

# Protocol of Mosquito Rearing for Evaluation of Different Aspects of Mosquito-Borne Diseases

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**Submitted:** 26 March 2021; **Accepted:** 10 March 2021; **Published:** 06 Apr 2021

**Citation:** Hassan Vatandoost (2021) Protocol of Mosquito Rearing for Evaluation of Different Aspects Of Mosquito-Borne Diseases. *J Mari Scie Res Ocean*, 4(1): 181-187.

## Classification of Mosquitoes

They belong to the Order: Diptera, Sub-order: Nematocera, Family: Culicidae, Genus: Anopheles, Aedes and Culex. (Fig.1). Around 3556 species of mosquitoes have been identified worldwide.

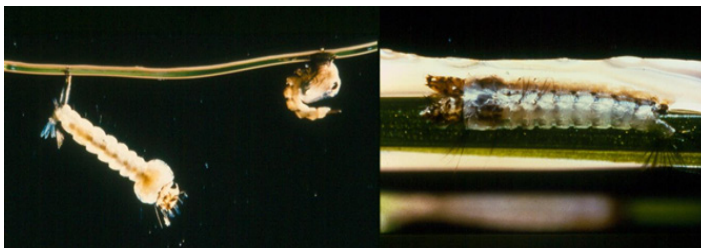


Figure 1: Larvae of Mosquito

## Breeding Site of Mosquitoes Are

small pools, fresh water, rice-land, drains, ditches, running water with shade, brackish water, salt water, stream, ponds, lakes, marshes, well, water container, discarded tin can, discarded tyre, hoof-print. (Fig.2)

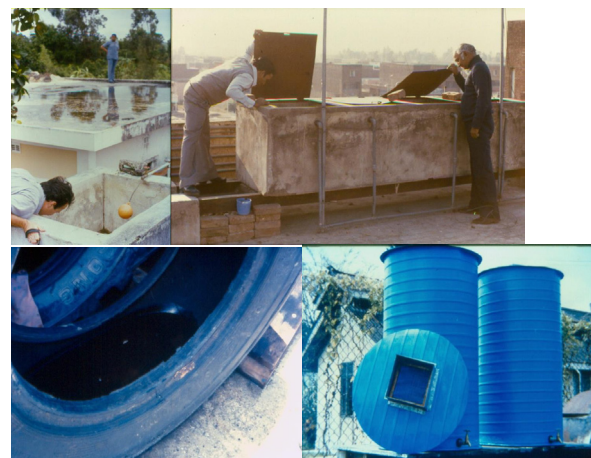


Figure 3: Breeding Places of Mosquitoes

Mosquito vectors can transmit several pathogens, including arboviruses, protozoans and filariae that cause infectious diseases of significant public health concern [1]. To a lesser extent, they may also transmit bacterial diseases [2]. Mosquitoes of medical importance belong to the family Culicidae and are widely distributed around the world. This large family currently encompasses 3556 valid species of mosquitoes distributed within the subfamilies Culicinae and Anophelinae [3,4]. The mosquito vectors mainly belong to three genera, Anopheles, Aedes and Culex.

Diseases transmitted by mosquitoes include malaria, dengue, West Nile virus, chikungunya, yellow fever, filariasis, tularemia, dirofilariasis, Japanese encephalitis, Saint Louis encephalitis, Western equine encephalitis, Eastern equine encephalitis, Venezuelan equine encephalitis, Ross River fever, Barmah Forest fever, La Crosse encephalitis, and Zika fever, as well as newly detected Keystone virus and Rift Valley fever [5, 6]. Nearly 700 million people get a mosquito-borne illness each year resulting in over one million deaths.

