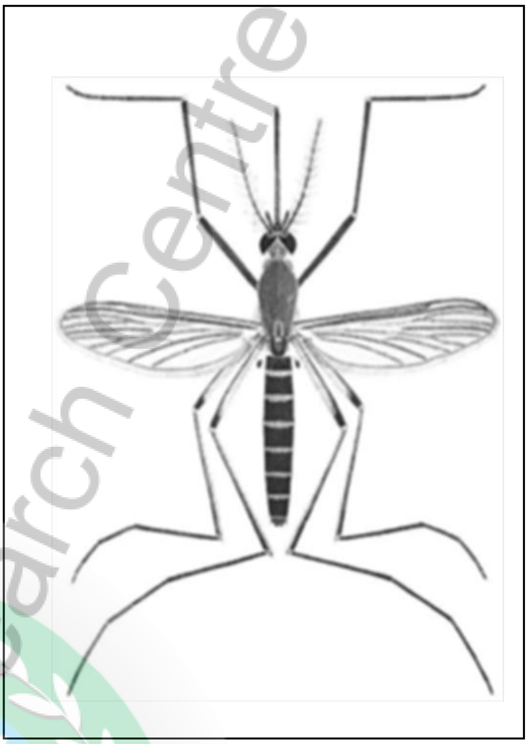


Figure.3.2.3. *Culex quinquefasciatus* Adult (female)

Host preference: Birds, Humans, Mammals

Larval habitat: Water with high organic content (such as sewage ditches), artificial containers

Flight range: Up to 1 mile

Biting activity: Night, dusk and dawn

Overwinter stage: Adult

Broods per year: Multiple

Diagnostic description:

• Palpi short

• Abdomen blunt or rounded

• Thorax, proboscis, tarsi, and wing scales entirely dark scaled

• Mid-lobe of scutellum with patch of long pale scales

• Abdominal segments with broad basal pale bands (bands narrow in width laterally)

• Abdominal segments 7–8 covered two-thirds or more with dark scales, with no copper scales

• Abdomen with black and white scales on bottom side (ventral)

Disease: West Nile Virus, St. Louis Encephalitis and Dog Heartworm.

3.2.4. *Anopheles stephensi*

(Malaria causing Mosquito)

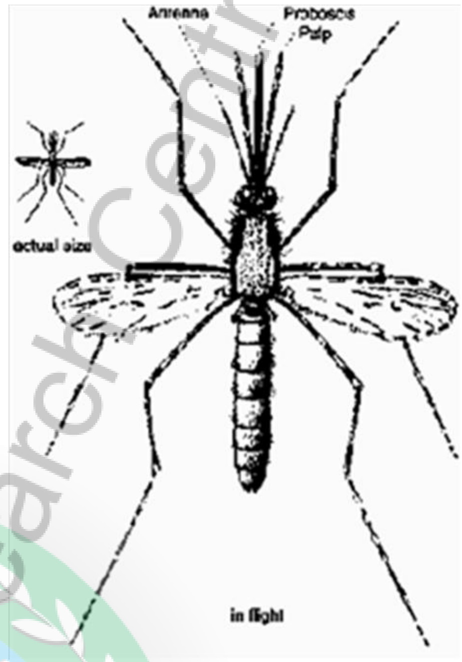


Figure.3.2.4. *Anopheles stephensi* Adult (female)

Host preference: Birds, Humans, Mammals

Larval habitat: Permanent water bodies

Flight range: Up to 4.3 km

Biting activity: Night, dusk and dawn

Overwinter stage: Adult

Broods per year: Multiple

Diagnostic description:

- Palpi long as length of probosics
- Abdomen pointed
- Thorax, proboscis, tarsi, and wing scales entirely brownish in colour
- Scutellum rounded
- Wings covered with setae
- Abdominal segments with broad basal pale (bands narrow in width laterally)

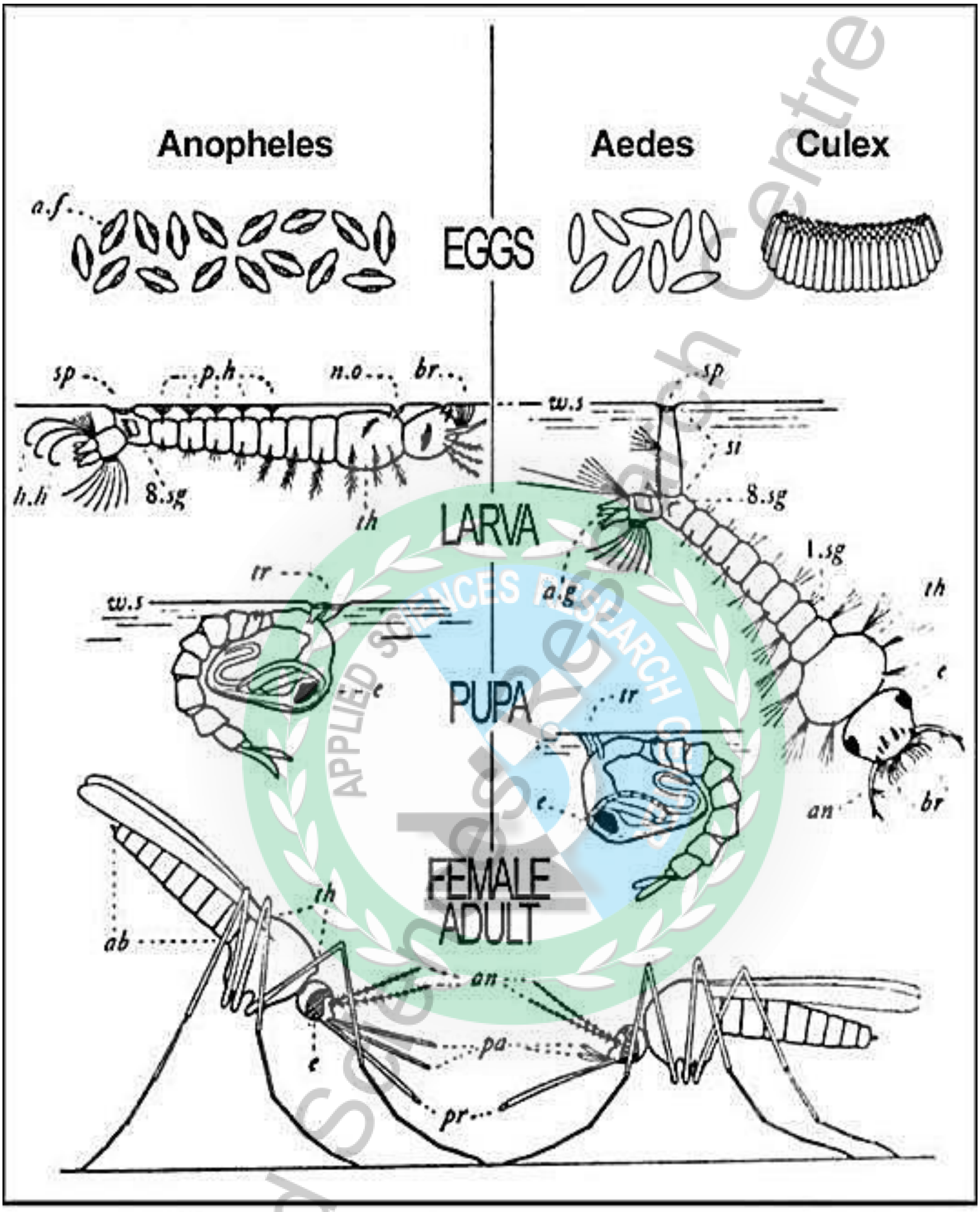
Disease: Malaria

4.1. Morphology

The mosquitoes are a family of small, midge-like flies: the **Culicidae**. Mosquitoes undergo complete metamorphosis, having egg, larval, pupal, and adult stages. The eggs vary among different species and their patterns are helpful for taxonomic identification. The mosquito larvae are commonly referred to as "**wigglers**" and pupae as "**tumblers**." Larvae and pupae of mosquitoes are absolutely aquatic. Breeding sites may be anything from water in discarded automobile tires and the axils of plants, to pools, puddles, swamps, and lakes. It is very important to note that mosquito species differ in their breeding habits, biting behavior, flight range, and so forth. There are two subfamilies in the mosquito family (Culicidae): Anophelinae and Culicinae. Most larvae in the subfamily Culicinae hang down just under the water surface by the siphon, whereas Anopheline larvae lie horizontally just beneath the water surface supported by small notched organs of the thorax and clusters of float hairs along the abdomen. They have no prominent siphon. Mosquito larvae feed on suspended particles in the water as well as microorganisms. However, there are a few species that capture and eat other species. They undergo four molts (each successively larger), the last of which results in the pupal stage. Adult mosquitoes of both sexes obtain nourishment for basic metabolism and flight by feeding on nectar. In addition, females of most species need a blood meal from birds, mammals, or other vertebrates for egg development. They suck blood via specialized piercing-sucking mouthparts (proboscis).

4.2.1. Eggs and oviposition

Mosquitoes are ovipositor, they lay egg. The morphology and behavior of egg laying very considerably from species to species. The simplest procedure of egg lying is followed by many species of *Anopheles*; just before laying egg female roaming over the water surface, boobing up and down to the water surface and lying eggs more or less singly. The bobbing behavior in insect is called as "dapping" and observed in various other aquatic insect species such as mayflies and dragonflies. The eggs of *Anopheles* species are elongated, roughly cigar-shaped and have floats down their sides. A single female lay about 100-200 eggs during her life cycle. Even with high egg and intergenerational mortality, over a period of several weeks, a single successful breeding pair can create a population of thousands.



Chief distinguishing features of Anophelines and Culicines, *a.f.*, air floats; *a.g.*, anal gills; *ab.*, abdomen; *an.*, antenna; *br.*, mouth brush; *e.*, eye; *h.h.*, hooked (or grapnel) hairs; *n.o.*, notched organ; *pa.*, maxillary palp; *p.h.*, palmate (or float) hairs; *pr.*, proboscis; *1.sg.*, 1st abdominal segment; *8.sg.*, 8th abdominal segment; *si.*, siphon; *sp.*, spiracles; *th.*, thorax; *tr.*, respiratory trumpets; *w.s.*, water surface.

Figure. 4.1. The comparative chart showing various life stages and diagnostic features of Anophelines and Culicines.

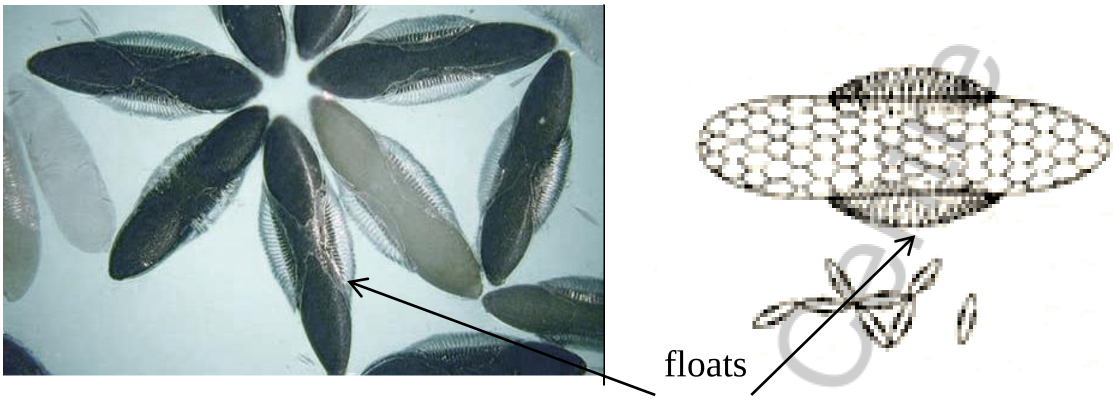
Source: http://www.infectionlandscapes.org/2011/03/malaria-part-2-vector.html

Occasionally *Anopheles* eggs (Fig 4.2.1) cluster together. These cluster loosely associated rather than compact glue rafts of eggs and showing a geometric pattern of laying eggs above the water surface.

Instead, the eggs form layers called "**rafts**" that float on the water (Fig 4.2.3). This is a common mode of oviposition, and most species of *Culex* are known for that habit, the female *Culex* laid her eggs in rafts. These egg rafts do not form adventitiously. Female *Culex* settles carefully on still water and lay egg one by one on water surface. To form rafts she twitches to arrange them in to a head-down array and attached together in a single clump, forming a floating egg rafts. *Culex* eggs have a cup-shaped corolla at one end, allowing them to sit vertically on the water surface in a raft; the upper ends have apical droplets with a chemical thought to maintain the raft upright. *Culex* mosquitoes lay their eggs on the surface of fresh or stagnant water. The water may be in tin cans, barrels, horse troughs, ornamental ponds, swimming pools, puddles, creeks, ditches, or marshy areas. Mosquitoes prefer water sheltered from the wind by grass and weeds.

Culex mosquitoes usually lay their eggs (Fig. 4.2.3) at night. A mosquito may lay a raft of eggs every third night during its life span. *Culex* mosquitoes lay their eggs one at a time, sticking them together to form a raft of from 200- 300 eggs. A raft of eggs looks like a speck of soot floating on the water and is about 1/4 inch long and 1/8 inch wide. Tiny mosquito larvae emerge from the eggs within 24 hours.

Aedes females generally laid their eggs (Fig 4.2.2) singly, much like *Anopheles* do. But unlike *Anopheles* they do not drop their eggs in to water. Instead *Aedes* females lay their eggs on dank mud or near the edges of water resources. The most common oviposition site is wall of a cavity such as resonating stump, plastic container, bucket or discarded tires. *Aedes* eggs withstand desiccation and hatched as they submerged with water. Straight after oviposition, they are not resistant to desiccation but must develop to a suitable degree first. Once they have achieved they can enter diapause and remain unharmed. In majority of mosquito species eggs hatched together at the same time, In contrast a batch of *Aedes* eggs do not hatched at the same time. This hatching behavior makes it much more difficult to control as compared to other species which hatched at the same time.



(A) (B)

Figure 4.2.1. Eggs of *Anopheles* spp. showing floats on both sides. **(A)** Microscopic view **(B)** diagrammatic representation.

(A) (B)

Figure 4.2.2. Eggs of *Aedes* spp. **(A)** *Aedes aegypti* eggs collected on filter paper **(B)** Microscopic view of *Aedes albopictus*.

(A) (B)

Figure 4.2.3. Eggs of *Culex* Spp. **(A)** showing raft of eggs **(B)** microscopic view of *Culex* eggs showing cigar- shaped eggs.

4.2.2. Larva

The mosquito larva has advanced morphological features. They have distinct well-developed head, mouth brushes for feeding, an elongated thorax without legs, and a segmented abdomen. They pass through four instar stages and resemble each other except their size. Larvae have spiracles or siphon for breathing. These spiracles are opening located on their eight abdominal segment. They frequently come to the water surface for breathing. Larvae feed on microscopic planktonic organisms such as bacteria, algae and other microbes. If they are disturbed they dive below. Larvae move to the water surface either through the propulsion movement created with the help of mouth brushes or through the jerk movement of their whole body, due to which commonly called as "**wigglers**" or "**wrigglers**".

Larvae exhibit clear taxonomic characteristics that can be easily observed on the slide mounted microscope (Fig. 4.3.1). Body is divided in to head, thorax and abdomen. The head is well-defined by a distinct capsule, bearing a pair of "eyes" comprise of clusters of lateral ocelli. A air of antenna of various size and shape is present on the head. Larvae have chewing mouth parts, having brushes and comb to create water current and assist in feeding. On labrum lateral brushes are present which create water current and draw suspended particles toward the mouth. Sweepers and brushes on the mandibles and maxillae collect the food particles from water and create a bolus of food in phyrnx. Behind the head, thorax is present. The thorax is wide and consist of legless indistinct segments.

The larva abdomen is cylindrical and narrow than thorax. It consists of eight apparent segments 8 and 9. A pair of spiracles opens on the dorsal side of the last segments. In some mosquitoes species such as Culicines the spiracles open at the terminal end of the respiratory siphon. Siphon is an elongated air tube. In anophelines the siphon is absent and spiracles are present on short spiracle plate. Segment 10, the anal segment, extends ventrally at an angle from the rest of the abdomen. On anal segment four anal papillae are present which are used for osmoregulation. The terminal region of larvae abdomen is so much important for classification as it contain various structures used for species identification (Fig 4.3.1). They include comb scales present on segment 8, a saddle sclerite encircling the anal segment, a spines present on the siphon, tufts and setae brushes.

Larva internal anatomy conforms to the general insect plan. The alimentary tract is straight, bearing eight large gastric caeca at the junction of the foregut and midgut in the thorax and

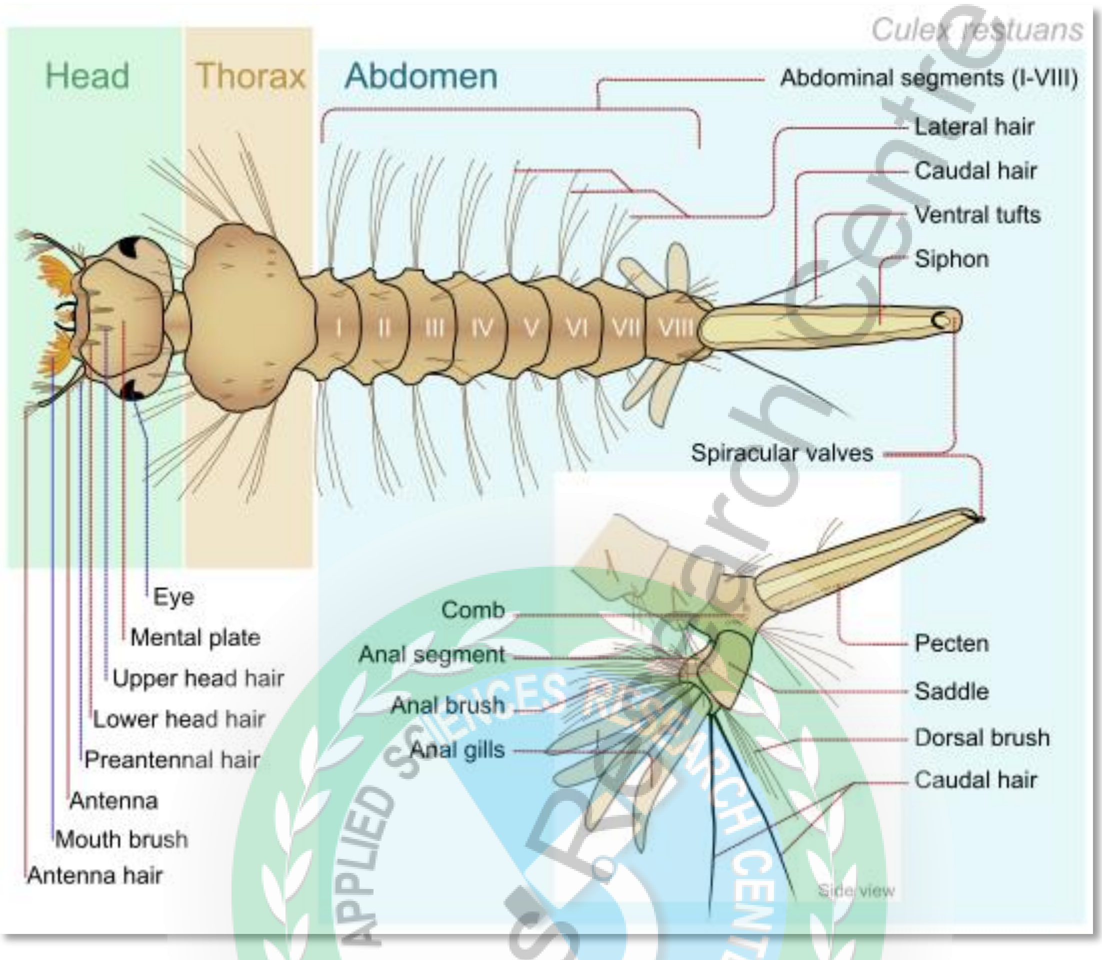


Figure 4.3.1. A representation of *Culex sp.* larvae showing its various morphological parts

five Malpighian tubules are present at the junction of midgut and hindgut. As most of the cuticle is semitransparent, two large tracheal trunks are obvious, extending forward from the spiracles to the thorax.

