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RESEARCH ARTICLE

Rearing Protocol for *Culex quinquefasciatus*

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Mosquitoes are vectors capable of transmitting various life-threatening diseases such as dengue, chikungunya, Japanese encephalitis, lymphatic filariasis, malaria, and so on. They are reared in the laboratories for conducting various studies such as vector biology, vector competence, mechanism of disease transmission, to check the efficiency and follow-up of various control methods, insecticide susceptibility, and vaccine trials. Moreover, mass rearing of mosquitoes is required for sterile insect technique (SIT) to control mosquitoes. Successful breeding of mosquitoes requires attention to detail and depends upon various factors such as quality and quantity of larval food, temperature, humidity, population size, mating, blood feeding, and egg laying. *Culex quinquefasciatus* is a vector of lymphatic filariasis (LF) and a cosmopolitan mosquito, abundant in tropical and subtropical regions. In this study, a simple and convenient rearing protocol for *Culex quinquefasciatus* has been discussed.

Keywords : *Culex quinquefasciatus*, mosquito rearing, protocol.

Introduction

Culex quinquefasciatus, an established vector of lymphatic filariasis is found throughout the year.[1] It breeds profusely in polluted stagnant water rich in organic matter and is abundant in nature. To combat the spread of diseases caused by *Culex quinquefasciatus*, the vector itself is targeted and kept under check through integrated vector management. This requires successful breeding of the vector to study the vector biology, vector competence, and mechanism of disease transmission, to check the efficiency and follow-up of various control methods, insecticide susceptibility, and vaccine trials. Mosquitoes are holometabolous organisms having four life stages namely egg, larva, pupa, and adult. The pre-adult stages are aquatic. Mosquito rearing requires careful attention to every aspect in detail and depends upon various factors such as the quality and quantity of larval food, temperature, humidity, population size, mating, blood feeding, and egg laying. This protocol provides simple and effective rearing of *Culex quinquefasciatus* which has been followed at our laboratory for the last four years.

Equipment and reagents

Adult mosquitoes or any life stages of *Culex quinquefasciatus*.

Rack: For holding enamel trays and mosquito cages and saving space. [Fig1(a)]

Enamel trays (Preferably upper surface in a rectangular shape) with dimensions of l×b×h of 30cm×25cm×6cm respectively have been found suitable in our experiment [Fig 1(b)]

Mosquito cage: The cage can be made of a mosquito net sewed in a cuboidal shape with a dimension of $(30cm)^3$ along with a circular window joined by a sleeve of the net long enough to be tied shut, on one side through which the mosquitoes, oviposition dishes and sugar solution/blood feed can be inserted or taken out. [Fig 1(c)]