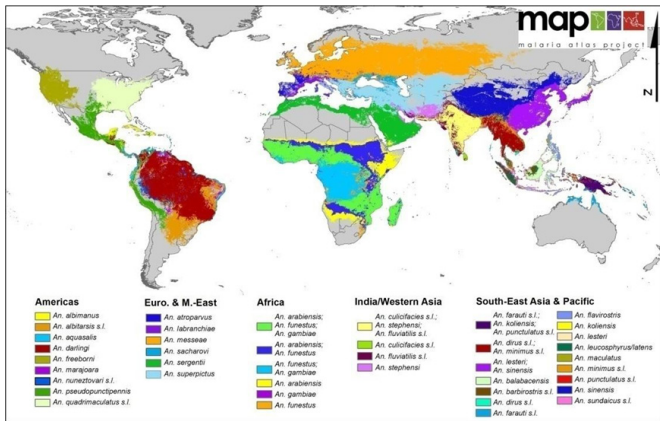


Figure 4: Global Distribution of Anopheles Species

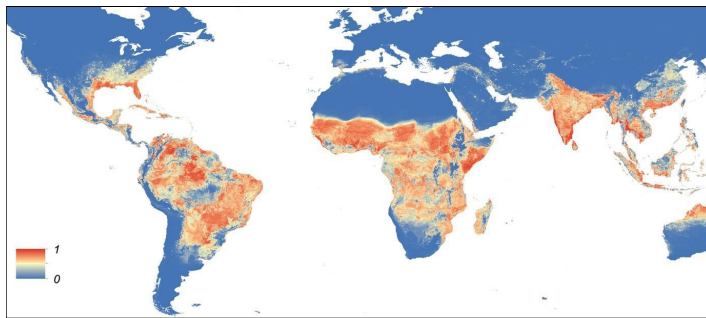


Figure 5: The Global Distribution of the Arbovirus Vectors Aedes aegypti

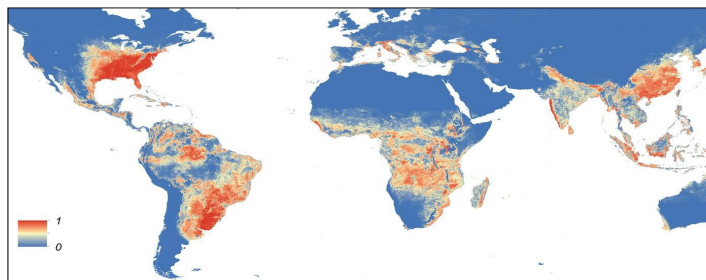


Figure 6: The Global Distribution of the Arbovirus Vectors Ae. Albopictus

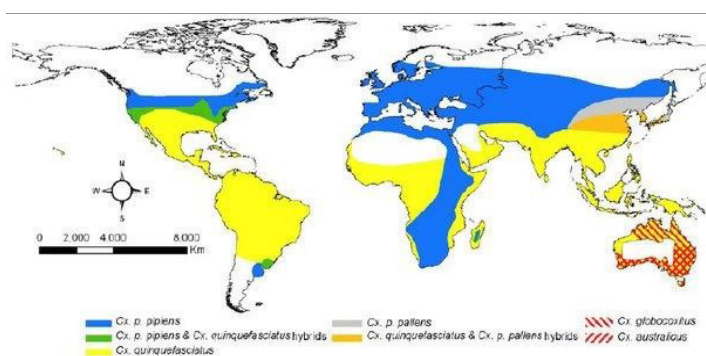


Figure 7: Global Distribution of Culex Species

**Different Method for Mosquito Collection are:**

Indoor hand catch, outdoor hand catch (shelter pits), animal bait,

human bait, larval collection, light trap.

Outdoor collection of mosquitoes are: on vegetable, on solid surfaces in shelter places, bank of stream, bank of ditches, hole in rocks, culverts, cracks, caves, animal burrows, on the trunk of trees, termite mounds.

Outdoor collection of mosquitoes are; collecting mosquito outdoors with a sucking tube, hand net, drop net, artificial outdoor shelters. Direct collection of mosquitoes from bait are; human bait indoors, human bait outdoors, animal bait outdoors, human-baited trap net, animal baited trap net.