The larvae of *Anopheles* and *Culex sp*. are not very active and stay on the surface even if alarmed by a passing shadow over them; but opposite with the *Aedes sp*. larvae as they respond to every alarmed situation which they receive from the outer environment and go underneath the water surface i.e. it is difficult to collect *Aedes* larvae from the field. If undisturbed, they soon float up again. The *Culex* and *Aedes* larvae have long siphon through which they hang vertically downwards below the water surface but the *Anopheles* larvae lacks a siphon and lies horizontally below the water surface. Larvae passes through four instar stages. At the end of each stage, larvae undergo molting, shedding their skin and grow in size, until they metamorphose in to pupa after last instar.

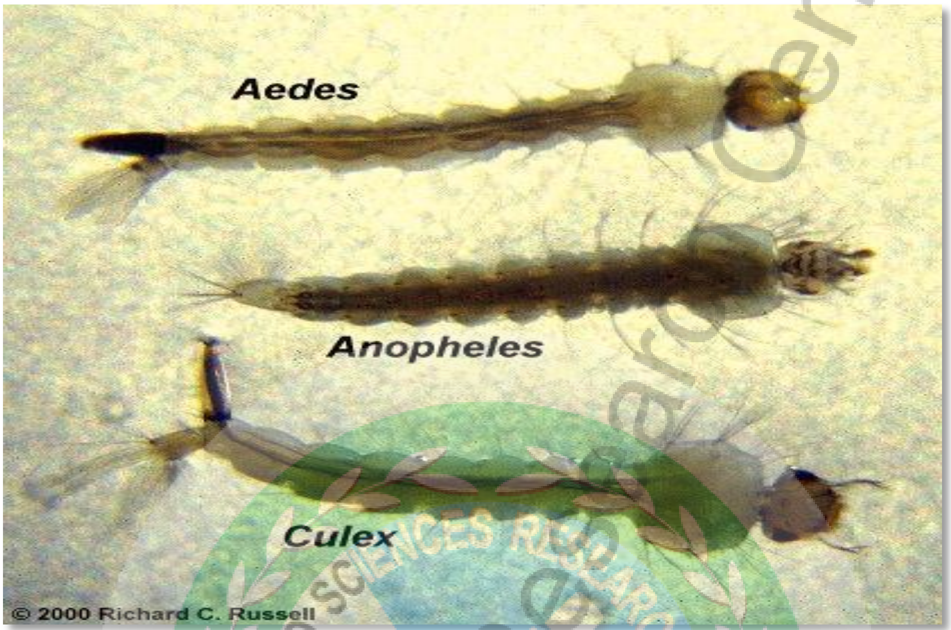


Figure 4.3.2. Showing *Aedes*, *Anopheles* and *Culex* Larvae

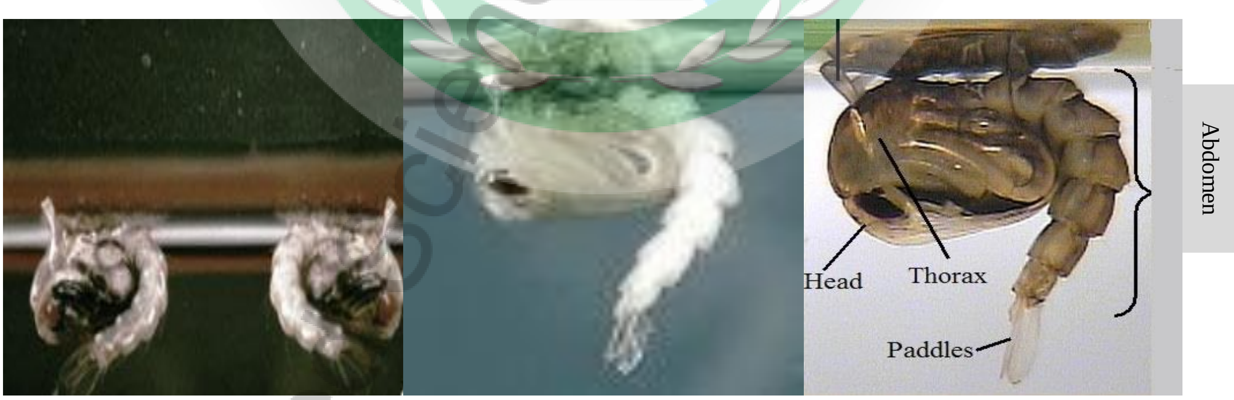


Figure 4.3.3. Showing *Anopheles sp., Aedes aegypti, and Culex sp.* pupa hanging with their air trumpets below the water surface.

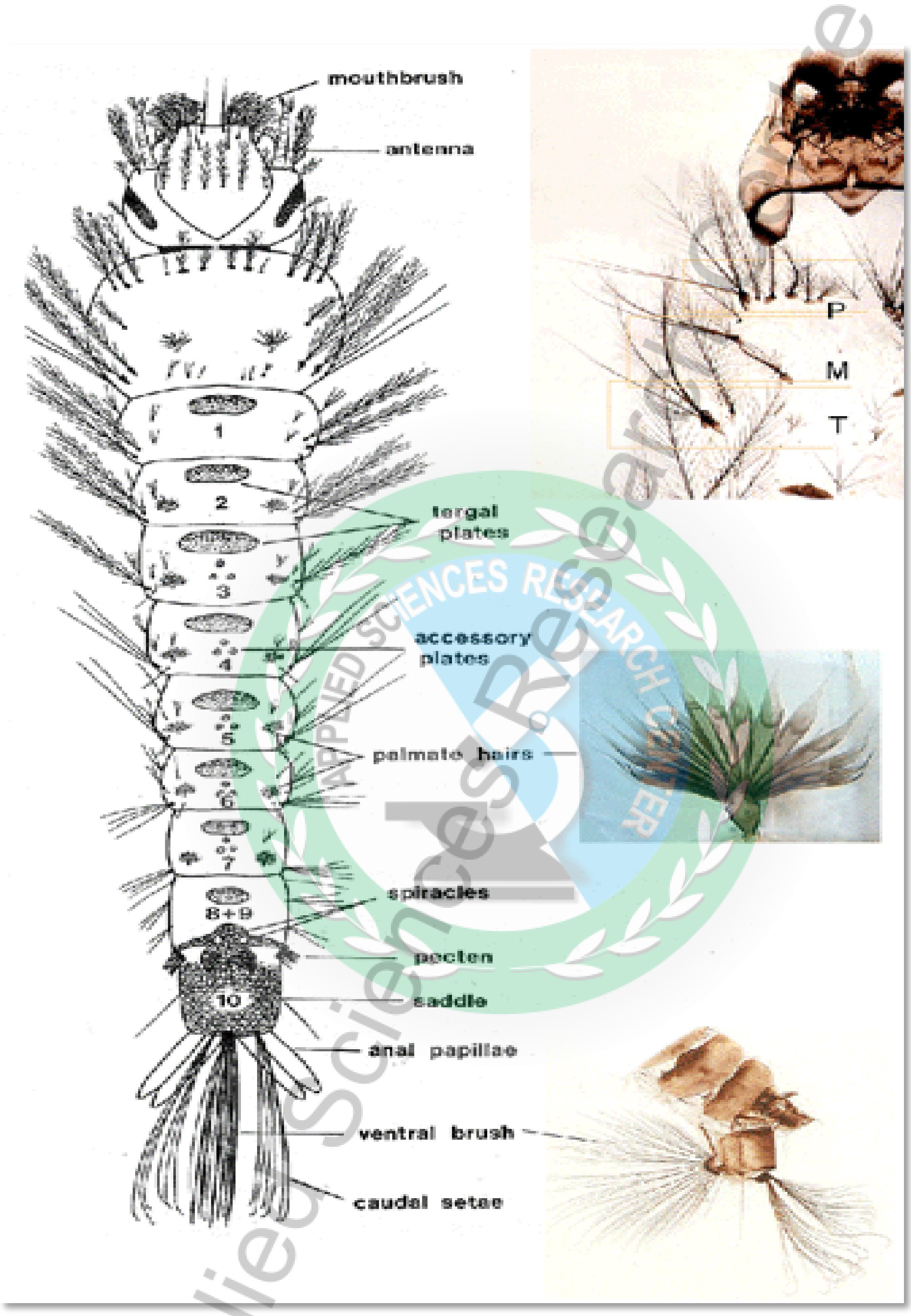


Figure 4.3.4. Diagrammatic representation of morphology of *Anopheles sp*. larvae with microscopic illustrations of head, palmate hairs, ventral brush and caudal setae (*J. P. Hervy et al. 1998, ORSTOM)*
Source: impact-malaria.com

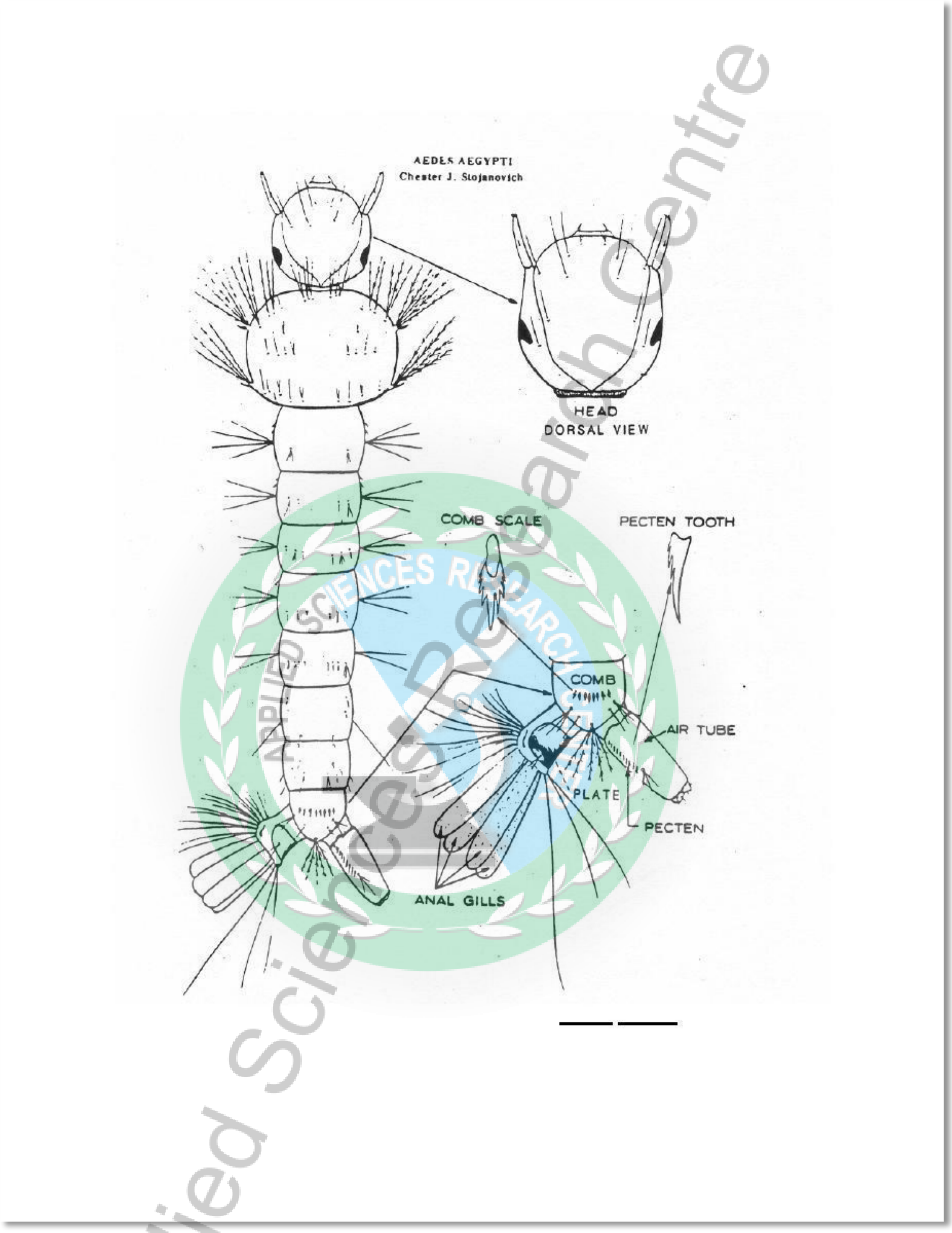


Figure 4.3.5. Showing the morphology of the *Aedes aegypti* larvae used for identification.
Source: http://armymedical.tpub.com/MD0170/MD01700121.htm

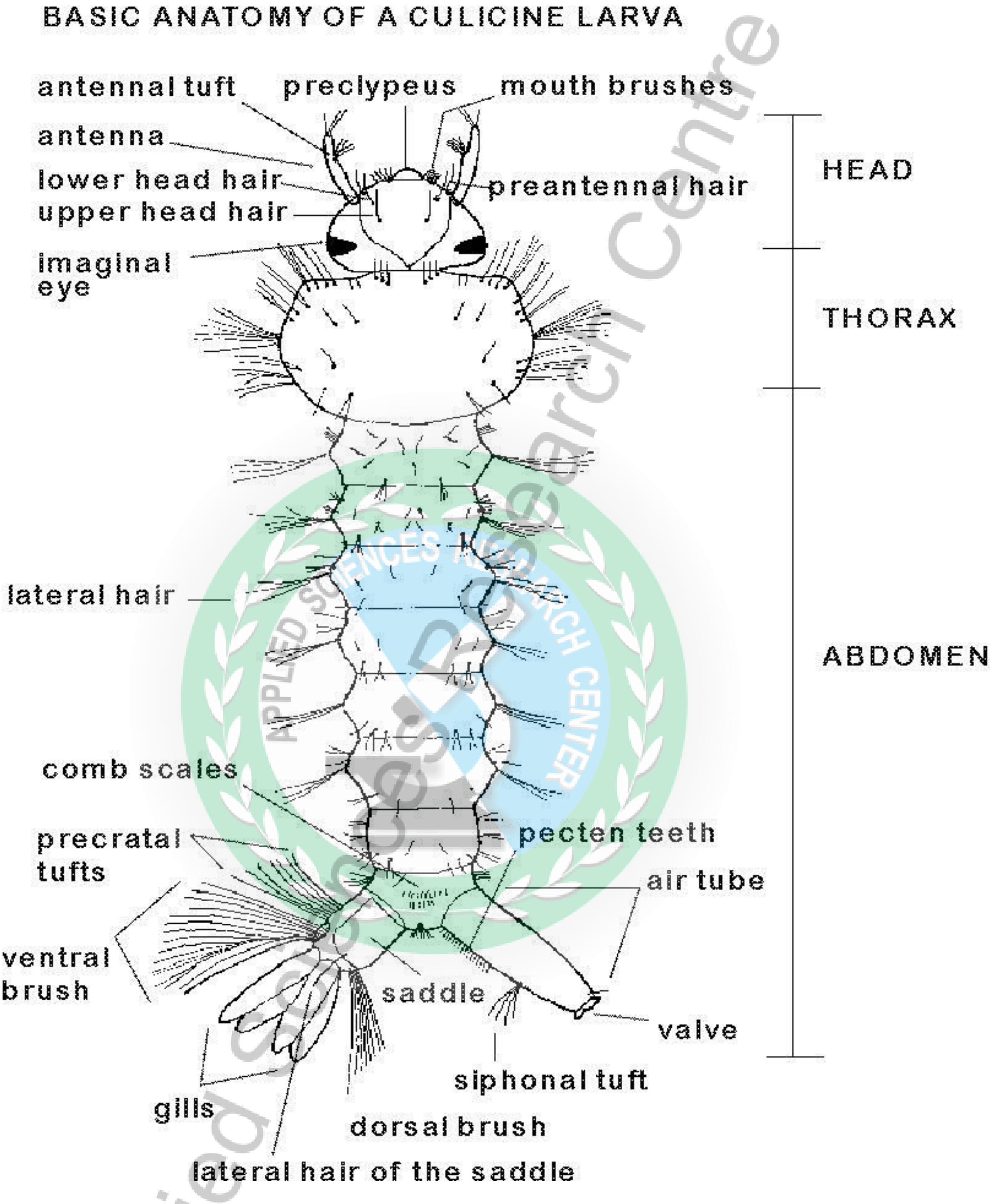


Figure 4.3.6. Diagrammatic representation of morphology of typical Culicine larvae. Source: www.glogster.com

4.2.3. Pupa

Pupa actively swims in water due to its swimming action, normally known as "**tumbler**". Just like larvae, pupae also come to the surface frequently for respiration to engulf the air. They have a pair of respiratory trumpets (fig 4.4) on their cephalothoraces for respiration. However, pupae do not feed during this stage; typically they pass their time hanging from the surface of the water by their respiratory trumpets. Within the cephalothorax the developing appendages of the adults head and thorax usually can be seen coiled ventrally; they envelop an air pocket, the ventral air space, which provides buoyancy to help maintain the pupa at the water surface when resting. At the end of the abdomen two broad paddles are attached to the eighth segment. If pupas are alarmed by passing shadow, they dive downwards by flipping their abdomen in the same way as larvae do. If they undisturbed, they soon move upward again. After a few days pupae rises to the water surface, cephalothorax split from dorsal surface and adult mosquito emerges. Emergence of adult from pupa depends on the ambient temperature and other circumstances. They are less active as compared to larvae, may be attributed to difference in feeding behavior of pupa and larvae. Pupa does not feed while larvae feed constantly (Spielman *et al.*, 2001).

4.2.4. Adult

Adult mosquitoes are slender, with thin legs and narrow, elongate wings (Fig. 4.5.1). The body surface is covered with scales, setae, and fine pile, creating the characteristic markings and colors of each species. The two compound eyes, each represented by 350-900 ommatidial lenses, wrap around the front and sides of the head. The antennae arise between the eyes, are long and filamentous, and are usually sexually dimorphic. In species in which sound is used to locate females in flight, the flagellum of the male antenna has whorls of much longer fibrillae, giving it a plumose appearance (Fig. 4.2.5). The pedicel at the base of the antennae is a large globular structure that contains, **Johnston's organ**, a mass of radially arranged mechanoreceptors that respond to vibrations of the flagellum induced by sound. In addition to the long fibrillae, the antenna has a variety of sensory structures, including those for detecting host odors.

Adults of *Aedes aegypti* commonly called as yellow fever mosquito shown in the fig male is on the left and females are on the right. Males have longer palp and bushy antennae. The developmental period from egg to adult varies among species and strongly correlated with environmental temperature. Some species of mosquito complete their life cycle within five days while in tropical conditions would be completed more or less within 40 days. The body size of adult mosquitoes varies depends on the larval population density and availability of food within the breeding site.

The mosquito proboscis is prominent, projecting anteriorly at least two-thirds the length of the abdomen. It consists of the basic complement of insect mouth parts: the labrum,paired mandibles, hypopharynx, paired maxillae, and labium. The first four structrues have evolved into fine stylets, forming a tightly fitting fascicle that in females is used to penetrate host skin (Fig. 4.5.2). The fascicle is cradled within the groove of the large and conspicuous labium, which comprises the bulk of the proboscis. The tip of the labium bears two small taste-sensitive labellar lobes and a short, pointed ligula (function unknown) between them. Of the fascicle of stylets, the hypopharynx and mandibles are narrowly pointed at their tips, whereas the maxillae end in serrated blades.

Both mandibles and maxilae puncture the skin and advance the fascicle into the host's tissue. A salivary channel runs the lengh of the hypoharynx, delivering saliva to the tissue during probing. The labrum is curled laterally to form a food canal for drawing the host's blood or a sugar solution up the proboscis. In males, and in female of nonblood-feeding species, the mandibles and maxillae have atrophied, so they cannot pierce skin. In both sexes of Toxorhynchites, the nonpiercing proboscis is curved downward. Maxillary palps arise at the base of the proboscis and bear several kinds of sensilla, though there are many exceptions, palps usually are short in female culicines, but longer than the proboscis in most male culicines and also in both sexes of anophelines (Fig. 4.5.2).