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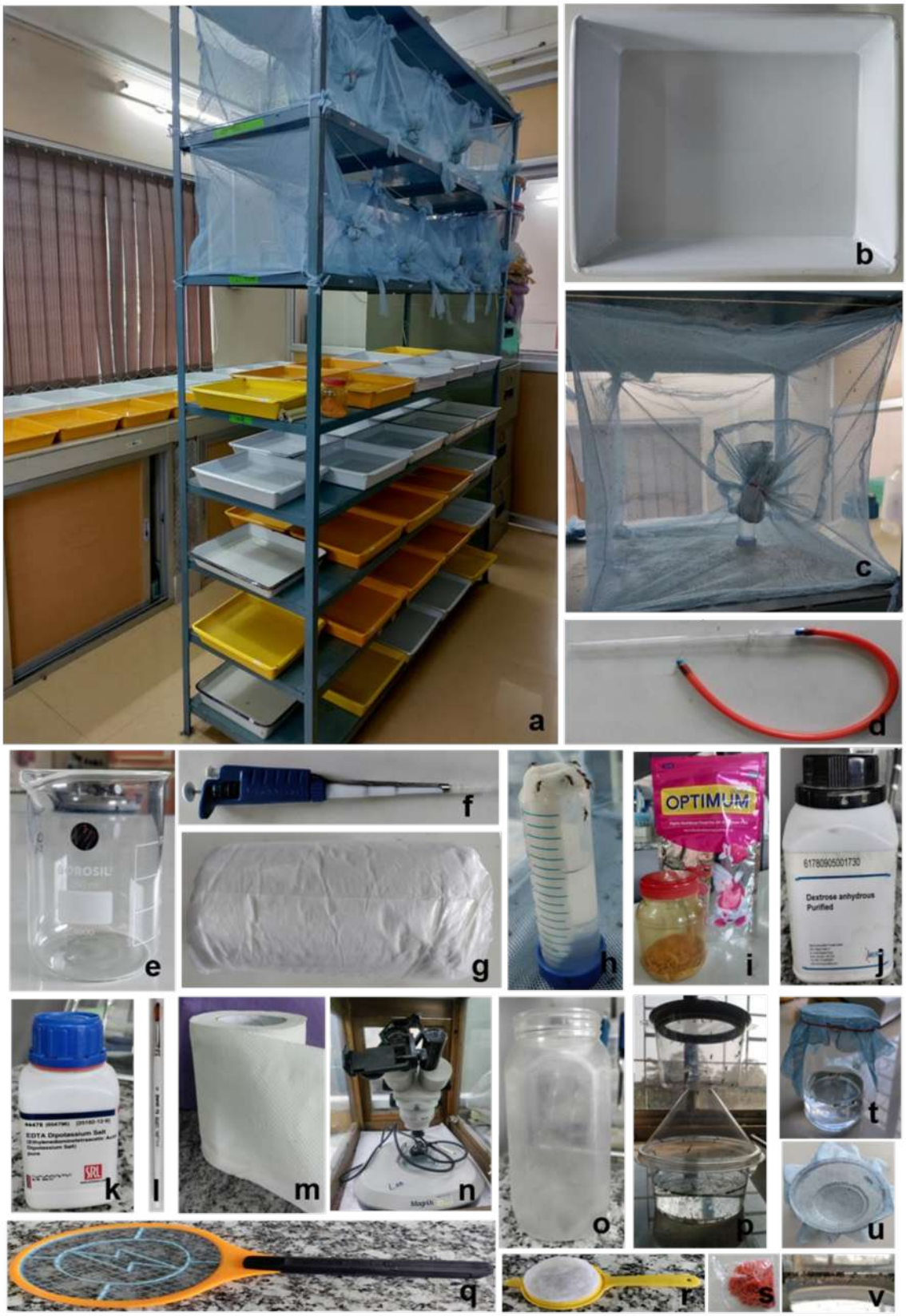

Figure 1. (a) Mosquito rearing rack with enamel trays and cages (b) Enamel tray (c) Cage (d) Manual aspirator (e)Glass beaker (f) 1ml Pipette (g) Absorbent cotton (h) Modified falcon tube (i) Fish food (j) Dextrose (k) EDTA (l) Paintbrush (m) Tissue paper (n) Microscope (o) Sample collecting jar (p) Adult emergence kit (q) Mosquito bat (r) Strainer (s) Rubber band (t) 250ml beaker with pupae covered with mosquito net (u) upper view of beaker showing the net (v) Larvae and pupae of *Culex quinquefasciatus*

Aspirator: Manual or mechanical (available at labitems.com) for collecting adult mosquitoes. [Fig 1(d)]

Glass beaker (250ml Borosil) [Fig 1(e)]

1 ml Pipette (Tarson) [Fig 1(f)]

Absorbent Cotton [Fig 1(g)]

50 ml Falcon tube (Tarson, 546041) [Fig 1(h)]

Fish food [Fig 1(i)]

Dextrose (Merck) or any carbohydrate source. [Fig 1 (j)]

EDTA Dipotassium Salt (Ethylenediaminetetraacetic Acid, SRL, 44478) [Fig 1(k)]

Chicken blood or any blood source.

Fine Painting brush (size 6) [Fig 1(l)]

Tissue paper [Fig 1(m)]

Microscope (40X magnification) [Fig 1(n)]

Plastic jar [Fig 1(o)]

Adult emergence kit (BugDorm, Taiwan, available at labitems.com) [Fig 1(p)]

Mosquito bat [Fig 1(q)]

Strainer [Fig 1(r)]

Rubber band [Fig 1(s)]

Sample collection

Culex quinquefasciatus breeds in water, rich in organic matter and an adequate amount of sample can be collected at once. In this study, larvae and pupae were collected from polluted drains. A strainer was attached to a stick and swiped through the upper surface of the water. The larvae and pupae collected in the strainer were gently transferred to a jar with clean water. They were then brought to the laboratory and the pupae were separated in an adult emergence kit [Figure 1(p)]. The adults formed were morphologically identified with the standard identification keys.[2,3] They were shifted into a $(30cm)^3$ cage with a density of 1 mosquito per $90cm^3$ of cage. 150 males and 150 females in a cage is ideal for successful mating and they were supplied with 10% dextrose solution. The females were provided blood meal as described in the section 'Adults'. Then the rearing procedure was followed as mentioned below.