Figure 8: Different Methods of Collection of Mosquitoes

**Establishment of Insectary for Rearing Of Mosquitoes**

An important factor for carrying out all the laboratory works are rearing and maintain the mosquito under insectary conditions. Equipment for Handling Mosquito Adults

**Cages**

A wide variety of rearing cages, Size is related to the numbers of mosquitoes to be caged. This density affects mating, feeding and longevity. A vertical resting surface of 1.8 cm<sup>2</sup> per mosquito,

Many species will not mate in cages less than 30 x 30 x 30 cm. Aedes, Culex, and Anopheles, Freshly colonized from the field will only mate successfully in cages of 1 x 1 x 1 m or more, Toxorhynchites and sometime some Anopheles species

### Cage Frame

wood, metal, brass, aluminum. The front of the cage should have a fabric sleeve wide enough to allow the introduction of small bowls (12 cm diameter) for egg laying. A solid base is preferable to one of netting as it has to support bowls containing pupae or water for egg laying (Fig.9)

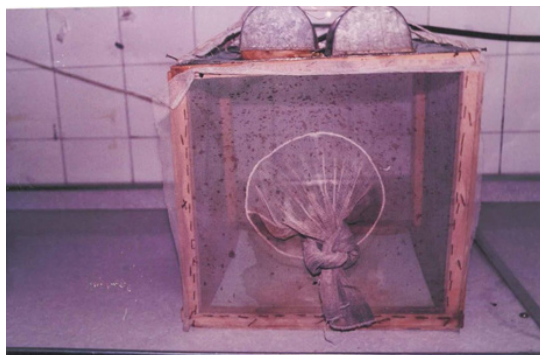


**Figure 9:** Cages for Mosquito Collection Under Insectary Conditions Adult Food

Water for adults may be provided in small bowls or in the form of water soaked cotton wool pads placed on top of the cage. 10% solutions sucrose in a cotton wool. Sliced apples or raisins or sultanas on the cage tops.

### Blood Meals

Blood meals are necessary for egg production in all but the few autogenous species. Colonies are usually fed blood twice a week. One of the simplest methods involves placing an anesthetized animal on the cage (Fig.10. Fig.11).



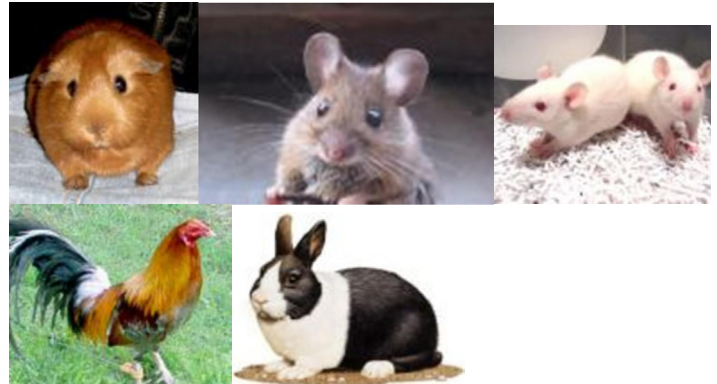
**Figure 10:** Blood Feeding of Mosquitoes On Guinea Pig



**Figure 11:** Blood Feeding of Mosquito

### Sources of Blood

Include guinea pigs, mice, rats, chicken and Rooster, rabbit, (Fig.12)



**Figure 12:** Sources Of Adult Food Of Mosquitoes

Mammals are usually shaved with hair clippers on the flank prior to feeding (Fig.13. Fig.14)



**Figure 13:** Shaved Mammals for Blood Feeding



**Figure 14:** Blood Feeding of Mosquitoes On Rabbit

### Artificial Feeding

Membrane feeding system, this technique is more usually reserved for infecting mosquitoes with malaria, filarial or arboviruses (Fig.15)



**Figure 15:** Apparatus for Artificial Feeding of Mosquitoes

For field use the smaller numbers of mosquitoes are often housed in cylinders of plastic or cardboard ice-cream cartons with netting end (Fig.16)



**Figure 16:** Cylinders of Plastic or Cardboard Ice-Cream

Adult mosquitoes are transferred between cages by means of an aspirator or suction device (Fig.17, Fig.18)



**Figure 17:** Aspirator or Suction Device



**Figure 18:** Mouth Aspirator with Filter

### Egg collection

The eggs of Anopheles are not drought resistance and are laid individually on the surface of free water, usually fresh, in a bowl. Sometimes filter paper is placed inside the bowl to prevent eggs sticking to the sides or to facilitate transfer of eggs. The eggs can sometimes be transported on damp filter paper although at room temperature they will hatch within a couple of days. Chilling may sometimes be used to delay hatching.