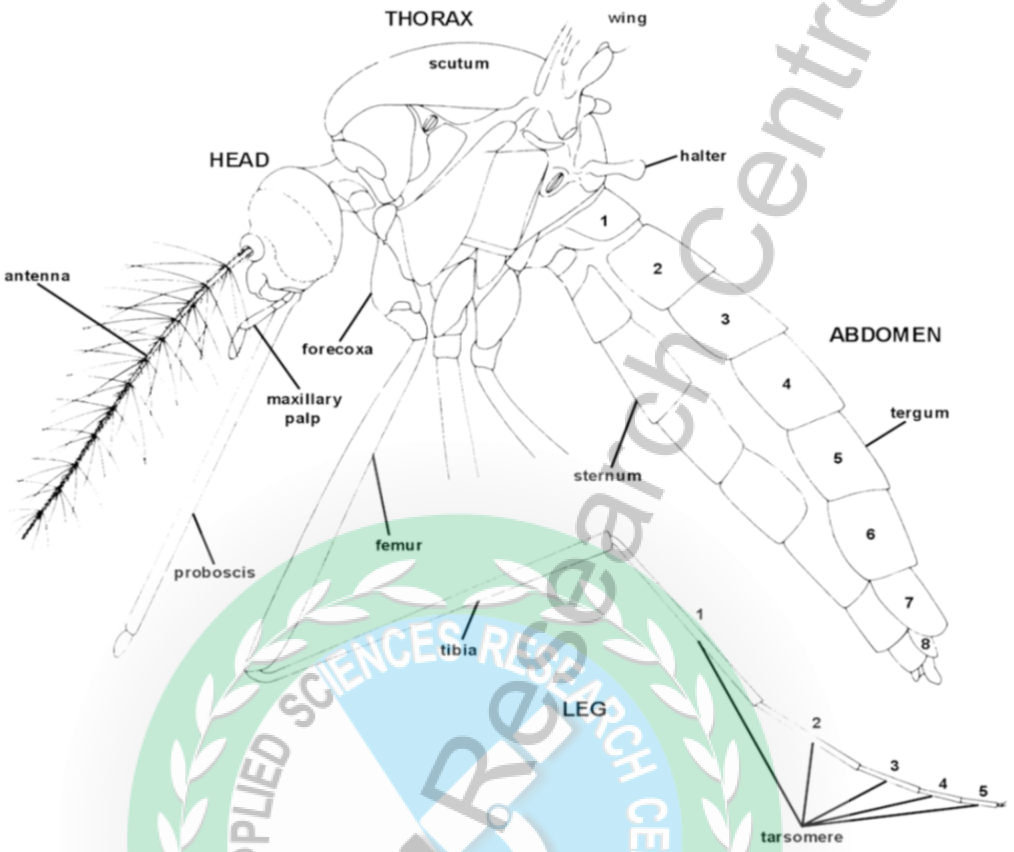


Figure 4.5.1. Morphology of Adult mosquito

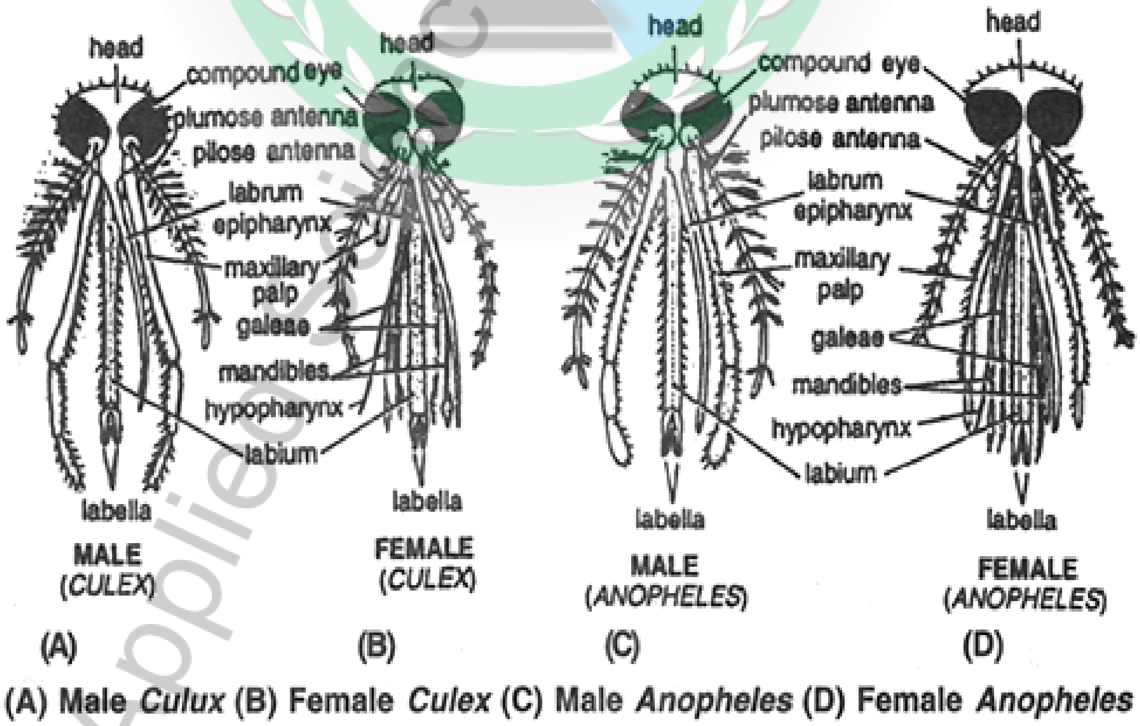


Figure 4.5.2. Showing the mouth parts of the male and female mosquitoes.

The mosquito thorax forms a single, relatively rigid muscle-filled locomotor unit with obscured segmentation. The meso and metathorax each has a pair of lateral spiracles. The slender legs are attached close together on the underside of the thorax by elongate, downward-projecting coxae; the tarsi are tipped with two claws and a central pad, the **empodium**. The wings are narrow, have a distinctive pattern of veins, and bear scales along the veins and the hind margin, the latter forming a fringe. The **halteres,** tiny modified hindwings used in fight control, are located right behind the insertions of the wings.

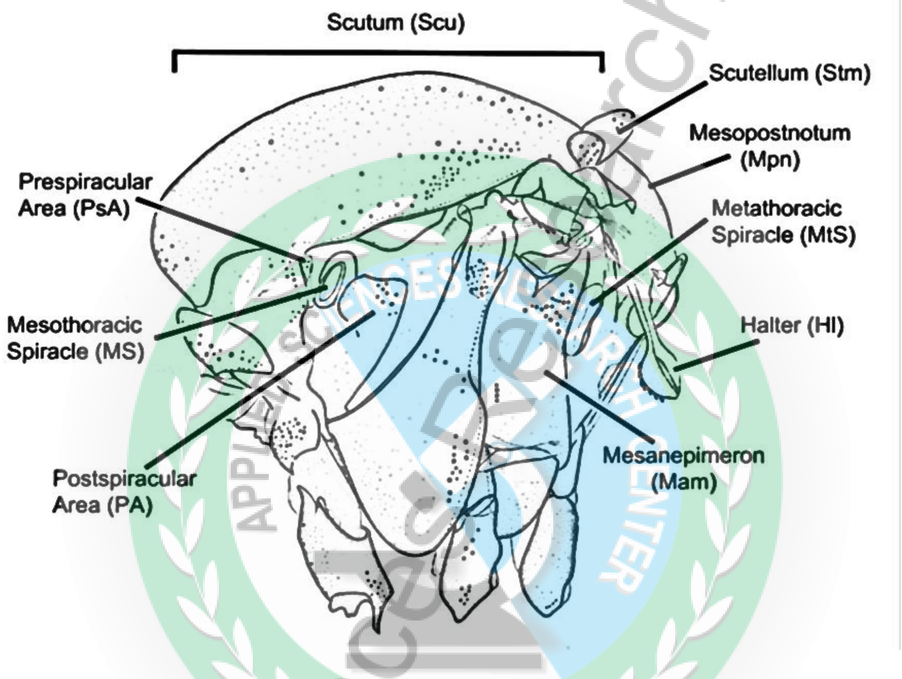


Figure 4.5.3. The lateral view of female mosquito thorax showing various areas, thoracic spiracles and halter.

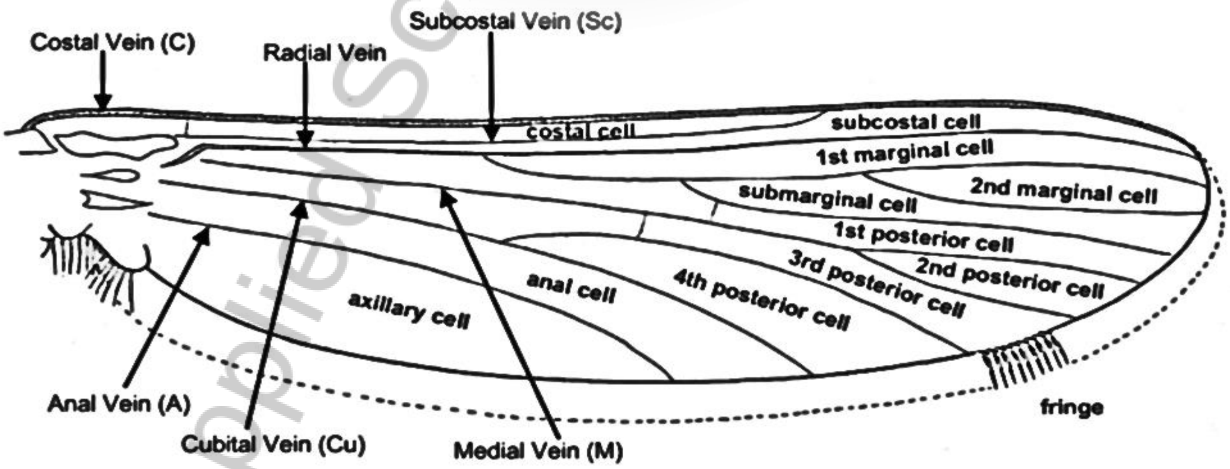


Figure 4.5.4. Showing the wing venation of adult mosquito.

The thorax is specialized for locomotion. Three pairs of legs and a pair of wings are attached to the thorax. The insect wing is an outgrowth of the exoskeleton. The Anopheles mosquito can fly for up to four hours continuously at 1–2 km/h (0.6–1 mph), [21] traveling up to 12 km (7.5 mi) in a night. Males beat their wings between 450 and 600 times per second [22].

The abdomen is clearly segmented and capable of extensive expansion and some movement, owing to the membranous area between each set of tergites and sternites (Figure 4.5.1). This allows for expansion of the abdominal wall to accommodate large blood and sugar meals and developing chutches of eggs. Abdominal segments 5 through 8 are progressively smaller so that the abdomen tapers toward the posterior end. Segment 9 is quite small and bears the cerci, the postgenital lobe of the female, and the claspers and other genitalic structure, or terminalia, of the male (Fig. 4.5.1). At emergence, the male genitalia are inverted, During the first hours of adulthood, segments 8 and 9 of males together rotate 180 to reach the mature position. The complex and varied male genitalia are a useful source of characters for species identification.

Located within the thorax are a pair of three-lobed salivary glands, whose ducts join anteriorly to form a common salivary duct that enters the hypopharynx. In males, these glands produce saliva used only in sugar-feeding; in females, some portions are devoted to sugar-feeding and other to blood-feeding. The foregut, which begins in the head with the muscular cibarium and pharynx, pumps food up the labral food canal. The tubular esophagus extends through the cervix, or neck, into the thorax. There it is modified to form three diverticula, including two small dorsal outpocketings and a large ventral crop; the crop extends through the thorax and expands to form a large sac within the abdomen. Imbibed sugar solutions are stored in these diverticular and pass, a little at a time, through the proventricular valve into the midgut. A blood meal, on the other hand, passes directly into the widened posterior Midgut, or stomach. There it becomes surrounded by a semipermeable, sac-like **peritrophic membrane** secreted by the midgut epithelium. The resulting blood bolus then is digested and absorbed.

The pyloric valve separates the midgut from hindgut; five Malpighian tubules empty into the hindgut just beyond the valve in the pyloric chamber. The anterior portion of the hindgut is tubular and loosely coiled; the posterior part is enlarged to form a bulbous rectum with large papillae projecting into it. The papillae probably are involved in resorption of salt ions. Paired gonads are located in the posterior one-third of the abdomen. The testes of males contain packets of sperm in various stages of maturation. A duct extending posteriorly form each testis widens to form a seminal vesicle, which stores mature sperm. The two seminal vesicles lie together and unite posteriorly to form the ejaculatory duct. Two large accessory glands open into this duct, which leads to the aedeagus. In females, the reproductive system consists of a pair of ovaries and accessory structures (Fig. 4.5.5). Each ovary includes a few dozen to over 200 polytrophic ovarioles, the egg-forming units. A duct from each ovary extends posteriorly, and the two unite to form a common oviduct, Opening into the genital chamber by tiny ducts are one to three sperm-storing spermathecae, a small accessory gland, and the seminal bursa (lacking in most *Anopheles*). Which receives semen from the male during mating.

After few days of emergence adult mosquitoes mate. In most species male mosquitoes form large swarms usually around down and dusk while female mosquitoes fly in to swarms to mate. Male mosquitoes feed on plant nectar and other food sources. Female mosquito feed on blood. After taking full blood meal, the female rest for few days and blood provide the essential nutrients required to mature the eggs. Maturation of the egg depends on the external temperature and blood availability, usually takes three to four days. Once the eggs fully matured female mosquito lay them and resume new host-seeking.

The cycle replicates itself until the female dies. Life span of females can be varied, in captivity she lives more than a month, while in nature they do not alive longer than one to two weeks. Their longevity of time depends on climatic conditions such as temperature, humidity, and availability of blood. Average length of the adult body varies, but is rarely more than 16 mm and weight up to 2.5 milligrams. All mosquitoes have elongated, slender divided in to three segments: head at proximal end, thorax at middle and abdomen at distal end.

Figure 4.5.5. *Aedes aegypti* adult mosquito **(A)** Male (left) **(B)** Female (right) **(C)** oviposition of female mosquito.

5.1.1. Feeding by adults

A mosquito has chemical, visual and heat sensors for tracking the prey. Typically, both male and female mosquitoes feed on plant juices. Male have sucking mouthparts while females have piercing and biting mouthparts. Female act as ectoparasite with mouthparts adapted for piercing the host skin and sucking their blood. Female required the blood for the development and maturation of the egg before laying them. She can produce more eggs after a blood meal. Both plant juice and blood are important source of energy in the form of sugar. While blood also provide the lipids and protein required for the production of the egg.

In most species, females are **anautogenous**. In **anautogenous** female mosquitoes the egg follicles remaining in the resting stage until a blood meal is taken and egg maturation started and develop one mature clutch of egg exhibiting what is known as **gonotrophic concordance.** While females of **autogenous** species do not require blood for egg maturation as **autogenous females** can produced the mature eggs without a blood meal; among these there are facultative and obligate types. A **facultatively autogenous** female typically develops only the first clutch of eggs without blood; she does so only if she emerges with sufficient reserves and cannot readily find blood. Thereafter, a blood meal is required for each **gonotrophic cycle**. On the other hand **obligately autogenous** females have atrophied feeding stylets, certainly not take blood meal and survive completely on their larval reserves food and plant sugar. Autogeny has been reviewed by O'Meara (1985). At the other extreme, there are some anautogenous species that take blood not only at the start of each gonotrophic cycle but also take additional blood meal during egg development. These supplementary blood meals can provide extra energy, perform function as a substitute for sugar (e.g., domestic *Aedes aegypti* and *Anopheles gambiae*).

Female mosquitoes pursue their blood host by sensing organic substances such as carbon dioxide (CO_2) and 1-octen-3-ol produced from the host. Mosquitoes favor some host over others. The chosen victim's sweat merely odors better than others because of the proportions of the carbon dioxide, octenol and other compounds that make up body odor [25]. The most powerful semiochemical that elicits the intense sense of smell of *Culex quinquefasciatus* is non-anal [26]. A mosquito has well developed olfactory system for sniffing out blood sources. About 72 types of odor receptors are present on the antenna, from which at least 27 are modified to perceive chemicals compounds found in perspiration. [27] In *Aedes*, the exploration for a target host takes place in two stages or phases. First, the mosquito reveals a nonspecific searching behavior until the sensitivity of host stimulants, and then it follows a targeted approach [28].

Usually mosquito species are crepuscular feeders, feed at the time of dawn and dusk. During the hotter part of the day, most mosquitoes respite in a shady, cool place and wait for the dusks, however if they are disturbed they may bite during day time [29]. Some species are known to fly and feed during day time such as the Asian tiger mosquito. Blood sucking mosquitos have anticoagulant substance in their saliva. Mosquitoes inject saliva into the body of their host prior to and during blood feeding. This saliva serves as an anticoagulant and prevents the clot to form. The saliva also is the key route by which mosquito transfer the disease causing pathogens to the hosts' interior body.

The bump left on the victim's skin after a mosquito bites is called a **wheal**, which is caused by histamines trying to fight off the protein left by the attacking insect [30].

When mosquito bite on the host body, histamines trying to fight against the protein left by biting of the mosquito. As a result of this immune reaction a red bump is left on the skin, these bumps are called as **wheal**.

Mosquitoes of the genus Toxorhynchites always feed on the blood [31] the larvae of Toxorhynchites prey on the larvae of other mosquitoes. They are mosquito eaters and successfully used in the past as biological control agents to control the population of mosquito [32].

5.2.1. Mouthparts

Mosquito mouthparts are modified according to feeding habitate. Females have well developed mouthparts as compared to males. As females bite and suck the blood of host. They have piercing and sucking mouth parts. Apart from blood, females also drink miscellaneous fluid rich in sugar content such as plant nectar and honey dew to obtained energy. Due to which their mouthparts show high degree of modifications. While males have simple sucking mouth parts because they feed just on sugary fluid [33].

Externally, in mosquito highly prominent feeding structure is the **proboscis**. They have **labium**, which forms the sheath encircling the rest of the mouthparts. Its mouthparts will be enclosed entirely in this sheath, and it will touch the tip of the labium to the skin in several places. Sometimes, it will bite the host directly, while other times, it will roam around the host, and apparently searching for a appropriate place. Sometimes, it will hover for a longer time, and ultimately fly away without biting. Probably, this probing behavior may be in search easily accessible host blood vessels, but the exact mechanism is dubious. At the tip of the labium there are two taste receptors, which may well play a role [34].

At the time of biting female mosquito bends the labium back ward but does not insert it in to host skin. Labium tip remains in contact with host skin as well as acting as a guide for other mouth parts. Beside the **labium** there are six other mouthparts including **two maxillae, two mandibles**, **labrum** and **hypopharynx**.

The mandibles and the maxillae are piercing mouthparts. The mandibles are sharp and pointed, while the maxillae ends have toothed "blades". To power them into the skin, the mosquito moves its head backwards and forwards. On one movement, the maxillae are moved as far forward as possible, and on the other movement the mandibles are pressed deeper into the skin by levering against the maxillae. The maxillae toothed blades grip the skin so, do not slip back while piercing the skin.

Structurally both hypopharany and labrum are hollow. **Saliva** with anticoagulant is propelled down the hypopharynx to inhibit blood clotting, and blood is drawn up from the host body to the labrum. In order understand the function mosquito mouthparts; it is helpful to make it comparison with a chewing insect, such as a dragonfly. A dragonfly has two mandibles for chewing the food and two maxillae used to hold the food the labrum forms the top of the mouth and labium forms the floor of the dragonfly's mouth, while the hypopharynx used in swallowing and present inside the mouth. Conceptually, then, the mosquito's proboscis is an adaptation of the mouthparts according to the feeding habit. The labium lies underneath the other mouthparts and extended into a proboscis. The maxillae "hold" the food while the mandibles "bite" it. The labrum has been developed into a channeled blade the length of the proboscis, with a cross-section like an inverted "U". Finally, the hypopharynx has been stretched into a cylindrical tube which delivers saliva at the end of the proboscis. The upper surface of proboscis is flattened to large extent so, when it is pressed against, the labrum forms a closed tube for carrying blood from the host body [35].

5.2.2. Saliva

To obtain the blood meal mosquitoes must circumvent the physiological responses of vertebrates. Just like other blood feeding insects, mosquitoes develop the mechanism to block the hemostasis mechanism with their saliva. Their saliva contained a mixture of saliva proteins which prevent the vasoconstriction, aggregation of platelets, blood clotting, immunity response and creates inflammation [36].

All blood feeding insects known as hematophagous arthropod contains at least one anticlotting, vasodilatory and antiplatelet substance. Mosquito saliva contained antimicrobial substances to prevent the bacterial growth in sugar meal. [37] as well as have several enzymes that help in feeding [38]. Biochemical analysis of mosquito saliva showed that it usually contains less than 20 dominant proteins [39]. Even though the pronounced progresses in information of these molecules and their importance in blood feeding achieved, researchers still cannot describe complete functions of the molecules found in saliva [39]. One promising application is the production of anticlotting drugs for cardiovascular disease, such as clotting inhibitors and blood vessel dilators.