Procedure

Eggs

Culex quinquefasciatus lays their eggs in rafts with 100-250 eggs glued together and floating on the upper surface of the water [Fig 2(b)]. An oviposition dish (250ml Borosil glass beaker) with 200ml of clean water can be placed in the cage after 4-6 days of blood feeding. Straw-boiled water can also be used as an oviposition stimulant imitating the dirty water sources that *Culex* prefers [Fig 2(a)]. The egg rafts are transferred gently to individual enamel trays with a fine paintbrush. If hatched within the oviposition dish, the content can be directly poured into the enamel tray. The eggs hatch within 24-48 hours and if failed to hatch within this time, they will never hatch. Those eggs are termed non-viable eggs.

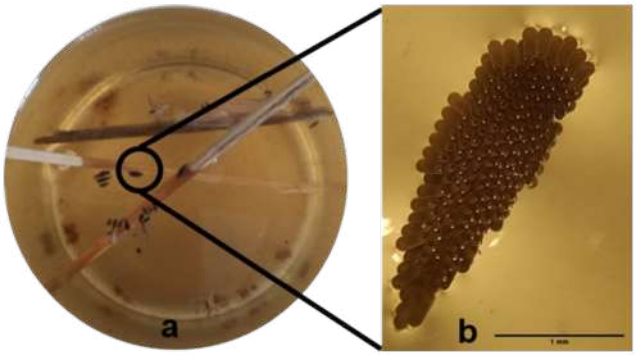

Figure 2: (a) Egg rafts in oviposition dish containing straw boiled water as a stimulant (b) Egg raft (40X Magnification)

Larva

Larvae are voracious eaters. They undergo four molting and are termed accordingly as first, second, third, and fourth instars. Just after hatching, they are termed as first instars and after the fourth instar, they develop into a pupa. The larvae stay in an inclined position from the surface of water through a siphon that helps in breathing [Fig 3(a), (b)]. Larvae shed their exoskeleton and grow larger during each molt. Each instar differs in its length and head size. 200-250 larvae in 2L of water per enamel tray of 30cm×25cm×6cm is best suited for proper growth and development. Overcrowding should be avoided as it leads to longer developmental times, competition for food and space, small body size of adults, and larval mortality as well.

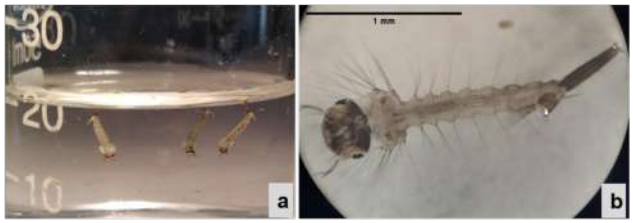

Figure 3. (a) *Culex quinquefasciatus* larvae in beaker (b) Larva (40X Magnification)

Pupae

Pupae are comma-shaped with the head attached to the abdomen. The head consists of two trumpets that help in breathing while the abdomen has flippers on the end for swimming [Fig 4(a), (b)]. They are active swimmers but do not consume food. The formation of pupae from the larvae of a single batch lasts for 5-6 days while they stay in the pupal stage for 1-2 days. As such the pupae must be separated in an adult emergence kit or beakers covered with a net to prevent the emergence of adults in the tray itself [Fig 1(p), (t), (u)]. Pupae can be separated manually with the help of a 1ml pipette attached with the microtip having a slight cut in its tip for broad space or with the help of a dropper [Fig 1 (f)]. When the number of pupae is greater than 20 along with the presence of larvae, it can be separated with the vortex in the ice water[4] or simply in the rearing water in a 1L conical flask.[5]

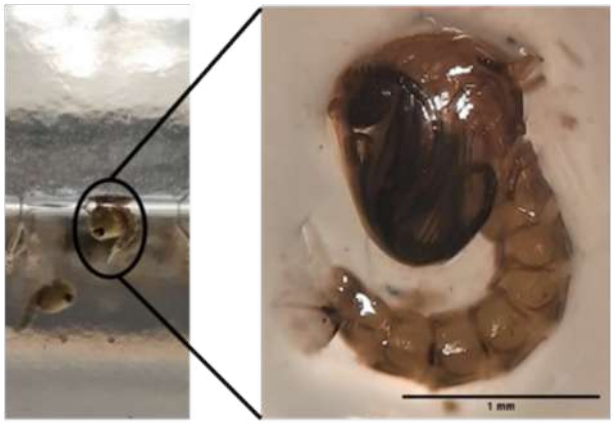

Figure 4: (a) Pupae in the beaker (b) Pupa (40X Magnification)

Adults

The pupae emerge into adults within 1-2 days. The adults rest on the surface of the water till their bodies dry and harden. A male and female adult can be easily separated with the help of antennae as males have plumose (bushy) antennae while females have pectinate antennae [Fig 5(a), (b)]. Moreover, males are relatively smaller in size than that of females. Males tend to emerge earlier than females. The number of males and females can be counted in the emergence kit and then transferred to the cage with the aspirator.