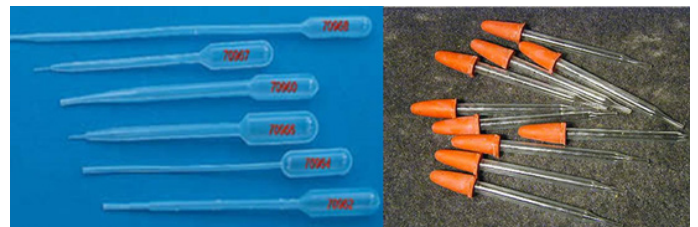
### Larval Rearing Procedures

Trays roughly 30 x 25 x 5 cm are suitable for holding water to depth of around 4 cm with 300 larvae per tray (Fig.19).



**Figure 19:** Trays for Larval Collection

Larvae are handled and transferred using glass pipettes, plastic pipettes or eye dropper (Fig.20)



**Figure 20:** Transferring Larvae by Glass Pipettes, Plastic Pipettes or Eye Dropper

### Larval Food

An extraordinary variety of materials have been used a larval food, ranging from guinea pig faces to well defined chemical diets. Commercial dog biscuit, yeast tablets, liver powder, dried blood, fish food flakes, baby food also are being used (Fig.21).



Figure 21: Larval Food of Mosquito

### Handling of pupae

Pupae may be picked with tent ended glass pipettes of the type used to handle larvae or with small netting lifters (Fig.22).

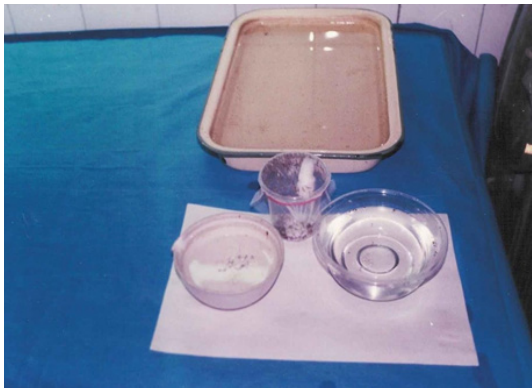


Figure 22: Collection of Pupae Insectary Design

The three main requirements for an insectary are an ability to control temperature, humidity, and photoperiod (Fig.23)

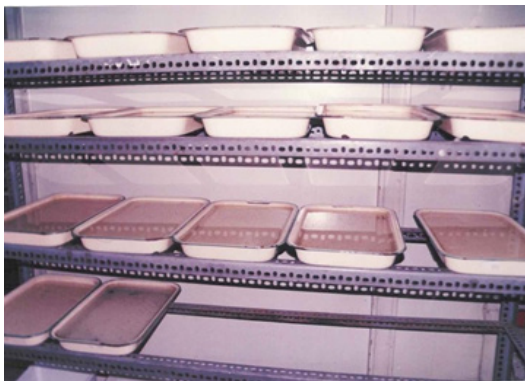


Figure 23: Rows of Insectary

### Temperature Control

Regular temperature records should be taken in the insectary. A maximum-minimum thermometer and/or recording thermo hygograph for this purpose. Temperature range 26-28 °C. Maximum and minimum- thermometer is required (Fig. 24)



Figure 24: Maximum and Minimum- Thermometer

### Humidity Control

provided by small commercially available mist/aerosol producing devices (Fig.25).



Figure 25: Equipment for Humidity Control of Insectary

### Photoperiod Control

Some species may require a crepuscular period to swarm and mate. This may necessitate insectary controls for automatic dimming of lights to provide a dusk effect and gradual increase in brightness to simulate dawn dimming of lights (Fig.26)



Figure 26: Down and Dusk Control in The Insectary

### Safety Correct Rearing Procedures

white painted walls, a white painted ante-room, drains may require, screening, oviposition traps Insectary should not be sited in close proximity to animal rooms.

## Using of Mosquito Reared for Evaluation of Insecticide

There are several guidelines for evaluation of susceptibility of mosquito to WHO recommended insecticide at the adult and larval stage as well as evaluation of bednet [7, 16]. (Figs 27,28,29,30,31)



Figure 27: Conical Bioassay Test



Figure 28: Tunnel Test



Figure 29: Larval Susceptibility Test



Figure 30: Irritability Tests



Figure 31: Adult Susceptibility Test

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