It is now well documented that blood sucking ticks, sandflies, and mosquitoes, have an capability to modulate the immune response of their host on which they feed.[36] The existence of this activity in parasite saliva is a reflection of the inherent corresponding and interrelated nature of the host inflammatory/immunological responses and the fundamental requirement to inhibit these host resistances mechanisms from distracting successful feeding. The mechanism through which mosquito saliva-induce alterations in the host immune response are vague. Scientist described that a factor in mosquito saliva directly suppresses TNF-α release, but not prevent the inflammatory histamine secretion from activated mast cells [40].

Experimentations by Cross *et al.* (1994) revealed that the addition of *Ae. aegypti* mosquito saliva in to mammalian cell cultures lead to a suppression of IFN-γ production and interleukin (IL)-2, while the cytokines IL-4 and IL-5 are unaffected by mosquito saliva.[41] Experimental studies showed that when activated splenocytes separated from mice fed upon by *Cx. pipiens* or *Ae. aegypti* mosquitoes produced high level of IL-4 and IL-10 and suppressed production of IFN-γ [42]. Surprisingly, this alteration in cytokine expression is detected in splenocytes for about 10 days after mosquito bite, proposing natural feeding of mosquitoes can have a intense, long-term, and systemic effect on the immune system and responses [42].

Mosquito saliva suppresses the activity of T cells as they are more susceptible to saliva attack.[43]. T cell populations are distinctly susceptible to the suppressive effect of mosquito saliva, showing increased mortality and decreased division rates.[43] Parallel work by Wasserman *et al.* (2004) revealed that T- and B-cell propagation was suppressed in a dose dependent manner with concentrations as low as 1/7 of the saliva.[44] Similarly, Depinay *et al.* (2005) detected a suppression or inhibition of antibody-specific T cell responses mediated by saliva and reliant on mast cells and IL-10 expression.[45]

A current study showed that expression of interferon−α/β mosquito saliva can also reduce by mosquito saliva [46]. Type I interferons (IFN) play important role in the recovery of viral infections and has been confirmed by in vivo administration of IFN-inducers or IFN [47]. More recent studies showed that mosquito saliva aggravates West Nile virus infection.[48] as well as other mosquito-transmitted viruses [49].

6.1. Egg development and blood digestion

Mosquito eggs become mature favorable temperature and after blood feeding. During maturation the egg follicle with in each ovarioles passes through a series of five physiological stages (Figure), demonstrated by Christophers (1911) and summarized by Clements (1992). Development of follicles take place synchronously, First stage is **previtellogenic phase** (stages G through II). The second stage is **resting phase** in which follicles usually stop further development at stage IIa or IIb. Resting stage end as female mosquito suck the blood, within hours of blood feeding, follicles undergoes the **initiation phase** (stage IIIa). During **initiation phase** body fats synthesize the vitellogenin (Yolk). After that follicles enter in to the **trophic phase** (stages IIIb and IV), the prominent stage of follicle development and yolk incorporation. After yolk incorporation **post-trophic phase** started, in which eggs fully mature and eggshell or **chorion** develop around the egg. After all these changes eggs are ready to be oviposited, by this time, the next follicle in each ovariole either has progressed to stage I or already has reached the resting stage (stage II). In the first case, it awaits oviposition before developing to the resting stage. Once oviposition has occurred and these new follicles are in the resting stage, the next blood meal is necessary for subsequent follicle development. The entire cycle of egg production, from blood meal through oviposition, is the **gonotrophic cycle**.

Sizes of the female body, energy reserves, and blood meal are the main factors that determine the number of mature eggs to produce at a time. The follicles that do not pass beyond stage II degenerate. As eggs become fully mature in the ovarioles, the ovaries may occupy approximately the entire volume of the distended abdomen. The gravid female fertilizes one egg at a time, as each passes down the oviduct to be oviposited on the water or a damp substrate.

When all eggs have been fertilized and expelled during oviposition, the ovaries return to their pretrophic size, but the tracheae on the ovaries, which had been tightly coiled as **tracheal skeins** before the eggs developed, become stretched and straightened, In Anopheles species, a swelling at the base of each lateral oviduct, the **ampulla**, becomes permanently stretched during the first oviposition. These sign serve to distinguish parous females, those that have completed at least one gonotrophic cycle, from nulliparous females, those that have not. The number of completed gonotrophic cycles also can be determined. According to current interpretation, each ovariole; ovulating a mature egg is left with an egg sac, which becomes reduced to a zone of granules in the calyx, the ovarioles connection to the lateral oviduct. Furthermore, a dilatation is formed in the stalk of each ovariole where a follicle has degenerated after a blood meal, instead of developing into an egg (Fig. 14.15). Thus, a count of the maximum numbers, per ovariole, of dilatations in the stalk and zones of granules in the calyx yields an estimate of the number of gonotrophic cycles completed. This physiological age grading can provide the medical entomologist with valuable information on the age of individuals and the age structure of a mosquito population and application are given by Detinova (1962), Sokolova (1994), Fox and Brust (1994), and Hoc (1996).

Female mosquitoes feed on two different food sources. Need energetic nectar sugar obtained from plants and blood as a source of protein for egg maturation and development. When female host find a suitable host, she takes blood as much as possible. However this generates another problem. To digest this large volume of blood female mosquito required large quantity of sugar solution at the same time. To overcome this problem, mosquito digestive system modified to store both types of food meals. When mosquito takes a sugar solution, it is store in crop and released from crop in to the stomach as needed. Due to this mosquito not becomes full of sugar solution so, remain empty to some extent and increased the chance of taking blood as she get.

Blood directly move in to the stomach rather than to store in crop. Mammalian hosts whose blood pressure is high, mosquito preferably feeds on active blood vessel where high blood pressure exists. High blood pressure assists in filling the stomach quickly. If, instead of slapping a feeding mosquito, one stretches one's skin so that it grasps the proboscis and the mosquito cannot withdraw it, the pressure will expand the gut until it rupture and the mosquito dies.[50][better source needed] In the unmolested mosquito, it will withdraw as the stomach fills up. The stomach lining secretes a peritrophic membrane that surrounds the blood. This peritrophic membrane keeps the blood isolated from anything else in the stomach.

However, like several other insects that survive on diluted, purely liquescent diet, particularly many of the Homoptera, adult mosquitoes excrete extra diluted liquid fractions even as they feed. (Figure See the photograph of a feeding *Anopheles stephensi*: Note that the excreted droplet clearly is not whole blood, being far more dilute). If they are undisturbed, allows the mosquitoes to feed continually, until they have acquired and accumulated a full meal of nutrient solids. As a result, a mosquito full with blood can remain to absorb sugar, even as the blood is gradually digest and processed over a period of several days [34]. The midgut of the female digestive system synthesizes proteolytic enzymes to hydrolyze the blood proteins into free amino acids. These amino acids are used as building blocks for the synthesis of egg yolk proteins.

In the Anopheles *stephensi*, activity of trypsin is limited completely to the posterior part of midgut. Before taking blood meal trypsin is inactivity but its activity enhances constantly up to 30 hours after feeding, and afterward reverts to baseline levels after 60 hours. Another enzyme Aminopeptidase is active in the anterior and posterior midgut portion before as well as after feeding. In the whole midgut, activity increases from a baseline of almost three enzyme units (EU) per midgut to a maximum of 12 EU at 30 hours after the blood meal, successively dropping to baseline points by 60 hours. A similar cycle of activity occurs in the anterior midgut and posterior midgut lumen, whereas aminopeptidase in the posterior midgut epithelium reduced in activity during digestion. Aminopeptidase in the anterior midgut is kept at a constant, low level, showing no major difference with time after feeding. Another important enzyme Alpha-glucosidase is active in anterior and posterior midguts before and after feeding. In whole midgut alpha-glucosidase activity rises gradually up to 18 hours after taking the blood meal, than increases quickly to a maximum level at 30 hours after the blood meal, whereas the subsequent decline in activity is less sure. All posterior midgut activities are bound to the posterior midgut lumen.

It has been documented that more than 25% of alpha-glucosidase activity is located in the anterior midgut. Protease enzymes required for digestion of protein present only in the posterior midgut part. Trypsin is primary hydrolytic enzyme and secreted in to the lumen of posterior midgut without the activation of posterior midgut epithelium. Aminopetidase activity may be luminal as well as cellular. Luminal activity of Aminopetidase observes only in the posterior midgut, while cellular Aminopetidase activity present in both anterior and posterior midguts. After taking blood meal Alpha-glucosidase activity is elevated in the posterior midgut, while activity in the anterior midgut is consistent with a nectar-processing role for this midgut section [51].

7. 1. Disease

Mosquitoes are vectors of several disease-causing infectious agents such as parasites and viruses. Infected mosquitoes transmit these microscopic agents from person to person without showing symptoms themselves. Mosquito-borne diseases include:

1. Viral infections (dengue fever, yellow fever and chikungunya) caused by viruses transmitted generally through biting of *Aedes aegypti*. Dengue fever is the most common cause of fever in travelers returning from the Caribbean, Central America, and South Central Asia. This disease is spread through the bites of infected mosquitoes and cannot be spread person to person.

2. Sometime severe dengue fever can be fatal, however with good care and medical treatment mortality rate can be let down, consequently less than 1% of patients die from dengue fever [56].

3. The mosquitoes of the genus Anopheles carry various species of Plasmodium and caused parasitic diseases collectively called malaria.

4. Elephantiasis caused by Lymphatic filariasis may spread by a wide variety of mosquito species [57]. West Nile virus is a alarm in the United States, but there are no trustworthy statistics on worldwide cases.

5. Encephalitis caused by Eastern equine encephalitis virus and most common in the eastern United States.

6. *Culex* and *Culiseta* are vectors of a bacterial disease caused by *Francisella tularensis*, is variously transmitted through viral bites [58].

7. Potential transmission of HIV was originally a public health concern, but practical considerations and detailed studies of epidemiological patterns suggest that any transmission of the HIV virus by mosquitoes is at worst extremely unlikely [59].

8. Epidemiological studies showed that several species of mosquitoes are estimated to spread abundant types of disease to more than 700 million people annually in South America, Mexico, Russia, Africa, Central America, and various regions of Asia, with millions of resultant deaths. At least about two million people annually die because of these diseases, and the infection rates are many times higher still.

9. Approaches used to inhibit the spread of disease or to defend peoples in regions where disease is endemic, include:

- Vector control aimed at mosquito eradication and elimination
- Disease control or prevention, using prophylactic medicines, drugs and vaccines
- Inhibition of mosquito bites, with insecticides, nets, and repellents
- Since most such diseases are carried by "elderly" female mosquitoes, some scientists have recommended concentrating on these to prevent the evolution of resistance [60].

8.1. Mosquito Surveillance

Mosquito surveillance is a prerequisite to an effective, efficient, and environmentally sound mosquito control program. Surveillance is used to define the nature and extent of the mosquito problem and to gauge daily mosquito control operations. It provides a basis for evaluating the effectiveness of control operations, the data needed to comply with state rules and regulations regarding the justification for treatments, and a basis for evaluating the potential for transmission of mosquito-borne diseases.

Mosquito surveillance is most effective when combined with an ongoing program for monitoring meteorological, astronomical, and environmental factors that may influence mosquito population change. For example, rainfall and ground water levels, temperature, relative humidity, wind direction and velocity, tidal changes, lunar cycles, storm water and wastewater management, and land use patterns are all factors that may influence mosquito population levels and adult mosquito flight behavior and dispersal. For effective mosquito surveillance, three things are important:

1. On-site inspection
2. Sample collection
3. Identification

Identification helps in locating breeding sites of mosquitoes that is marked on the map. Mapping is essential requirement for monitoring the breeding sites of mosquitoes during epidemics. Standardized methods for surveillance exists (Services, 1993) and consists of larval and adult sampling. For sampling following traps/apparatus are used.

1. Light traps
2. Baited traps
3. Gravid traps
4. Oviposition traps
5. Larval sampling
6. Aspirator

Every mosquito species has its own biology, behavior and ecology. In order, to have effective control measures; it is very important to understand it.

8.2.1. Surveillance of *Aedes* spp. in Pakistan

In 1934, *Aedes aegypti* was reported in the following cities of Pakistan: Peshawar, Dera Ismail Khan, Larkhana and Karachi (Barraud, 1934). In 1960, Qutubuddin reported the surveillance of *Aedes aegypti* in Kohat Hangu Valley of Pakistan. In 1950, Malaria Eradication Program started in Pakistan and as a result of this many mosquito species declined. *Aedes aegypti* surveillance regained in 1986 in the city of Karachi (Kamimura *et al.*, 1986), in 1993, Landikotal (Border area of Peshawar and Afganistan) (Suleman, 1996) and in 2010, Karachi and its surrounding reported by Tarq *et al*, 2010.

8.2.2. Surveillance of *Anopheles* spp. in Pakistan

Surface irrigation system in the Punjab province of Pakistan has been carried out sinc 19th century after the development of Indus Basin Irrigation System.

Various field has been carried out to understand how these irrigation systems related with the adult anopheline mosquitoes (Diptera: Culicidae) population dynamics and related with transmission of malaria in rural areas.‘In this paper we present our observations from three villages located along an irrigation canal in South Punjab. The study was carried out from 1 April 1999 to 31 March 2000. Mosquitoes were collected from bedrooms using the pyrethroid spraycatch method and from vegetation and animal sheds using backpack aspirators. Overall, *Anopheles subpictus* Grassi *sensu lato* predominated (55.6%), followed by *An. stephensi* Liston s. *l.* (41.4%), *An. culicifacies* Giles s. *l.* (2.0%), *An. Pulcherrimus* Theobald (1.0%) and *An. peditaeniatus* Leicester (0.1%). Most mosquitoes (98.8%) were collected from indoor resting-sites whereas collections from potential resting-sites outdoors accounted for only 1.2% of total anopheline densities, confirming the endophilic behaviour of anophelines in Pakistan. *Anopheles stephensi*, *An. culicifacies* and *An. subpictus* populations peaked in August, September and October, respectively. High temperatures and low rainfall negatively affected seasonal abundance in our area. There were interesting differences in anopheline fauna between villages, with *An. culicifacies* occurring almost exclusively in the village at the head of the irrigation canal, where waterlogged and irrigated fields prevailed. Monthly house-to-house fever surveys showed that malaria transmission remained low with an overall slide positivity rate of 2.4% and all cases were due to infection with *Plasmodium vivax*. The most plausible explanation for low transmission in our study area seems to be the low density of Pakistan's primary malaria vector, *An. culicifacies*. The role of other species such as *An. stephensi* is not clear. Our observations indicate that, in South Punjab, irrigation-related sites support the breeding of anopheline mosquitoes, including the vectors of malaria. As our study was carried out during a year with exceptionally hot and dry climatic conditions, densities and longevity of mosquitoes would probably be higher in other years and could result in more significant malaria transmission than we observed. To assess the overall importance of irrigation-related sites in the epidemiology of malaria in the Punjab, more studies are needed to compare irrigated and non-irrigated areas (Herrel *et al.*, 2004).

The Pakistani Punjab experienced several devastating malaria epidemics during the twentieth century. Since the 1980s, however, malaria has been at low ebb, while in other areas of Pakistan and neighbouring India malaria is on the increase. This raises the question of whether transmission in the Pakistani Punjab may have been influenced by a change in vector species abundance or composition, possibly induced by environmental changes. To investigate this question, routinely-collected government entomological data for the period 1970 to 1999 for the district of Bahawalnagar, in the Indus Basin irrigation system in the southern Punjab, was analysed. Our findings suggest that *Anopheles stephensi* has increased in prevalence and became more common than *A. culicifacies* during the 1980s. This shift in species dominance may be due to the large-scale ecological changes that have taken place in the Punjab, where irrigation-induced waterlogging of soil with related salinization has created an environment favourable for the more salt-tolerant *A. stephensi*. Some biotypes of *A. stephensi* are suspected of being less efficient vectors and, therefore, the shift in species dominance might have played a role in the reduced transmission in the Punjab, although further research is needed to investigate the effect of other transmission-influencing factors. (Klinkenberga *et al.*, 2004).

8.2.3. Surveillance of *Culex* spp. In Pakistan

Gadahi *et al*, 2012 investigated the prevalence of mosquito genera in Tandojam and its surroundings from May to June 2010. A total of 2316 mosquito samples were collected which belongs to five genera viz; *Culex*, *Anopheles*, *Aedes*, *Uranotaenia* and *Psorophora* comprising 49%, 16%, 1.4%, 11.7% and 21.8% respectively.

9.1. Control

Numerous approaches are used for mosquito control. The most important usually include:

1. Destruction of feeding sites (e.g., take out stagnant water in ditches)
2. Use bio-control agents e.g. introducing natural predators such as insectivores fishes dragonflies
3. Use traps and/or larvicides and insecticides to kill larvae or adults
4. Exclusion such as mosquito nets and window screening

9.1.2. Source Reduction

Source reduction is one of the most effective strategy of Integrated Pest Management program. It is also known as permanent control. This technique consist of eliminate the possible mosquito breeding sites. These strategies can be as much simple as to removal of water containers which are mosquito breeding site, as complex as Open Marsh Water Management techniques to control saltmarsh mosquitoes. Source reduction technique is an important path in mosquito control programme as it can eliminate the application of insecticides.

Since the early 1980s, intensive struggles to bring back or rehabilitate salt marshes destroy by mosquito control have been a current management initiative. Source reduction in freshwater habitats (e.g., floodplains, swamps, and marshes) usually includes constructing and conserving channels. These channels can perform the double functions of dewatering a region before the emergence of mosquito and as harborage for larvivorous fish. Storm water/waste water is most important breeding sites for mosquitoes. These habitats can be managed by keeping the area free of weeds through an aquatic plant management program and by the introduction of larvivorous fish in these habitats. Lastly, vehicles tyres are a challenging mosquito generating habitat which can be managed by appropriate discarding.

9.1.3. Biocontrol

The **biocontrol** means to control a population of a pest using its biological predator. For example, dragon flies are predators of adult mosquitoes and larvivorous fishes (guppy fish) are predators of the mosquito larvae. In 2011, when dengue epidemic was its peak; predacious dragon fly species were introduced in various parks and gardens in Lahore city, Pakistan to prey upon the dengue vectors *Ae. aegypti* and *Ae. albopictus*.

The Chief Minister of Punjab Mr. Shahbaz Sherif, during a meeting held on 14.09.2011, desired for biological control of dengue vector via introducing larvivous fish in different water bodies in and around Punjab. Tilapia and Grass Carp were the two species introduced in the different water bodies of in various cities of Punjab identified as hot spots for dengue mosquito. About 48 to 450 fishes of both species have been released at 61 sites. The impact of such biological control included the eradication of eggs and larvae of mosquito in ponds and ditches of water. The Grass Carp consumes the ingrown aquatic vegetation, which otherwise serves to anchor larvae and mosquitoes.

9.1.4. Insecticides and Larvicides

Larviciding is a general term for killing immature mosquitoes by applying agents, collectively called **larvicides**, to control mosquito larvae and/or pupae. Larvicides are used as a source reduction method in Urban Malaria Control Programme. This method is also effective against controlling the vectors of filariasis and dengue. The **Larval Source Management** (LSM) involves both the alteration of water habitats, known as Source Reduction, and the application of larvicides to control mosquito production. Various mosquito species spend most of their life time in the larval stage when they are highly susceptible to both predation and control efforts. They are concentrated within defined water boundaries, immobile with little ability to disperse, and accessible. while adult mosquitoes fly in pursuit of mates, blood meals, or water sources for egg laying and are often inaccessible and widely distributed.

• Insect growth regulators (IGRs)

• Microbial larvicides

• Organophosphates (OPs)

• Surface oils and films

1. Insect Growth Regulators (IGR)

IGRs inhibit the insect growth ultimately lead to larval or pupal mortality. From last three decades, the most important IGR methoprene, Altosid, has been a extensively used mosquito larvicide. Methoprene is specific to immature insect larvae, particularly mosquitoes.

2. Microbial Larvicides

Usage of microbial larvicides is most important approach of biological control. These formulations are used to deliver a natural toxin to the target organisms. *Bacillus thuringiensis* (Bt) is the most commonly used soil bacterium used as agricultural microbial pesticide in the world. Environmental Protection Agency (EPA) registered various products as effective microbial pesticides. The Bt serovar kurstaki (Btk) is also registered microbial pesticide, and effectively used against Lepidoptera (butterflies and moths) larvae. It was originally isolated from natural Lepidopteran die-offs in Germany and Japan. Bt are second most widely used registered microbial pesticides (de Barjac, 1990).