The adults feed on any carbohydrate sources. In our study, we provided 10% dextrose solution filled in an inverted 50 ml Falcon tube with its bottom cut and a cotton wick placed that worked through capillary action [Fig 1(h)]. The dextrose solution and cotton wick were changed every 3 days. The females need a blood meal to produce eggs.[6] *Culex quinquefasciatus* is an opportunistic feeder, preferably feeding on avian

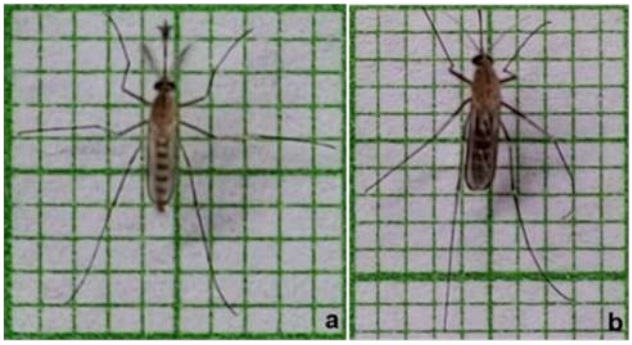

Figure 5: *Culex quinquefasciatus* (a) Male (b) Female

as well as mammalian blood. Hopken et al.[7] reported that 81.2% of the blood meal of Culex *quinquefasciatus* was from avian taxa, 17.9% from mammalian taxa, and 0.85% from reptilian taxon through analysis of DNA extracted from mosquito blood meals. In this study, fresh broiler blood collected directly from the local chicken shop was used. 5% EDTA was added to the blood-collecting vessel to prevent blood clotting. The blood was soaked in the cotton, spread in a Petri dish (Fig 6), and put inside the cage containing 3-5 days old, 24 hours-starved female mosquitoes after successful mating. The blood meal was provided in the evening. The number of engorged females was noted down to check the blood-feeding rate.

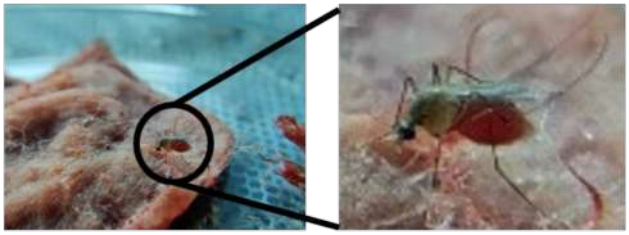

Figure 6: Female *Culex* feeding on blood

Day-wise summary of *Culex quinquefasciatus* rearing:

- *Day 1:* Keep 3-5 days old female in starvation for 24 hours
- *Day 2:* Feed them with blood
- *Days 3-5:* Continue feeding with sugar solution
- *Day 6:* Place the oviposition dish
- *Day 7:* Collect egg rafts and place them individually in enamel trays
- *Day 8:* Check the hatchability of egg rafts
- *Days 9-13:* Feed the larvae and separate if overcrowded

- *Days 14-17:* Collect pupae
- *Day 18:* Starve the females
- *Day 19:* Feed them with blood and then continue the cycle

Insectary maintenance

- The temperature and humidity are critical factors for mosquito rearing and it is suggested to maintain a temperature of 25±5°C and 70-80% humidity. In this study, the rearing was carried out in the ambient room temperature with a fluctuation according to the seasonal variation and was found still suitable for mosquito colonization.
- The light : dark hours should be maintained at 14:10 hours respectively.
- The rearing water should not be cloudy, and dirty.
- The enamel trays, adult emergence kit, and aspirator should be washed at regular intervals.
- The cages should be cleaned before the introduction of the next generation of mosquitoes.
- Adults if escaped during transfer must be killed with a mosquito-killing bat or trap set.
- The excess larvae and pupae no longer required for rearing can be sieved through a strainer and then shifted to tissue paper before disposal.
- The excess adults are collected in a beaker covered with a mosquito net and put in the freezer for 1 hour before disposal. The adults may also be starved till death or let them die naturally and disposed later.

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