Bacillus thuringiensis israelensis

Bacillus thuringiensis is a naturally occurring soil and aquatic bacterium. In 1976, Goldberg and Margalit (1977) isolated *Bti* from *Culex pipiens* collected in an Israeli riverbed. It is noted that B. *thuringiensis* is toxic to mosquitoes and other noxious insects such as black fly larvae. In 1977, de Barjac designated this *Bt* strain as H-14. In last three decades, various new strains have been investigated. SA3A and FM65-52 are two important *Bt*-strains as they are used in commercial products. The delta endotoxin (d-endotoxin) crystals are active ingredient of *Bt*-toxin commercial product. These toxic crystals are incorporated in to *Bt*-formulations and allow them to release in to water at the target site, so that ingested by mosquito.

As *Bt*-toxin ingested in to mosquito body, the d-endotoxin crystals are activated in the gut alkaline environment in the presence of various enzymes. Midgut enzymes and alkaline environment cause the hydrolysis of the crystal protein coating and release of pro-toxin. Enzymes facilitate the binding of pro-toxin to mosquito gut epithelium. As the pro-toxin bind to midgut epithelium, cells become rupture leading to release of cellular fluid and ultimately cause death. *Bacillus thuringiensis israelensis* is listed by the EPA as a biopesticide (http://www.epa.gov/oppbppd1/biopesticides/ingredients/index.htm).

Bti products are harmless to non-target organisms. The crystalline d-endotoxins are activated only in basic environment, but not in the acidic gut of the human and other mammals. As acid inactivate them. *Bt*-toxin also requires mid gut enzymes for their proper functioning and activity. So it is also not activated in the alkaline gut of the animals ehich does not contain this enzyme. This specificity is account for highly selective nature of *Bt*- formulations, which is limited to Dipterans such as mosquitoes, midges and black flies. It is effective on most mosquito species in a very wide variety of habitats; *Bti* formulations are thus ideally suited for integrated pest management (IPM).

"Bti product labels show the potency of the product as the number of International Toxic Units (ITU) available. This value is more meaningful than the weight percent of the active ingredients, as it characterizes the formulation's effectiveness. ITU values are determined by a standardized laboratory bioassay which uses 4th instar *Culex quinquefasciatus*. Prepared volumes of toxins are applied to living mosquito larvae and the resulting mortality data provide a numerical measure of activity. *Bti* labels bear the "CAUTION" signal word".

Table. 2.1. WHOPES-recommended compounds and formulations for control of mosquito larvae

Insecticide compounds and formulation(s)[1]	***Class group*** [2]	***Dosage (active ingredient)***	
		General (g/ha)	***Container breeding mg/L***
Bacillus thuringiensis israelensis, strain AM65-52, WG.	BL	125-750[3]	1-5[3]
Diflubenzuron DT, GR, WP	BU	25-100	0.02-0.25
Novaluron EC	BU	10-100	0.01-0.05
Pyriproxyfen GR	JH	10-50	0.01
Fenthion EC	OP	22-112	-
Pirimiphos-methyl EC	OP	50-500	1
Temephos EC, GR	OP	56-112	1
Spinosad DT, EC, GR, SC	SP	20-500	0.1-0.5

[1] DT = tablet for direct application; GR = granule; EC = emulsifiable concentration; WG = water-dispersible granule; WP = wettable powder.
[2] BL = Bacterial Larvicide; BU = Benzoylureas; JH = Juvenile Hormone Mimics; OP = Organophosphates; SP = Spinosyns.
[3] Formulated products

ADULTICIDES AND ADULTICIDING

Organophosphates - General Description

Organophosphates (OP) are acutely poisonous and they preventing the working of vital enzymes of the nervous system that play a important role in the transmission of nerve impulses. Electrical signals generated as nerve impulses travel along neurons (nerve cells), at the point of synapse which is junction between two neurons (a synapse) and neuromuscular junction which is present between a neuron and a muscle, the impulse is transmitted in the form of a chemical substance (neurotransmitter). In central nervous system Acetylcholine is an important neurotransmitter function in autonomic nervous system and neuromuscular junction. Acetylcholine generates the nerve impulse. After generation of nerve impulses acetylcholine is broken down and inactivated by the enzyme cholinesterase in milliseconds. With exposure to OPs, activity of cholinesterase is inhibited, and a build-up of acetylcholine occurs. If acetylcholine is remain active and does not broken down, the nerve impulse continuously generated and does not stop, eventually causing paralysis of the insect and ultimately death. The organophosphates used in Pakistan include chlorpyrifos, malathion, and naled.

Malathion

Malathion is used for both ground and aerial applications.

Mode of Action: Malathion is most commonly used to control insects belong to various orders Coleoptera, Diptera, Hymenoptera, Hemiptera and Lepidoptera in wide range of crops. Malathion is nonsystematic stomach poison. It is used to control the insects of various vector diseases, ecto-parasites and noxious insects. They have low mammalian toxicity. One of the chief problem with the use of malathion is that if it is used for long period of time, it will develop the resistance.

Chlorpyrifos

Mode of action: Just like malathion Chlorpyrifos is a non-systemic contact and stomach poison with respiratory action. It is used to control various insects belonging to order Homoptera, Diptera, Coleoptera and Lepidoptera on vegetation and in soil. It also is used in the control of noxious pests in houses, for mosquito control, in animal hoses. It is rarely used in mosquito control in Pakistan and worldwide.

Pyrethroids – General Description

Pyrethroids are synthetic pesticide. They mimc the structure of natural insecticide pyrethrum and Pyrethrins. Pyrethrin is naturally found in the flower of some plants belonging to the family Asteracae (e.g., chrysanthemums). Pyrethrin have the ability to knockdown insects rapidly. However under the influence of sun light Pyrethrums can be quickly degraded by ultraviolet light. Overall, this rapid degradation phenomenon leads to lesser environmental hazard. Research showed that Pyrethroids cause significant toxic hazards to organisms living in aquatic habitat. In comparison to OPs pesticides, Pyrethroids are extremely poisonous to anouxious pests at very low dose. Synthetic pyrethroids have been chemically and structurally improved to make them more stable and less toxic and safer to mammals and human beings. Pyrethroids show their effect as axonic poisons; when they enter in to the body they inhibit the closing of the voltage-gated sodium channel. Under normal conditions these protein gates opens and generated the action potential and closes at the end of the signals. Pyrethroids bind to these channels and inhibit it from closing, consequently generate continuous nerve stimulation. Nervous system disturbed and control is vanished, generating uncoordinated movement and eventually cause death of the target insect.

Pyrethrum

Mode of Action: Pyrethrum is non-systematic contact insecticide. It effect the nervous system. As pyrethrum enter in to the body of target insect, it shows the toxic effect on the sodium channels. Pyrethrum bind to the sodium channels and remain them open for a long period of time. As sodium channels remain open they create the multiple nerve impulses. Due to generation of excessive nerve impulses it cause paralysis and eventually death of the insect. Pyrethrum is used for control of various vectors or pests. Usually it is mix with synergists to enhance it toxic effect to the target insect and preventing the detoxification by the insect. From environmental point of view it does not accumulate in to the ecosystem, because it is derived from natural sources and degrades rapidly in to less toxic metabolites under sunlight.

Permethrin

Permethrin is commonly used adulticide in Pakistan. It is usually used for ground applications. Permethrin can also be used for aerial adulticiding.

Mode of Action: Permethrin is effective for a broad range of insects. it is a non-systemic insecticide with contact and stomach action. They have good residual activity on treated vegetation, stable under sunlight and have low mammalian toxicity. Furthermore, it is one of the least expensive compounds available for adulticiding. But it is highly toxic to fishes.

Resmethrin

Resmethrin is used for both aerial and ground applications. In FY 2004-05, it was applied to 4.5% of the total acreage for ground adulticiding and 1.5% of the acreage for aerial adulticiding.

Mode of Action: Remethrin is broad spectrum insecticide. It is a non-systematic, contact pesticide. It is recommended that remethrin is used along with more persistent pesticides. They have low mammalian toxicity and large knockdown effects against target insects. It is sensitive to sunlight and does not persist for the long time. Like Pyrethrin it is highly toxic to aquatic organisms.

GROUND ADULTICIDING

Barrier Treatments

These pesticides spray on the plants in such a way that if mosquito land on the treated plants it will pick up enough quantity of pesticide active ingredients and cause the death through direct pesticide contact. Typically these type of spraying or treatments done in the restricted areas to protect the public during night time outdoor events such as weddings and parties in the parks. Barrier treatments are effective for days and even weeks depending on the pesticide formulation and weather conditions.

The pesticide can be applied to the vegetation by following three methods:

1. Drenching Sprays

Drenching sprays are applied to the vegetation as a very dilute aqueous formulation. usually by means of a vehicle mounted larvicide spraying equipments such as pesticide filled tank, pump, hose pipe, and spray-gun or nozzels. It produces very coarse minute "raindrop" droplets of 500 to 1000+ microns in diameter. Vegetation is treated with a spray-gun attached at the end of the hose. Normally, it would take 50 gallons or more of pesticide formulation to treat an acre.

2. Mist Sprays

Insecticides are less heavily diluted for mist sprays than for drenching sprays. Mist sprays are used for the application of aqueous based pesticide formulations. It creates the fine mist type droplets ranging in size from 100-150 microns in diameter. Mist is created through a air blast type sprayer either as backpack or vehicle/trailer mounted equipment such as a "Buffalo Mist Turbine." In comparison to drenching sprays, Pesticide formulation is concentrated in mist sprays. The smaller droplets have large kinetic energy (100+ miles per hour) rapidly absorbed on the foliage and the surfaces through which they contact.

3. Electrostatic Sprays

Electrostatic sprays are generating electrically charged pesticides that more efficiently "sticks" to the surface to which it is applied. Even though this method is not extensively used for mosquito control in Pakistan, however this procedure is very efficient due to the low volumes required for effective coverage and the minimal waste/contamination to the ground. The amount of barrier treatment sprays used in Pakistan mosquito control programs is insignificant when compared to the amount of ground adulticiding, but barrier treatments getting popularity day by day and perform a central role in the mosquito control program presented by commercial vector control companies to public.

4. Thermal Fog

Thermal foggers were developed largely from smoke generators built principally for Concealing military maneuvers. The pesticide is mixed into a fog-oil, usually with #2 Diesel or a light petroleum distillate, which is injected into a heated, often double walled nozzle. Heat vaporized the mixture. Extreme high temperature about 1000^{o}F is used to generate thermal fog. A source of forced air pushes these insecticide vapors out of the nozzle. As these hot vapours move outside, the outside cooler air condenses it into fog with droplets ranging in size from 0.5 - 1.5 microns. If the insecticide flow does not overwhelm the vaporization capacity (sufficient BTUs/gallon/hour) of the machinery, all of the droplets will be in this near sub-micron range and often are referred to as a dry fog. Large size droplets will be produced if the pesticide released rapidly or the heat decrease as some of the mixture will not be fully vaporized.

9.1.4. Exclusion

To prevent the exposure of mosquitoes window screening and netting is important. In the course of an epidemic period generally the mosquito netting is immersed in mosquito repellent to prevent them. Pyrethroids are the only insecticides presently recommended for treatment of mosquito nets. Symptoms of poisoning due to insecticide treated mosquito nets are sporadic, in the same way if large dermal contact occur it cause only a brief numbness or tingling. Depending on the rate of washing, mosquito nets retreated with insecticides every 6–12 months.

At the time of treatment protective clothing should be worn. Several kits are available for the treatment of mosquito net for home usage. Such kits are designed for untrained adults and children. A lay man treats the mosquito net with minimum exposure to the insecticide. Insecticides are present in any form; they may be in the form of dry tablets or in liquid formulations. Dry tablets are easy to handle as compared to liquid formulations.

When the kit used pictorial instructions in the kit should be carefully followed. Disposable hand gloves should be wearing before diluting and mixing concentrated insecticide in water, dipping the net in this solution then putting it out to dry and disposing of any unused insecticide. After treatment used gloves should be thrown out. If no gloves are available at the time of treatment, the insecticide should be mixed with a wooden stick or tool rather than with the naked hands. Care should be carried out to avoid splashes on the skin. If insecticide splashes fall on the skin they should be rinse with water immediately. Precaution should be taken in disposing of remaining insecticide, as pyrethroids are poisonous to aquatic life. Insecticide kits and chemicals should be kept away from the young children.

WHO has published a general risk assessment model for pesticide treatment and use of treated mosquito nets. These models can be used to predict the insecticide exposure risks during net treatment or from use of the nets by adults, children and infants. A full risk assessment model of the use of the pyrethroid, deltamethrin on mosquito nets has also been published, which is generally reassuring about the absence of risk for persons treating nets with 'do-it-yourself' kits or using such nets.

10.1 MOSQUITO CONTROL BENEFITS AND RISKS

The usage of several chemicals in an attempt to control pests of humans, animals and plants, has been documented since ancient times. Homer described that Odysseus fumigated a house with burning sulfur used to control pests (Ware 1994). The Chinese used arsenic sulfide to eliminate insects (Pimentel and Lehman 1993). However the uses of chemicals considerably altered with the development of synthetic pesticides a little over fifty years ago. The Swiss chemist Paul Müller discovered the insecticidal properties of the organochlorine pesticide dichloro- diphenyl-trichloroethane (DDT), and the United States Department of Agriculture (USDA) laboratory in Orlando developed it for field use by the armed services. An arsenic compound (Paris green) was used as larvicide in Florida for larval control during the 1960s.

Pyrethroids: pyrethroids are synthetic insecticides of naturally derived compound pyrethrum. Pyrethrum is naturally derived from chrysanthemum flowers. Resmethrin, Permethrin, and Sumethrin are synthetic pyrethroid used to control mosquito population in Florida. Pyrethroids are persistent insecticide as compared to pyrethrum (WHO, 1989). They are toxic to aquatic organisms. Pyrethroid is broad spectrum insecticide. Due to their broad spectrum activity, they may affect the beneficial species and increase the need for further chemical control (Edwards 1993). They show low mammalian toxicity (EPA, 2002).

Carbamates: Carbamates are broad spectrum insecticide, derived from physostigmine, a naturally occurring alkaloid sperated from Calabar bean (WHO 1986b). It is more persistent insecticide then OPs (Edward, 1993). However microorganisms degrade carbamate in the soil (WHO 1986).

IGR: Insect growth regulator interferes with the normal developmental pattern of insect and caused mortality. Methoprene and Altosid® are widely used IGR for Dipterans insects. Methoprene have low mammalian toxicity. Diflubenzuron (Dimilin®) is a chitin inhibitor, it inhibit the normal growth of chitin. However due to broad spectrum activity and high toxicity to aquatic organisms, they are restricted to certain areas.

Biologicals: For biological control soil bacteria *Bacillus thuringiensis israelensis* (*Bti*) and *B. sphaericus* (*Bs*) are most commonly used. These bacteria act as larvicides. They are specific for mosquitoes acting as stomach poisons. *Bti* formulations are used in all over the world but *Bs* is only effective in freas water habitat (Bauman *et al.*, 1991). *Bti* and *Bs* toxin are nontoxic to humans and other mammals. They exhibit few or no non-target effect (WHO, 1999; Ware, 1994; Boisvert and Boisvert 2000).

Surface film: Petroleum products are most commonly used for the control of mosquito pupa population. They act as pupacides, suffocate mosquito pupa before the adult emergence. The oil is toxic to predatory Hemiptera and coleopteran but harmless to protzoa and rotifers (Mulla and Darwazeh 1981; Tietze *et al.*, 1993, 1995). Alcohol ethoxylated surfactants are most commonly used as larvicide and pupacides. Due to oil surface tension disturb and pupa drown in to water bottom. Monomolecular films are also used as pupacides, however these are not effective under high wind (Nayar and Ali, 2003).

11.1 MOSQUITO CONTROL RESEARCH

To effectively control a mosquito population causing epidemics or playing role as the vector of specific agents; following points should be considered:

• **Surveillance**: Efficient mosquito-borne pathogen surveillance methods are the soul of an effective mosquito control program. Effective and precise methods to survey mosquito populations to detect and predict mosquito outbreaks and to identify the existence of mosquito-borne pathogens are compulsory so that the most effective and environmentally sound control approaches can be carry out. Study is required to advance present surveillance practices.

• **Mosquito Biology:** Mosquitoes have been around for millions of years and have learned ways to persist – in spite of habitat manipulation and insecticide control.

Research on the biology of many of the species has provided much of the information that led to today's control methods. These methods include understanding of mosquito biology, ecology, reproduction, and habits. As we get more and more knowledge about the mosquitoes, the better the potential for developing and modified techniques for their eradication without damaging the environment.

• **Wetlands Ecology:** Originally, mosquitoes inhabiting salt marshes and aquatic habitat. Various chemicals and draining systems used to control saltmarsh mosquitoes. However, these solutions caused some ecological problems. Exploration has confirmed that mosquito control and wetlands conservation are not mutually exclusive goals. Advance field research is necessary to modify and improve mosquito control programs.

• **Human-made Mosquito Problems:** Rain water, stagnant water and water collecting bottles and tanks thrown away by humans are the main cause of mosquito problems. Research is desired to produce solutions to mosquito problems made by humans. For example, research is needed on the biology and control of *Aedes agepti* and other invasive species introduced into the Pakistan.

• **Repellents:** Safe and effective personal protection is required to repel the biting insects, unluckily, most repellents that are oil base and have an unpleasant smell. In certain persons these products cause allergic reactions. Various plants, bug zappers and buzzers are available as mosquito repellents. To date, all of these devices have proven useless. Research should be carried out for the development of new repellents.

• **Attractants:** In contrast to repellents, mosquito attractants also present. Experimental study has shown that mosquitoes are attracted to some gases such as CO_2 and octenol. These gases act as attractant and used to s to attract the mosquitoes into killing zones treated with a pesticide. Through research, we are capable to advance new integrated procedures for the mosquito control.

Resistance Definition:

"When an organism modifies itself genetically against some chemical, insecticide or any climatic situation than it is said to become resistant to them. This phenomenon is termed as Resistance."

Resistance Mechanism

Pesticide resistance

uses of insecticides or pesticides have been the keystone of vector-borne disease control for the past 50 years; yet, practices of chemicals on a massive and increasing scale has led to the pervasive development of resistance as a consequence of selection for certain resistant genes. The number of insecticide-resistant insects of public health importance rose from 2 in 1946 to 150 in 1980 and 198 in 1990. Several insect have developed resistant to numerous multiple pesticides, making their control by chemical methods tremendously problematic and costly. Vectors of public health importance such as tsetse flies, trombiculid mites, triatomine bugs, and snail hosts of human schistosomiasis are only ones in which resistance does not play important role for control. The status of resistance of most important vectors to pesticides and insecticides in several geographical zones was last reviewed by WHO in 1992.

Inspection and monitoring of insect resistance to insecticides should be a fundamental constituent of the planning and assessment of vector-bone disease and control programmes. Such monitoring should be standardized to make sure the comparability of data from different various sources. The utilization of standard test kits and procedures, including ‘discriminating concentrations’ are suggested. Discriminating concentrations (dosages) of pesticides are proven under standardized research laboratory conditions, through which a wide range of pest population susceptible to pesticides. Discriminating concentrations are not intended to mimic the doses applied in the field but are the concentrations found reliably to kill strains that have never encountered pesticides and are therefore assumed to be susceptible.

Reported resistance of a specific vector species in a precise area does not in itself justify a direct alteration in strategy for vector control programmes. Planning for substitute approaches and insecticides should be initiated. If present control measures and methods are insufficient to overcome disease, the stratagem or insecticide should be changes. It has been demonstrated that even insects develop resistance against pesticides, but still susceptible to pesticide applications, either vectors susceptible to higher concentration of insecticide or resistant gene that decrease the pesticide irritability is not so much developed. In a recent years, a molecular study showing high level of insecticide resistance to Pyrethroid by susceptibility testing of Anopheles *gambiae* Savannah in Benin and Gene analysis reveals high frequency of *kdr* gene. Results of experimental studies showed that pyrethroid-treated mosquito nets were killing wild mosquitoes, and malaria occurrence continued to be reduced.

This highlights the requirement to document resistance and its influence on the effectiveness of interventions prudently before implementing curative measures. Resistance checking and monitoring should be a vital part of vector control programmes. The vulnerability of disease causing insect should be determined before assortment of a pesticide and for further resistance monitoring provides baseline data. Surveillance during the whole course of a programme will permit initial detection of resistance, so that resistance management stratagems can be implemented, or, in the case of late detection, indication of control failure can validate replacement of the insecticide. Insecticide resistance can be detected easily by means of the standard WHO test kits.

Insecticide resistance management programme consists of inhibiting or delaying the development of resistance to insecticide whereas maintaining an effective level of disease control at the same time. It is prerequisites a trustworthy method and system for disease surveillance and resistance detection and management. It is essential to detect that very few insecticides are present for use in public health programmes. Hence, the susceptibility of vectors to effective pesticides should be measured a valuable resource that must be conserved as long as possible. The following recommendations might be considered in management of resistance and managing vector control with optimal cost–effectiveness:

• Non-chemical control techniques should be used, either as a supplementary measure or alone in the regions in which they are applicable and cost-effective

• Restriction of pesticide use to regions with considerable levels of disease transmission

• To kill adult femal mosquito use only adulticides rather than to larvicides, which kill both sexes, resulting in around half the selection pressure for resistance

• Rotation among unrelated insecticides according to a pre-arranged plan created on knowledge of the probability of resistance developing to each compound

• Select a compound that develop narrow spectrum resistance rather than broad spectrum

• apply mosaic treatments with distinct chemical composition, so that vector population resistant to only one of the chemical constitute are killed by the other. This principle is used regularly to avoid the generation of drug resistance in treatment of various infectious diseases such as HIV infection, tuberculosis and hepatitis etc. and should be more systematically investigated for pesticides.

So far, switching among distinct pesticides in response to revealing of resistance has been the main method used. It is very significant that: (1) nontoxic, safe, operative substitutions are prepared before the recognition of serious resistance; and (2) resistance management is realized preventively to preserve the efficiency of the few pesticides utilized for public health purposes.

11.2. Selecting an appropriate chemical control strategy

Clear understanding of behavior and binomics of mosquito is prerequisite for efficient mosquito control program. it require careful training, regular supervision of control strategies, periodic evolution of targeted vector and epidemic of disease. Chemical control measures should be considered only as a complementary addition to basic sanitation. In choosing a insecticide and the suitable formulation, attention should be given to its biological effectiveness against the target vector or pest, the susceptibility of the target vector and pest, the mode of application, possible toxicity to humans, poisoning to non-target organisms, the registration status of the insecticide use and its price. It is recommended that, small experimental trials on the effectiveness of a pesticide formulation and application method should be conducted under local conditions before purchasing large quantities. The concentration of active ingredient per unit area must be known for determining the quantities of pesticide formulation required.

Special attentions should also be given to the impact of the pesticide on the environment, including fish, birds and beneficial invertebrates. These aspects should be discussed with the representatives of potential suppliers so that informed choices can be made on the most appropriate pesticide for the local context. WHO guidelines for the purchase of pesticides for public health use provide general guidance on selection of appropriate, good quality pesticides and formulations[5].

Pesticide formulations

Technical-grade (active ingredient) is a pure form of pesticide. Pure form of pesticide rarely used. For field application the technical-grade material (active ingredient) usually mixed with non-pesticide ingredients. These ingredients or agents known as'inerts' and perform various imporatant functions.

The key function is to assist in the delivery of the insecticide to the target pest; increase pesticide stability, increase effectiveness or simplify formulation handling and management. The type of pesticide formulation, and in some cases the choice of product of the same formulation type, can markedly affect the results obtained in practical use. When porous or absorbent surfaces such as mud are sprayed, suspensions of water-dispersible powders, water-dispersible granules or diluted suspension concentrates often have a longer residual effect than emulsions or solutions, which tend to be absorbed below the surface.

Microencapsulated products tend to offer long-term control and are more effective in outdoor environment. Frequently used pesticide formulations are described briefly below.

•***Bait (ready-for-use)*****:** Bait is a formulation which is prepared as an attractant and be eaten by the target insect.

•***Capsule suspension (slow- or controlled-release)*****:** a capsules suspension in a fluid, usually intended for dilution with water before use.

The formulation active ingredient is wrapped in microscopic polymer capsules, which discharge the pesticide active ingredient slowly, increase the insecticide half-life. They guarantee that at the time of application extreme surface concentration is not produced and are comparatively easily wash away from the skin if unintentional contamination occurs during application. When formulations apply through the capsule, the active ingredient does not rapidly absorbed by porous or absorbent surface but they efficiently adhere to insects, enhance insect–insecticide contact. They have minute odor and a good residual effect, as the active ingredient is protected from sunlight and air. The diluted product might require agitation during application

•***Dustable powder:*** For this formulation a free-flowing powder is suitable for dusting. Dustable powder mainly used to control bugs, lice and fleas.

•***Emulsifiable concentrate*****:** This type of formulation is a solution of insecticide active ingredient and surfactants in a water immiscible solvent, which diluted with water or kerosene oil and form stable emulsion. They are readily mixed with aqueous solution such as water to form an emulsion, nearly, which then needs merely a slight agitation to sustain a formulation appropriate for application. These emulsifiable concentrate leave little visible deposits on the treated surface; these formulations usually readily absorbed by porous surface and have intense smell. Emulsifiers formulations can injure plants and assist absorption of the active ingredient through the skin, thus enhances the hazard to operators. Emulsifiable concentrates also used for space spraying.

• ***Emulssion, oil-in-water*****:** This emulsifiable formulation comprises of an active ingredient dissolved in a water-immiscible solvent, in the existence of surfactants, is distributed as fine oil-phase droplets in water. This emulsion is just like to a diluted emulsifiable concentrate but is generally stable for longer time and comprises minor concentrations of surfactants and solvent. The concentration of water-immiscible liquid active ingredients can be as high as 500 g/l. The formulations of oil-in-water emulsion can be prepared for preparation only by dilution with water.

• ***Granules*:** Granules are solid formulation of well-defined grains, readily available for use. These formulations are prepared by impregnating, coating coarse inert carrier particles with, about 10–100 grams of the active ingredient substances per kilogram (1–10%). it is observed that for the control of mosquito larvae granules provide better diffusion and penetration as compared to liquid formulations, as well as persistence and bioavailability of the active ingredient in the treated region may be improved. granules are easy to handle and can be applied through hand rather than to use specific equipment.

• ***Suspension concentrate (flowable concentrate*)**: Suspension concentrate is a formulation of active ingredient dissolved water before use. These insecticide formulations are similar to water-dispersible granules or wettable powders dissolved in water. Suspension concentrate have active ingredient is in the form of small crystalline particles. Comparable to emulsifiable concentrate these particles are not penetrate into porous surfaces and the active ingredient does not readily absorbed in to the dermis, as compared to wettable powder they leave fewer hazardous residues, as the particles are so small.

• ***Technical grade*:** The purest commercial form of insecticide active ingredient is called *Technical grade.* It is used for the preparation of formulations.

• ***Ultra-low-volume liquid*:** It is a solution generally in a water-immiscible solvent, use with cold fogging (ultra-low-volume) equipment for space spraying. These can be prepared by dilute with oil or kerosene or ready-to-use formulations.

• ***Water-dispersible granules*:** An insecticide formulation consisting of granules which disperse in water and applied by spraying. The particulates in suspension consist of the active ingredient. They are safer to use as there is less danger of inhalation of airborne particles than from wettable powders or water-dispersible powders. Various water-dispersible granules are available in high concentrations, thus decreasing the expenses of transport and storage.

• ***Water-dispersible tablets*:** Tablet insecticidal formulations used independently to form a dispersion of the active ingredient after dissolution in aqueous solvent such as water. The tablets can be effervescent to aid dispersion. Just like water-dispersible granules they can easily use and the risk of contact by inhalation is usually lower. These insecticidal formulations have been used for treatment of mosquito nets by dipping.

• ***Wettable powders and water-dispersible powders*****:** Formulations that have insecticide active ingredient, wetting agents and inert carrier, used to formulate water-based suspensions solution. the powders generally hold the active ingredient at a concentration of 100–500 g/l (10–50%) for public health use. These suspensions have been extensively used for indoor residual spraying; however, the particles in suspensions made from wettable and water-dispersible powders are larger than those in suspension concentrates. visible residues may be left on sprayed area or surface because particles in suspensions made from wettable powder larger in size as compared to suspension concentrates. Moreover, there is a high risk of inhalation of airborne particles, so care should be taken during mixing. Such as masks should be wearing at the time of mixing water soluble envelopes should be placed in the spray tank, thus inhibiting the release of airborne droplets

Pesticide application equipment

The choice of accurately designed application equipment is an essential part of vector control strategy. Most commonly hand-operated equipment used. For the applications of larvicide and molluscicide compression sprayer are most commonly used. The handling of compression sprayers is fairly simple, but it is essential that one should know how to change worn-out nozzles, linings and pump washers. The operation and repairs of electrically powered equipment have need of extra skills and required full trained person to handle it. A series of application equipment for carrying insecticide to the target site is discussed in manual. Brief explanations of generally used equipment are given below.

Hand-operated compression sprayer

These sprayers are designed for applying pesticides onto surfaces with which the vector or pest will come in contact or to breeding sites. A pesticide and water mix is either added to the tank or mixed within it. Air is forcing in to tank with a hand-operated plunger and pressure is created. A lever is present on the sprayer arm to control the release of spray through nozzle. Filter water should be used while filing the sprayer otherwise dust and soil particles in unfiltered water can deteriorate the sprayer. Appropriate maintenance, replacement of damaged nozzle tips is crucial for maintaining sprayer efficacy. A drawback of the sprayer is that the pressure inside the falls as the tank is emptied, with an accompanying decrease in the rate of delivery.

To counteract this, a pressure control valve should be present at the nozzle. Keep in mind the cost of pesticides and the dose required to control the target organism, it is vital to maintain nozzle tips in good condition and to check the calibration of this equipment to ensure the correct rate of application.

Mist blowers (power-operated)

Mist blowers known as power-operated. Mist blowers can be either movable or vehicle-mounted. Portable knapsack mist blowers produced a high velocity air stream and produced a fine mist of insecticide. They are powered by a two-stroke engine. The engine must have a protector to avoid someone touching the hot exhaust. They have restrictors to regulate the volume emitted, but large size droplets are produced at high flow rates. Large size droplets are absorbed onto the treated surfaces, while small size droplets stay in the air as airborne particles and affect insects in flight as well as at rest. Even though water is mixed to the pesticide concentrates, the overall pesticide volumes applied with mist blowers are somewhat small. With the aid of smallest restrictors ultra-low-volume sprays can be applied. Knapsack mist blowers is more efficient as it can cover a large area in a relatively short time and can be functioned and easily handle in areas through which heavy vehicle-mounted equipment cannot pass, such as narrow lanes. Difficulties occur most frequently in starting these machines, because the fuel mixture (oil and petrol) left in the engine after use evaporates, leaving an oily residue over the spark-plug. After using these equipments the fuel mixture such as oil and petrol left over spark-plug.

This can be avoided if the technique used to stop the machine at the completion of spraying is to turn off the fuel, resulting in combustion of all the fuel in the carburetor. Which does not left any residue on spark-plug. To increase its life nozzles, air filters, fuel filters should be washed on a regular basis, and the water used in the preparation of insecticide formulation mixture should be free of debris or filtered, as dirt particles can block the nozzle opening. The drawbacks of the knapsack mist blower are the danger of burns from the hot engine and the distress produced by heat, pulsation, vibration and sharpe noise.

Aerosol generators (power-operated)

Cold fogging or aerosol generators are used for the application of pesticide, either in their technical-grade form or, more commonly, formulate in water or oil as space treatments, frequently at ultra-low volume. The equipment can be hand-held, but larger forms are truck-mounted. The volume sprayed per unit area is much smaller and cover larger areas more speedily. Portable ultra-low-volume aerosol generators are more effective when access by road is challenging as well as at the time of indoor spraying. The huge truck-mounted aerosol machines can cover widespread metropolitan areas where develop main road access is reasonable. The most important concern in usage of ultra-low-volume cold foggers is the standardization and precision of droplet size. For mosquitoes and flies, droplet size should be 15–25 μm. Aspects that should be taking in selecting a method for ground application of aerosols include the target insect behavior and daily activity times, the trained staff for maintenance of equipments, market price and safety of operation. Only pesticide formulations suggested for ultra-low-volume use by the manufacturer company should be used in ultra-low-volume application apparatus or equipments.

Safe use of pesticides

The following suggestions are proposed as a guide for the safe use of pesticides in vector, pest and public health control programmes.

Common principles of safety measures

All insecticides are poisonous to humans or other mammals to some extent; but, the acute toxic doses to humans are generally far higher than those mandatory for killing vectors and pests. The key to non-hazardous use of insecticide is to minimize the risks of unsafe exposure during handling and treatment of toxic chemicals. Hence, precaution in handling of insecticides, mainly by spraying staff and individuals living in the treated houses, must be a repetitive practice and form an vital aspect of any vector control programme including the pesticide or insecticide application. The general principles on which safety measures are based are discussed below, with distinct consideration to indoor use of residual sprays.

Toxicity and hazard

The most important principle of safety measures is that, both the pesticide nature, its formulation, and the suggested way of application must be taken in to consideration.

Determination of acute LD_{50} value is one of the important safety measures for the estimation of potential toxicity of pesticide to humans and other mammals after oral or dermal applications, which provide an assessment of the number of milligrams of pesticide active ingredient per kilogram of body weight required to kill 50% of a large population of test animals. For new pesticides, this measure has been replaced by one requiring use of considerably fewer test animals. While these numbers show the relative acute toxicity of different compounds to test animals, they do not characterize the actual or real risk when pesticide is used in the field. Moreover, the effects of long-term exposure or contact to low doses are not tested or measure for acute toxicity.

Factors that influence toxicity are: nature of pesticide concentrate formulation, sort of packaging, concentration or amount of pesticide active ingredient in the final formulation, way of application, area to be treated, required dose, contact or exposure of human or other organisms with treated surface or area, and the animal species exposed, their age, gender and condition. In selecting pesticide formulations, the oral and dermal acute toxicity for rats should be checked, because the values for the available formulations might differ markedly from those for the active ingredient, which are quoted in the tables of this guide.

Organochlorine and organophosphates are persistent and intensely toxic even at low doses. Therefore, use of pyrethroid insecticides has increased over the past two decades. After oral adminstartion the acute LD_{50} values for pyrethroids are relatively low, demonstrating a comparatively high intrinsic toxicity, thus they are less toxic insecticides. They are effective against target insects even at low dose as the ratio of insect: mammalian toxicity is large. If pyrethroid insecticides enter in to systematic circulation they are rapidly metabolized in to less toxic metabolites. In field applications it is necessary to follow recommended concentrations and precautionary measures. In order to evade irrtation, skin should be covered during application of pyrethroid concentrates.

Toxicity is the intrinsic property to cause a harmful effect. Risk is the chance that a detrimental effect might outcome from exposure to a specific hazard. For hazard to occur there must be contact to a poisonous chemical. Hazard is not the similar as risk, which depends on the quantity and exposure route. Usually hazardous exposure due to pesticide during occupational setting comprises direct contact with skin, eyes, nasal and respiratory tract, chiefly in airborne particles or aerosols. Hence, in order to minimize the exposure of pesticide, consideration must be paid to application equipment and to the training of workers.

Supplies and equipment

Harmless safe and sound transport as well as packaging of insecticide concentrates is an important consideration of vector control campaign. These should not be kept in rooms in which individuals live or where food is kept. They should be put in storage out of direct sunlight and secure from moisture and rain. Protection against misuse and reached by children should be assured. Suitable qualified people take full concern for the safekeeping of stocks and for the dumping or management of vacant or nearly empty pesticide containers.

Insecticides that have satisfactorily completed the WHOPES10 and for which either an interim or final specification has been suggested should be used in preference to compounds that have not been evaluated inside this criteria.

All insecticide vessels should be adequately labeled to identify and recognize the contents and display in an easy understandable form by the worker or person, the nature of the material and the precautionary measures must be mentioned. Labels should always be printed in the native language. All application equipment used for the application of insecticide should follow to the general and specific recommendations issued by WHO. All parts of applicator equipment's and machines must be examined regularly and systematically to confirm that there is no leakage of insecticide.

Responsibility for safety

The expert witness that approves insecticide uses, together with replacement of a novel material for one already in usage, must make certain that it is applied under suitable trained supervision. It is necessary to provide particular training, instructions and guidance to local people advisers and technical specialists might have to be recruited. It also include the appropriate training of pesticide spray teams, setting up some diagnostic and analytical measures, establishing facilities for treatment, including the delivery of antidotes in case of unintentional poisoning. While the ultimate responsibility for the health of spraying staff and persons living in treated places must rest with a medical officer, the regular accountability for warranting that safe and sound application techniques are practiced can be given to any experienced field worker. The leader of the field team and other workers should also follow guidelines about the rates of application that will certify accurate dosages delivery.

Safe Use of Pesticides

Safety training

For the safe and sound usage of pesticides trainings should be provided: (1) for medical consultants, engineers, entomologists and public health programme supervisors on the mode of action of the insecticide, the importance of analytical measures, identification of the signs and symptoms of poisonous effects, and the medical facilities necessary for treatment of poisoning cases; and (2) for field team leaders and workers in spraying practices, precautionary measures, safe pesticide spray equipment, first-aid methods, including resuscitation. Handbooks on dealing insecticide poisoning cases are offered to doctors and health care workers.

Safety staff must be ordered into squads, in which each person knows exactly what are his or her responsibilities and duties. Before the use of toxic material training is essential, during which the operator should work in the required protective clothing, to certify that it is suitable and that they can work appropriately while wearing it. All workforces should know the possible hazard of the work. They should recognize the actual risks and should not be led off target by inaccurate preconceptions.

Medical surveillance

Measures must be made to make sure that any exposed person can simply report any symptoms to a supervisor, who will then convey the complaint to the consideration of a health officer. Any unwarranted incidence of infection not related with well-recognized signs and symptoms of poisoning by the specific insecticide should be noted and informed to the appropriate health authorities. A watch should be kept for understanding neurological effects, such as loss of ability to understand written material and to concentrate. Apart from clinical surveillance, qualitative and quantitative biochemical tests can be carried out to evaluate the extent of exposure. Measurements of the exposure of spray operators have been described.

Protective equipment

The stuffs of protective outfit that might have to be used are:

• ***Hats.*** These should be of water-resistant material with a broad rim to shield the face and neck, as well as should be able to tolerate regular cleaning.

• ***Veils and visors or screens*** A plastic mesh net should be used as they shield the face from pesticide spray droplets and allow sufficient visibility. On the other hand, a transparent plastic visor can also be used.

• **Capes** Short light weight capes should be used for the protection of shoulders. These light plastic caps are suspended from the hat to protect the shoulders.

• ***Overalls*** These should be made of light weight and resilient cotton fabric. They must be washed able, and should be washed regularly after insecticide being used. Washing with soap and washing detergent or washing soda is suitable for Carbamate and Organophosphate compounds. A wash in light kerosene oil might be required for Organochlorine insecticides, and then washed with detergents.

• ***Aprons*** Rubber or polyvinyl chloride (PVC) aprons should be wearing before pesticide application as apron will shield from spills of liquid concentrates.

• ***Rubber boots*** These will complete the safety gave by the apron.

• ***Gloves*** Polyvinyl chloride or rubber gloves should be used during formulation treatment. It is recommended that during handling of pyrethroids PVC gloves should not be used, as they can be absorbed by PVC; rubber gloves should be used to handle concentrates with an organic solvent base. Impervious gloves must be washed and cleaned regularly from both inside and outside.

• ***Face masks*** During the application of water-dispersible powder spray masks of wire gauze or other similar material should be used. They can filter the particles as well as worn to lessen the chance of inhalation of the spray and dermal exposure of the face. They must be washed regularly, in some cases; new masks might be required to be used for the second half of a day's spraying, so that the face is protected and not contaminated.

• ***Respirators*** (masks with cartridge or canister). These are manufactured to protect operators who do fogging with very toxic powder formulations. After using respirator cartridge or canister must be changed frequently. Respirators are not usually required for vector control.

Personal hygiene

Considerable attention to personal hygiene is a vital factor for the safe use of pesticides. For professional spraying staff operating in the tropics, the safety precautions might depend chiefly on personal hygiene, together with washing and changing clothes. A practice for carrying out and directing personal hygiene, regular washing of protective clothes and cleaning of application equipment should be organized along the following lines:

• Formulation spraying staff should be provided with at least two costumes to permit for regular changes.

• Laundry facilities with adequate water and detergent should provide in the field at suitable places.

• All working clothes must be changed after spraying and a shower or bath taken.

• Working dresses must be washed frequently with detergents or soap, depending on the toxicity of the pesticide formulation used.

• Specific care should be given to gloves, as wearing contaminated gloves can be more hazardous than not wearing gloves at all.

• Spray operators must wash before eating.

• Drinking, eating, and smoking during work must be rigorously prohibited.

• When work involves pesticides of fairly high toxicity, the times of work must be set in such a way exposure to the toxic material or component is not excessive; transport should be arranged at working place, so that there is not a long delay between the end of treatment time and return to base for washing.

Safe Use of Pesticides

For the safe use of pesticide following guidelines should be followed

Disposal of empty or nearly empty containers

Vacant or empty containers should be disposed carefully. They must be put away from the unauthorized persons who may be used them for putting the drinking water or food stuffs. Such re-use of containers cause serious pesticide poisoning. To minimize the risk of pesticide poisoning it is important to ensure that pesticide containers will not be re- used. Used containers can be efficiently cleansed by washing them with tap water and thoroughly scrubbing the insides with dish wash or detergent. Containers in which organophosphate was present should also be wash with washing soda at 50 g/l (5%). This solution should be present in the container overnight. During washing and scrubbing rubber gloves should be wearing. All pesticide containers and vessels should be labeled 'not for storage of food or water for human or animal consumption'.

Operational procedures

Preparation of spray materials

Pesticide exposure risk is increase during pesticide formulation and handling. Suitable facilities should be provided during pesticide handling. The compounds should be provided in diluted form when are used by common man or non-commercial operators. Water dispersible powder formulations should be prepared in deep vessels and mix with long handle wooden stick or spatula.

Power appliances are most suitable for the dilution of solid pastes and allow preparation of the dilution in a closed vessel. No vessel should be filled to a level at which the operator risks being splashed. During mixing vessels should not be filled at which splashing started. Long handle wooden scoops should be used to transfer the pesticide formulation from one container to other container. All fill containers should be tightly seal and packed to withstand transportation in the area of application. The person handling the insecticide formulation should cover with protective clothing (see section 2.1.6) and suitable washing facilities should be available so that spills on the skin can be immediately removed.

House treatment with residual sprays

Spraying staff will inevitably be exposed to insecticide spray, and absolute protection of the skin and respiratory tract would impose physical limitations that would make such work impossible in hot climates. The skin can be protected to a considerable degree by cotton clothing and by regular washing with soap and water.

Residents must inform before the insecticide application. They must be informed about the purpose of application, timing of spray and should be given all instructions to what to do before and after their houses have been treated. During spraying all house members and children should be stay out of house. Wash the floor thoroughly after insecticide application. Drinking water and food stuffs should be cover and placed away from insecticide splashes.

During pesticide treatment precautions should be followed, as when opening, weighing and mixing the insecticide gloves and mask should be wear and stand in a position that wind blows the dust away from them. Operators must wear the protective clothing. Appropriate facilities must be provided for handling the of insecticide formulations, both as emulsifiable concentrates and water-dispersible powders. Operators must worn protective clothing and use application equipment. If pesticides fall on skin, it should be washed with water and soap. If insecticide spill on the floor it should be washed or removed, particularly for the protection of children, domestic animals and chickens. Floors with tiles should be washed with water while to clean earth surfaces, damp soil should be remove and then buried. During indoor spraying operations large numbers of house insects, such as flies, moths and bedbugs, might be killed and fall on the floor. Thus, these dead insects are presenting a hazard particularly to chickens when they consume these insects. Floors should be swept.

Workers should wear protective clothing and head covering, in the application of *organochlorine* insecticides such as DDT. After completing the spray they should wash and change. Operators spraying organophosphates should wear clean wash-able overalls, broad-rim hats, gloves and PV shoes. Mixers and any other person handling the formulation should also wear protective clothing and rubber gloves. They should wash the clothes and take a shower at the end of a day’s work.

In handling of *fenitrothion* and *diazinon*, firm protective procedures should be follow, including daily washing of working clothes, wear face masks, broad-rim hats and PV shoes or boots. Mixers and baggers handling the concentrate should also wear rubber boots, gloves and aprons. If insecticide falls onto the skin, it should be washed off with in no time. Clothes that are contaminated or wetted with the pesticide should be changed instantly. Field workers should not be exposed to the insecticide for longer time usually 5–6 h. Bath should be taken at the end of the treatment or spraying. Transport should be arranged to lessen delays between the end of the treatment and taking shower. From medical point of view all worker should be examined regularly and their cholinesterase activity checked. Workers should be inhibited from insecticide exposure if their cholinesterase activity decreases to 50% or more. The simple tintometric method is suitable for determining blood cholinesterase activity, and the commercially available field kit14 contains an instruction sheet.

With reference to the *carbamate* insecticides, regular precautionary procedures should be taken in applying carbaryl, but for propoxur stricter precautions measure follow. Spraying workers should wear water-proof overalls, masks, broad-rim hats and plastic boots. Mixers should wear the protective clothing as well as PV hand gloves and aprons. Consideration should be placed on personal hygiene, with all the precautionary measures mentioned above, except for determination of cholinesterase activity, since when the enzyme is inhibited by a carbamate it reactivates too rapidly for this technique to be of operational use. For bendiocarb handling the similar precautions are to be taken as for propoxur with the exception of that the pesticide should be mixed in the sprayer: the opened the outer packet, the inner soluble sachet is added to the sprayer containing the required quantity of water, and then closed the sprayer, pressurized it and shaken well.

The blank outer sachet should be give back to the supervisor for dumping. For the *pyrethroid* pesticides, regular precautionary measures, such as wearing protective clothes, canvas shoes or rubber boots and brood-rim hats, and washing the body and changing clothing after spraying should be taken in to account. The disposable face masks should be wearing. visors shoud be wear especially during the handling of irritating pyrethroids.

Whenever possible, exposure to the pesticide should be reduced to fewer than seven pump charges per day, and workers should obey firmly with the hygiene procedure such as washing the hands and face with water and soap after spraying a pump charge. As now pyrethroids applied to mosquito nets are an essential part of pesticide use for vector control worldwide, a more comprehensive explanation is given below.

Larvicide treatments

Persons applying larvicides are generally much less exposed than staff engaged in indoor house treatment, and exposure is confined mainly to the hands and arms. For the majority of larvicides, care must be taken to avoid contamination of drinking water and waters inhabited by non-target organisms of value, such as fish and crustaceans.

Temephos, methoprene, *Bacillus thuringiensis* H-14 (*Bacillus thuringiensis israelensis*), pyriproxyfen and permethrin can be used to control mosquitoes that breed in drinking water containers. Such treatment should always be made with pesticide formulations that ensure accurate and reliable dosing.

Diagnosis and treatment of pesticide poisoning

Symptoms of poisoning

The symptoms of organochlorine poisoning are excitation of the nervous system. In the beginning, the victim complains of headache and dizziness and might seem nervous and excited. Later, he or she may start vomiting and feel feebleness in the arms and legs, and the hands might shake. The victim can become disoriented in time and space, and fits can follow.

The sign and symptoms of organophosphate and carbamate pesticides are similar, but the signs of pesticide poisoning appear more rapidly as compared to organochlorine. The early symptoms of toxicity include excessive perspiring, headache, dizziness, constriction of pupils, sickness, vomiting, salivation or bronchial secretion, stomach pains, incoherent speech and jerking. Later, there may be diarrhoea, convulsions and coma.

The most common sign and symptoms of pyrethroid poisoning include paraesthesia which is burning sensation of the skin especially on the more exposed parts such as face and hands. If it is inhaled it cause upper respiratory tract irritation and sometime cause dermis allergy. If these insecticides ingested they disturbed the digestive system and cause nausea, vomiting and crams. In sever toxic effects patients have fits and become nervously unconscious. It also causes respiratory spasm, paralysis and eventually death.

The symptoms of fumigant poisoning are highly diverse. Fumigants such as chloroform, hydrogen cyanide, carbon disulfide and naphthalene have serious toxic effects on central nervous system. They disturbed the normal functioning of the nervous system. Aluminium phosphide and Methyl bromide cause pulmonary oedema , in the same way hydrogen cyanide causes severe hypoxia.

First aid and decontamination

Once insecticide poisoning has been diagnosed, A doctor should be consulted immediately: All information such as name of the pesticide, (on packaging), route of exposure (e.g. skin, mouth) the time of poisoning as well as the reason for poisoning either it is intended, unintentional, overexposure during application or other information about the circumstances.

Before the providing first aid, should know the type of chemical or active ingredients present in the insecticide. The common procedures for treatment based on signs and symptoms should be applied. First of all respiratory, heart beat and pulse rate should be checked. If either is absent, resuscitation should be started. If the patient is unconscious, the passage of air should be freed by drawing the chin up and rearward. The patient should lie on his or her side or front downwards, and turned the head to one side. In order to prevent vomitus from entering the lungs this posture should be used if the patient is transfer to hospital from accidental site.

The person giving first aid must be safe from the solvents and active ingredient by wearing gloves and masks; if for normal breathing mouth-to-mouth respiration is necessary remove all vomit and saliva from the patient’s mouth and a clean handkerchief placed between the mouth of the patient and the mouth of the person giving first aid. Affected skin area should be clean with soap and rinse with water. If the eyes are contaminated, with the help of fingers eye lids should be opened and washed with sterilize excessive water.

Antidots should be given to the patients and transported to the patients as soon as possible.Freash air or oxygen should be given to the patient after pesticide poisoning. In cases of ingestion, gastric lavage should be performed within 1 h. for treating poisoning with organophosphorus pesticide activated charcoal can be effective.

For treating organophosphate and carbamate poisoning, 1–1.5 mg injection of of atropine should be administrated intravenously or intramuscularly for every 5–10 min until signs of atropinization such as dilated, fixed pupils, heart rate > 140 beats/min, blushing of the face and loss of salvation appear. Similarly in case of Carbamate poisoning Oximes such as obidoxime chloride (Toxogonin) or pralidoxime chloride (Protapam, 2-PAM) must not be given. Automatic injectors are present for administration of atropine17, and it is suggested that these should be at hand and easily accessible where organophosphate or Carbamate pesticides are being used. They should not be distributed to the workers, but field supervisors should be trained in emergency treatment of insecticide poisoning and resuscitation where a doctor does not exist.

for pyrethroid poisoning specific antidote is not available. Treatment is principally suggestive and supportive after decontamination to inhibit more absorption. For persistent paraesthesia Vitamin E oil preparations is recommended. There is no particular antidote for organochlorine poisoning. The patient is treated symptomatically and supportively to continue ventilation and control convulsions. Activated charcoal is recommended if the pesticide has been ingested. If the compound has been thrown on the skin, it should be wash thoroughly with soap and water. Diazepam should be administrated intravenously at a dosage of 0.3 mg/kg for children and 5–10 mg for adults to inhibit convulsions.

Washing of the skin and eyes must be instantly after exposure to a fumigant. The skin and eyes should be rinse with excess of water for at least 15 min. Special medical treatment should be taken after washing. Specific processes are needed for different agents.

Mosquitoes are the main cause for transmitting the most important vector-borne diseases such as malaria, lymphatic filariasis, Yellow fever, West Nile fever and dengue, as well as, various types of encephalitis. A complete knowledge on the behavior and physiology of the target insect should be known before using the specific chemical substance

Protection from mosquito attack can be got by the use of repellents or insecticide treated mosquito nets. This chapter deals mainly with chemical control of mosquito and does not address the control of some important nuisance species.

***Anopheles* spp.**

Some species of *Anopheles* are anthropophilic and prefer to bite humans. It is responsible for various vector borne diseases such as malaria and in some areas, of lymphatic filariasis as well. Interior spraying is effective in the regions where the mosquitos are strongly endophilic mean tend to rest inside houses. Pesticide-treated mosquito nets are significantly recommended to inhibit the *Anopheles* species. Mosquitoes that are primarily exophilic i.e. living outside the houses, but feed or rest indoors briefly can also be controlled successfully by indoor pesticide spraying, especially with fumigant (see Table 1). In mosquito affected areas where they are strongly exophilic, which strictly rest and bite outdoors, individual protection such as application of repellents on exposed body parts and other precautionary measures, such as space spraying or larval control, should be taken.

Due to excessive use of pesticide many mosquito vector species have developed resistance to organochlorine compounds as well as develop resistant to various organophosphate, carbamate and pyrethroid insecticides. For long-term use of pesticide, regular monitoring of resistance should be carried out.

Indoor residual spraying

For indoor residual spraying well develop application techniques and equipment should be used. Indoor residual spraying is very efficient and effectively used for the control of malaria vector control. It enhances the risks for a mosquito each time as it move in a house for a blood meal, which it usually does every 2–3 days, so that few will live the just about 12 days that are required for malaria parasites to complete part of their life cycle in the vector.

The efficiency of indoor spraying for mosquito control depends on various factors such as insecticide active ingredients, the application technique, public or community acceptance of spraying, the accessibility of well-efficient equipment, well-trained spraying personnel, well-organized supervision and strong financial support.

(a) *Target area*

Usually all interior premises of the house such as interior walls and ceilings should be spray. Not only permanent living residencies sprays but also temporary hurts in field areas in which people sleep during the planting, harvesting, construction of roads, animal houses. On the basis of target species behavior, pesticide treatment might be carried out on ceilings, on the upper half or lower half of the walls. For the complete eradication of the mosquito all possible resting places such as furniture, drenches and outside porches should be treated. It has been estimated that residual effect of applied pesticide usually short on some places such as mud wall, alkaline whitewash and surfaces exposed to the excessive sun radiations or light.

(b) *Insecticides*

The factors that should be taken in to account in choosing an indoor spraying insecticide include availability, market price, residual efficacy, safety, repellency and susceptibility to target insect.

Insecticides appropriate for interior residual treatment are listed in Table

1. The use of DDT is discussed in section 1.1.

(c) *Application procedures*

Hand-operated compression sprayers fitted with a flat fan nozzle are widely used to apply insecticide. Water-dispersible powders are usually used as they are cost effective and have longer residual effects.

1. Emulsifiable concentrate formulations are not suggested for indoor residual spraying because of their short lived and non-persistent.

(d) ***Treatment cycle***

The frequency of repetition of treatment depends on the residual effect of pesticide on treated surface, vector behavior, weather and climatic conditions. Insecticide should be spray in the hoses before the starting of malarial and dengue season. The average duration of effectiveness of the various insecticides is given in Table 1. Treatment cycle may be repeated earlier if insecticide applied on the surface remove due to whitewashing, plastering or smoke deposits.

Insecticide-treated nets

Generally malaria vector tend to bite late at night, mosquito nets impregnated with insecticide would be used successfully to repel or kill them. Nets should be treated with the material that is safer to humans and other domestic animals. Nets treated with pyrethroids are fairly effective in inhibiting mosquito biting, even if they are torn. As with indoor residual spraying, enhances the risk of mosquitoes when they move in a house to bite, means that fewer will survive long enough to transmit malaria.

Mosquito Management Strategy

Besides all the measures and steps taken above the ideal mosquito management strategy is

1. Awareness at public or community level
2. Preventive measures
3. Monitoring and inspecting the methods

For this purpose there must be cooperation among individual efforts, Government and Business Zone. Spraying should be the last remedy. Punjab government is approving an effective plan by devising public awareness at media sector, campaign among students of schools, colleges and university levels. However primarily it is the responsibility of community to utilize all forms of educational tools such as banners, posters, pamphlets etc. In Pakistan people are using mosquito sprays due to absence of adequate information regarding public health. It is essential that the policy makers should formulate suitable rules and guidelines for Mosquito Management Programs at public level, than decide whether or not to use pesticides and health hazards related with pesticide exposure should not be ignored. Third and most vital part of ideal Mosquito Management Strategy is Monitoring. It supports us to get the idea about egg, larval and adult mosquito population dynamics, species type, and possible breeding sites.

From all this information it should be decided whether there is a need for spraying or not. However it is cost effective so in my view it should be carry at school/college level with in that a science teacher should be trained in mosquito Identification. For that a simple microscope is needed which is present in any school/college science laboratory. To kill the them larvicides and adulticides should not be used normally as their excessive use develop the resistance in mosquito against them.

For and satisfactory spraying, we must follow the guidelines as below:

1. After monitoring, identify mosquito susceptible areas.
2. Follow WHO guidelines and pesticides.
3. Studying and explore the biology and behavior of mosquitoes, spray the insecticide only when mosquitoes are active.
4. Watch and monitor weather forecast of the target area.
5. Train the staff and operators for spraying
6. Read and understand all labeled guidelines instructions and there should be strict follow them.
7. Try to avoid aerial spraying.
8. Before spraying issue proper notice to public at least 72 hours in advance.
9. Be careful about drinking water contaminated bodies.
10. Each Dengue Incharge/Focal person must circulate strategies on how to lessen pesticide exposure.
11. Ask school and college students and hospital patients to take additional precautionary measures.

What can we do at individual level:

1. Leave the affected area
2. shut the windows and door
3. switch off air conditioners and fans
4. Take household furniture and toys inside the house
5. Covered the swimming pools
6. prohibit the children to move and play in that area
7. Legal action should be taken against untrained pesticide applicators
8. Attention should be given to possible breeding sites and monitoring immatures before they hatch into adults.

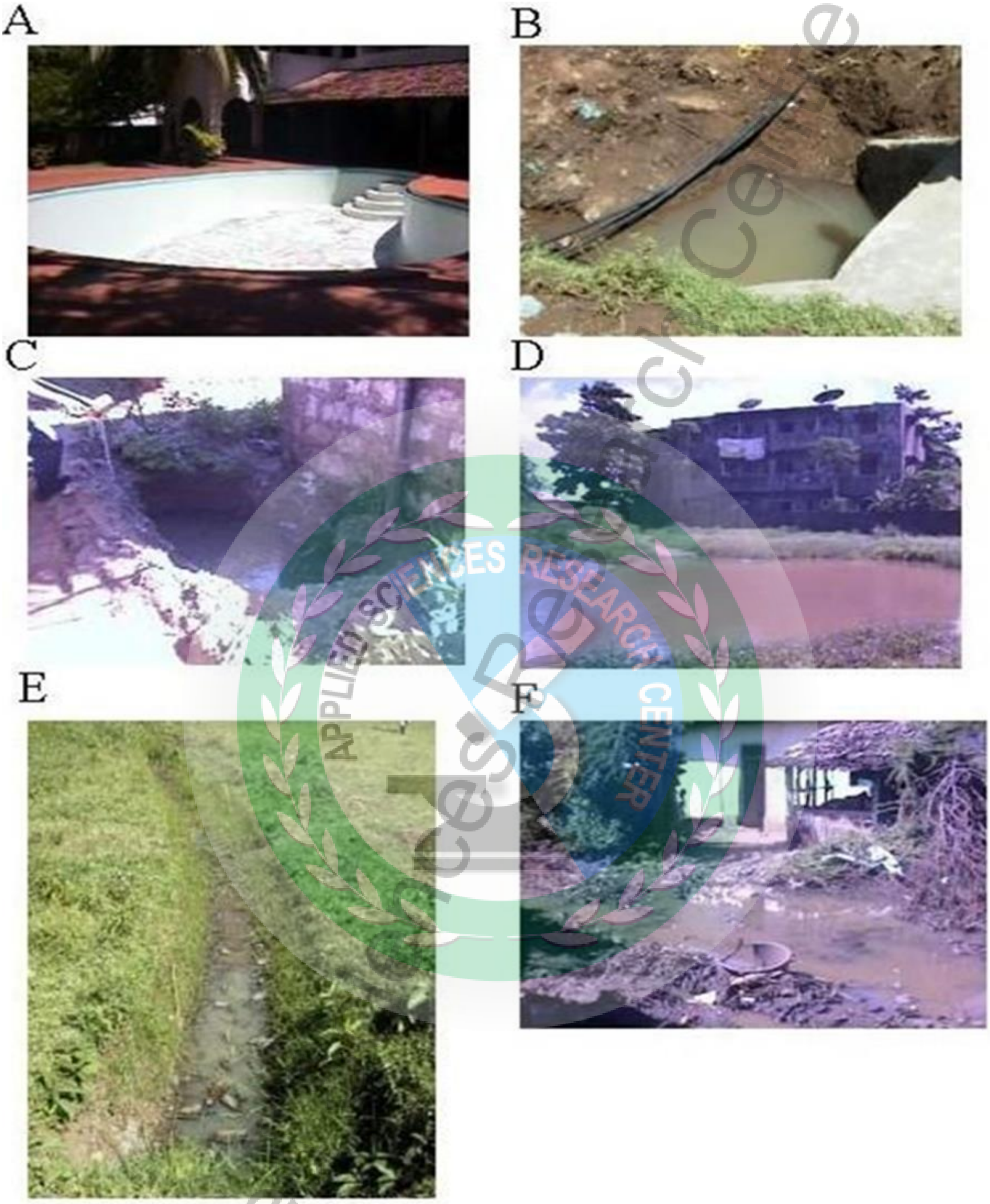


Figure A. breeding sites of female *Anopheles* spp. Mosquito

Figure B. Showing *Aedes aegypti* breeding sites at Samanabad, Lahore

Figure C. Showing the breeding site of *Aedes aegypti* at Allama Iqbal Town, Lahore

Figure D. Showing the *Aedes aegypti* breeding sites with dense population of larvae at jail road, Lahore

Figure E. Showing breeding sites of *Aedes aegypti* at Samanabad and Allama Iqbal Town, Lahore

MOSQUITOES (DIPTERA: CULICIDAE) OF THE PUNJAB

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Punjab, the biggest province of Pakistan, consisting circa 60 % of the population, primarily lies in the Oriental zoogeographical region, however, it has culicidae fauna of Palaearctic, Mediterranean and Ethiopeen regions also. Thus Punjab has a large diverse fauna of mosquitoes, some of which are vicious blood sucker while some are notorious vectors of disease, e.g., malaria, dengue, filariasis, etc. The earliest literature on mosquitoes is Fauna of British India, describing Anophilini (Christophers, 1934) and Culicini (Barraud. 19s5). After 1947 scanty work is available, particularly on anopheles mosquitoes because malaria has been taking heavy toll of human life (Ansari, 1958.). In 1971, Aslamkhan prepared a checklist of mosquitoes of Pakistan describing 91 species from West wing and 89 from East wing. Aslamkhan (1972) described all the taxa of Culicidae described from the area of Pakistan. Since 1965, our work on genetics, bionomics, control, taxonomy, etc., has revealed many new records of mosquito fauna of Pakistan (For details please see selected bibliography).

The present work is the first effort to produce a current list of mosquitoes of the Punjab. The mosquito family Culicidae consist of 3 subfamilies: Anophilinae, Culicinae and Toxorhynchitinae, which are represented in the Punjab, with 81 species belonging to 12 genera: ***Anopheles 16, Aedes 18, Armigeres 1, Coquillettidia 1, Culex 23, Culiseta 3, Mansonia 2, Mimomyia 1, Ochlerotatus 12, Uranotaenia 1, Verallina 2, Toxorhynchites 1***. These have been recorded from various districts of the Punjab, including Murree Hill. The names of species are as follows.

Family **CULICIDAE** Meigen 1818

Subfamily **ANOPHELINAE** Theobald 1901

1. Genus ***Anopheles*** Meigen, 1818
2. Subgenus ***Anopheles*** Meigen, 1818
3. ***Anopheles (Anopheles) barbirostris*** Van der Wulp, 1884
4. ***Anopheles (Anopheles) barianensis*** James, 1911
5. ***Anopheles (Anopheles) gigas*** **var.** ***simlensis*** (James) 1911

6. ***Anopheles (Anopheles) nigerrimus*** Giles, 1900

Subgenus **CELLIA** Theobald, 1902

7. ***Anopheles (Cellia) annularis*** Van der Wulp, 1884
8. ***Anopheles (Cellia) culicifacies*** Giles, 1901
9. ***Anopheles (Cellia) fluviatilis*** James, 1902
10. ***Anopheles (Cellia) maculatus*** Theobald, 1901
11. ***Anopheles (Cellia) pallidus*** Theobald,1901
12. ***Anopheles (Cellia) pulcherrimus*** Theobald, 1902
13. ***Anopheles (Cellia) splendidus*** Koidzumi, 1920
14. ***Anopheles (Cellia) stephensi*** Liston, 1901
15. ***Anopheles (Cellia) subpictus*** Grassi, 1899
16. ***Anopheles (Cellia) theobaldi*** Giles, 1901
17. ***Anopheles (Cellia) turkhudi*** Liston, 1901
18. ***Anopheles (Cellia) willmorei*** (James), 1903

Subfamily **CULICINAE** Theobald

19. Genus *Aedes* Meigen, 1818

Subgenus **AEDIMORPHUS** Theobald, 1903

20. ***Aedes (Aedimorphus) caecus*** (Theobald), 1901
21. ***Aedes (Aedimorphus) culicinus*** Edwards, 1922
22. **Aedes (*Aedimorphus*)** gouldi Reinert, 1972
23. ***Aedes (Aedimorphus) pallidostriatus*** (Theobald), 1907
24. ***Aedes (Aedimorphus) pipersalatus*** (Giles), 1901
25. ***Aedes (Aedimorphus) taeniorhynchoides*** (Christophe rs), 1911
26. ***Aedes (Aedimorphus) vexans*** (Meigem), 1830

Subgenus **CHRISTOPHERSIOMYIA** Barraud, 1923

27. ***Aedes (Christophersiomyia) thomsoni*** (Theobald), 1905.

Subgenus **DICEROMYIA** Theobald, 1911

28. ***Aedes (Diceromyia) micropterus*** (Giles), 1901
29. ***Aedes (Diceromyia) periskelatus*** (Giles), 1902.

Subgenus **FREDWARDSIUS** Reinert, 2000

30. ***Aedes (Fredwardsius) vittatus*** (Bigot), 1861.

Subgenus **INDUSIUS** Edwards, 1934

31. ***Aedes (Indusius) pulverulentus*** Edwards, 1922.

Subgenus **NEOMELANICONION** Newstead, 1807

32. ***Aedes (Neomelaniconion) lineatopennis*** (Ludlow), 1905.

Subgenus **STEGOMYIA** Theobald, 1901

33. ***Aedes (Stegomyia) aegypti*** (Linnaeus), 1762.

34. ***Aedes (Stegomyia) albopictus*** (Skuse), 1894.

35. ***Aedes (Stegomyia) patriciae*** Mattingly, 1954.

36. ***Aedes (Stegomyia) unilineatus*** (Theobald), 1906

37. ***Aedes (Stegomyia) w-albus*** (Theobald), 1905

Genus **ARMIGERES** Theobald, 1901

Subgenus **ARMIGERES** Theobald, 1901

38. ***Armigeres (Armigeres) subalbatus*** (Coquillett), 1898

Genus **COQUILLETTIDIA** Dyar, 1905

Subgenus **COQUILLETTIDIA** Dyar, 1905

39. ***Coquillettidia (Coquillettidia) crassipes*** (Van der Wulp), 1881

Genus **CULEX** Linnaeus, 1758

Subgenus **BARRAUDIUS** Edwards, 1921

40. ***Culex (Barraudius) modestus*** Ficalbi, 1889

Subgenus **CULEX** Linnaeus, 1758

41. ***Culex (Culex) barraudi*** Edwards, 1922.

42. ***Culex (Culex) bitaeniorhynchus*** Giles, 1901.

43. ***Culex (Culex) epidesmus*** (Theobald), 1910
44. ***Culex (Culex) fuscocephala*** Theobald, 1907
45. ***Culex (Culex) gelidus*** Theobald, 1901
46. ***Culex (Culex) mimeticus*** Noe, 1899
47. ***Culex (Culex) mimulus*** Edwards, 1915
48. ***Culex (Culex) pipiens* ssp. *quinquefasciatus* Say**, 1828
49. ***Culex (Culex) pseudovishnui*** Colles, 1957
50. ***Culex (Culex) sitiens*** Wiedemann, 1828
51. ***Culex (Culex) theileri*** Theobald, 1903
52. ***Culex (Culex) tritaeniorhynchus*** Giles, 1901
53. ***Culex (Culex) univittatus*** Theobald, 1901
54. ***Culex (Culex) vagans*** Wiedemann, 1828
55. ***Culex (Culex) vishnui*** Theobald, 1901
56. ***Culex (Culex) whitmorei*** (Giles), 1904

Subgenus **CULICIOMYIA** Theobald, 1907

57. ***Culex (Culiciomyia) pallidothorax*** Theobald, 1907
58. ***Culex (Culiciomyia) viridiventer Giles,*** 1901

Subgenus **EUMELANOMYIA** Theobald, 1909

59. ***Culex (Eumelanomyia) malayi*** (Leicester), 1908

Subgenus **LOPHOCERAOMYIA** Theobald, 1905

60. ***Culex (Lophoceraomyia) minutissimus*** (Theobald), 1907

Subgenus **LUTZIA** Theobald

61. ***Culex (Lutzia) fuscanus*** Wiedemann, 1820
62. **Culex (Lutzia) halifaxii** Theobald, 1903
63. Genus ***Culiseta*** Felt, 1904

Subgenus **ALLOTHEOBALDIA** Broelemann, 1919

64. ***Culiseta (Allotheobaldia) longiareolata*** (Macquart), 1838

Subgenus **CULISETA** Felt, 1904

65. ***Culiseta (Culiseta) alaskaensis* ssp. *indica* (Edwards), 1920**

66. ***Culiseta (Culiseta) neveitaeniata* (Theobald), 1907**

Genus **MANSONIA** Blanchard, 1901

Subgenus **MANSONIOIDES** Theobald, 1907

67. ***Mansonia (Mansonioides) Indiana*** Edwards, 1930

68. ***Mansonia (Mansonioides) uniformis*** (Theobald), 1901

Genus **MIMOMYIA** Theobald, 1904

Subgenus **MIMOMYIA** Theobald, 1903

69. ***Mimoyia (Mimomyia) chamberlaini* ssp. *clavipalpus*** (Theobald), 1908

Genus **OCHLEROTATUS** Llynch Arribalzaga, 1891

Subgenus **FINLAYA** Theobald, 1901

70. ***Ochlerotatus (Finlay) albolateralis*** (Theobald), 1908

71. ***Ochlerotatus (Finlay) christophersi*** Edwards, 1922

72. ***Ochlerotatus (Finlay) oreophilus*** Edwards, 1916

73. ***Ochlerotatus (Finlay) pseudotaeniatus*** (Giles), 1901

74. ***Ochlerotatus (Finlay) pulchriventer*** (Giles), 1901

75. ***Ochlerotatus (Finlay) shortti*** (Barraud), 1923

76. ***Ochlerotatus (Finlay) sintoni*** (Barraud), 1924

77. ***Ochlerotatus (Finlay) versicolori*** (Barraud), 1924

Subgenus **MUCIDUS** Theobald, 1901

78. ***Ochlerotatus (Mucidus) scatophagoides*** (Theobald), 1901

Subgenus **OCHLEROTATUS** Lynch Arribalzaga, 1891

79. ***Ochlerotatus (Ochlerotatus) caspius*** (Pallas), 1771

80. ***Ochlerotatus (Ochlerotatus) pulchritarsis*** (Rondani), 1872

81. ***Ochlerotatus (Ochlerotatus) pullatus*** (Coquillett), 1904

Genus **URANOTAENIA** Lynch Arribalzaga, 1891

Subgenus **PSEUDOFICALBIA** Theobald, 1912

82. ***Uranotaenia (Pseudoficalbia) unguiculata*** Edwards, 1913

Genus **VERRALLINA** Theobald, 1903

Subgenus **HARBACHIUS** Reinert, 1999

83. ***Verrallina (Harbachius) yusafi*** Barraud, 1931

Subgenus **NEOMACLEAYA** Theobald, 1907

84. ***Verrallina (Neomacleaya) indicus*** (Theobald), 1907

Subfamily **TOXORHYNCHITINAE**

Genus **TOXORHYNCHITES** Theobald, 1901

Subgenus **TOXORHYNCHITES** Theobald, 1901

85. ***Toxorhynchites (Toxorhynchites) albipes*** (Edwards), 1922

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87. The WHO Guidelines for drinking-water quality (http://www.who.int/water_sanitation_health/dwq/gdwq3rev/en/) provides authoritative guidance and should be consulted for application of insecticides in potable water for mosquito larviciding; and

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GLOSSARY

Abdomen – the most posterior of the three main body regions, divided into segments called tergites (dorsal half) and sternites (ventral half)

Acrostichal – referring to the median longitudinal area of the scutum, from which setae and/or scales often arise

Alula – a small lobe along the posterobasal margin of the wing

Anal Vein – the most posterior wing vein

Anepisternum – the region on the side of the thorax composed of the postspiracular area, subspiracular area, and the hypostigmal area located below and behind the spiracle and separated from the katepisternum by the anepisternal cleft

Antenna (pl., **antennae**) – a paired appendage arising from the head and divided into segments including the narrow, ring-like scape, the enlarged pedicel and the distal flagellum which is in turn divided into articles called flagellomeres

Antepronotum – a setose lobe located along the anterior margin on the side of the thorax, in front of the postpronotom and above the proepisternum

Anterior – part of a structure that is toward, or in the direction of, the front of the mosquito

Anterodorsal – referring toward or in the direction of the front and top of the mosquito

Apex (pl., **apices**) – the end or tip of a structure furthest away from the main body

Apical – at or referring to the apex of a structure or appendage

Apicolateral Patches — the patches of pale scales on the sides of the abdomen that are larger on the apical side of the abdominal segment, usually contiguous with apical transverse bands when present on the tergites

Appressed – in reference to scales, meaning lying flat on and possibly touching the body

Band – usually refers to a pattern of pale scales such as rings around or transversely across the legs and abdomen

Basal – toward, or in the direction of, the base or point of attachment to the main body

Basolateral Patches – the patches of pale scales on the sides of the abdomen that are larger on the basal side of the abdominal segment, usually contiguous with basal transverse bands when present on the tergites

Cercus (pl., **cerci**) – a small, paired appendage towards the apex of abdomen (in females only) which may or may not be visible, depending on the genus

Claw – tiny, paired structure at the apex of each leg, the shape of which can be used as a distinguishing character among species

Costa – first of the six major wing veins, located along the anterior margin and extending from the base of the wing to the apex

Coxa (pl. **coxae**)- the most basal segment of the leg, attached to the thorax

Cubitus – one of the six major wing veins, located between the media and anal vein, beginning at the base and splitting into two branches, the scaling pattern of which can b be used as a distinguishing character among species

Desiccated – referring to the drying out of specimens after death, which causes anatomical structures, such as the thorax and abdomen, to fold in on itself, making identification difficult

Distal – near or toward the end of an appendage; farther away from the main body

Dorsal – the upper surface of any structure as viewed from above

Dorsocentral Area – the longitudinal area of the scutum located between the submedian and sublateral regions

Exserted – visible; protruding from the body

Femur – the second segment of the leg located between the coxa and the tibia

Flagellomere – an individual unit of the antennal flagellum

Flagellum – the third and most distal segment of the antenna, divided into thirteen segments called flagellomeres

Fore – towards the front; anterior

Fringe – the row of long scales along the posterior margin and the apex of the wing

Genitalia – the structures involved in mating located at the end of the abdomen (may or may not be visible)

Genus (pl., **genera**) – a taxonomic classification including one or a group of species having one or a combination of characters

Gonocoxite - the largest (and basal) section of the male genitalia

Halter (pl., **halteres**) – a small, paired, club-shaped structure arising from the posterodorsal region of the thorax, just behind and below the wing base (a reduced, modified wing)

Head – the most anterior of the three major body regions

Hind – towards the back; posterior

Hypostigmal Area – a small area of the anepisternum located immediately below the spiracle (part of the anepisternum)

Inserted – not visible; not protruding from the body

Integument – the outer layer of the mosquito body from which scales and setae arise

Katepisternum – a large, relatively central section of the thorax located laterally below the anepisternum and above the fore and mid legs

Lateral – pertaining to either side of the body; when referring to scutal scaling patterns, located along the margins of the scutum

Lateral Patches – in reference to abdominal scale pattern, the pale, often triangular-shaped patches of scales located on the sides of the abdomen

Lower Mesepimeral Setae – the setae arising from the mid to lower regions of the mesepimeron, usually one to three and aligned in a row

Media – one of the six major wing veins, located between the cubitus and radius, beginning at the base and splitting into two branches

Median – in the middle; in reference to scutal scaling patterns, the middorsal area of the scutum from which the acrostichal setae arise

Mesepimeron – located laterally on the thorax, the relatively large area behind the katepisternum and above the hind coxa

Mesomeron – located laterally on the thorax; a triangular-shaped area between the mesepimeron and hind coxa

Metameron – located laterally on the thorax; a small, narrow area behind the mesepimeron, mesomeron, and hind coxa

Occiput – the posterodorsal part of the head, not distinctly separated from the vertex

Palpomere – one individual segment of the maxillary palpus

Palpus (pl., **palpi**) – the paired, segmented appendage (telopodite) of the maxilla arising from the head between the proboscis and the antenna

Paratergite – a small, oblong structure along the margin of the scutum between the spiracle and wing base

Pedicel – the enlarged, second segment of the antenna

Pleuron – the side of the thorax

Pleural – pertaining to the pleuron

Plumose – feather-like; as in the long, dense setae of the male antennae

Pollinose – having a covering of submicroscopic outgrowths (like pollen)

Posterior – pertaining to or near the rear of the mosquito or particular body parts of the mosquito

Postprocoxal Membrane – the membrane located between the fore coxa and the katepisternum and below the proepisternum (it may be difficult to see if the specimen is desiccated)

Postpronotum – located laterally on the thorax; the round area between the spiracle and the antepronotum with setae along the posterior margin

Postspiracular Area – located laterally on the thorax; the raised, rounded area immediately behind and below the spiracle (part of the anepisternum)

Postspiracular Setae – setae arising from the postspiracular area

Prescutellar Depression – the round, concave area anterior to the posterior edge of the scutum, usually without scales or setae

Probasisternum – the area on the anterior surface of the thorax, visible above and between the fore coxa and below the region where the head joins the thorax

Proboscis – the long, slender mouthparts arising from the head below the palpi

Proepisternum – located on the side of the thorax, below and anterior to the subspiracular area, above and anterior to the katepisternum and the fore coxa

Radius – one of the six major wing veins, located between the subcosta and the media; beginning at the base of the wing, it divides three times and terminates in four branches

Recumbent - in reference to scales, it means to lie flat on the surface of the integument

scale – a tiny outgrowth from a microscopic socket in the integument, the color and pattern of which is often used to distinguish among species; may be long and narrow (sometimes even resembling setae) or short and broad, sometimes triangular-shaped

Scutal – pertaining to the scutum

Scutum – the dorsal surface of the thorax

Scutellum – a transverse lobe on the posterior margin of the scutum

Seta (pl., **setae**) – a hair-like growth from the integument, the presence or absence of which is often of taxonomic importance

Setose – having setae

Species – the basic rank of taxonomic classification; a group of individuals similar in structure and appearance and able to reproduce with each other and bear fertile offspring

Spiracle – a small opening into the side of the thorax through which the mosquito breathes; while there are actually more than one, the one located directly behind the postpronotum is of taxonomic importance and for simplicity in this key is referred to as the spiracle

Spiracular Setae – the setae that arise from the small, triangular-shaped area directly in front of and slightly above the spiracle (not to be confused with the setae arising from the nearby postpronotum)

Sternite (pl., **sternites**) – one of the segments on the ventral side of the abdomen

Sternum – the ventral surface of the abdomen

Subbasal Tooth – a minute structure whose shape and presence or absence as part of the tarsal claw is of taxonomic importance

Subcosta – the second of the major wing veins, located between the costa and the radius

Sublateral – pertaining to scutal scale patterns, it refers to the longitudinal area between the lateral and submedian areas

Submedian – pertaining to scutal scale patterns, it refers to the longitudinal area between the median and sublateral areas

Subspiracular Area - the area laterally on the thorax below the spiracle and postpronotum and above the katepisternum (part of the anepisternum)

Tarsomere – one of the five units of the tarsus (numbered 1 to 5 basal to apical)

Tarsus (pl., **tarsi**) – the distal portion of the leg, divided into five segments called tarsomeres, and connected to the tibia

Tergite (pl., **tergites**) - one of the segments on the dorsal surface of the abdomen

Tergum – the dorsal surface of the abdomen

Terminalia – the apical abdominal segments that form the genitalia

Thorax – the second major body region, between the head and the abdomen, from which the legs and wings arise

Tibia – the third segment of the leg, situated between the femur and tarsus

Transverse Suture – a faint division of the scutum, dividing it into anterior (presutural) and posterior (postsutural) areas

Upper Calypter – the distal portion of the lobe along the posterior margin of the wing close to the base which may or may not have a fringe of setae, depending on the genus

Ventral – pertaining to the under surface of the mosquito or structure

Vertex – the region of the head between the eyes and the occiput

Wing – the paired, membranous appendage arising from the thorax; the scaling patterns on the veins are taxonomically important

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