

REARING AND COLLECTION OF MOSQUITO LARVAE FROM NATURAL
BREEDING POINT FOR LABORATORY BIOASSAYNeeraj Yadav¹ and Archana Yadav^{2*}¹Ph. D. Scholar, Department of Zoology, Kamla Nehru Institute of Physical and Social Sciences, Sultanpur Uttar Pradesh.²Assistant Professor & HOD, Department of Zoology Kamla Nehru Institute of Physical and Social Sciences, Sultanpur Uttar Pradesh.***Corresponding Author: Dr. Archana Yadav**

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ABSTRACT

Controlling mosquitoes is essential for public health since they are important carriers of many infectious diseases. For laboratory bioassays, this study explores the capture and raising of mosquito larvae from natural nesting areas. The study shows a number of collecting techniques, such as gravid traps, CO₂-baited traps, and human landing catches, highlighting their effectiveness and adaptability to many ecological settings. In addition, the study looks at mosquito breeding site dynamics and larval rearing methods, which sheds light on the ecological preferences and life phases of vector species. Laboratory bioassays on these larvae make it easier to monitor resistance and assess larvicidal activity, both of which are essential for creating efficient vector management plans. In order to lower the prevalence of mosquito-borne diseases and improve public health outcomes, the research's findings help develop novel mosquito control strategies, deepen our understanding of the dynamics of disease transmission, and guide integrated vector management strategies.

KEYWORD:- Gravid traps, CO₂-baited traps, Mosquito-borne diseases, Disease transmission.**INTRODUCTION**

Mosquitoes continue to be vital insect species in spite of changing ecological conditions, host species, and climatic and environmental factors. Despite having some aquatic components in their life cycle, mosquitoes are biological carriers of numerous infectious diseases, both known and unknown.^[1-5] Reducing mosquito populations and avoiding contact with them are the most effective ways to prevent the spread of agents.^[6,7] When one considers their size, movement, diversity, and reproduction capacity, this becomes difficult. By altering the site's maintenance conditions and implementing protective equipment, nets, and repellents, mosquito populations can be decreased.^[8,9] Resistance may arise when larvicides of chemical and microbiological origin, along with growth regulators, are used to inhibit mosquito larvae. Unplanned and unregulated use of these substances can exacerbate resistance. Resistance to inorganic pesticides was documented in 12 arthropod species even prior to the development of organic pesticides. H6 Resistance was found in 198 insect species in 1990, 150 insect species in 1980, and two insect species in 1946.

The World Health Organization (1992) reported that resistance was found in 20 species of mosquitoes belonging to the genus *Culex*, 19 species of mosquitoes belonging to the genus *Aedes*, and 56 species of mosquitoes belonging to the genus *Anopheles*. Larvae and adults were shown to be resistant.^[11] 553 arthropod species have resistance by 2008, with 202 of them species being significant for public health.^[12]

Numerous mosquito species, some of which are the main carriers of malaria and some arboviral illnesses, can grow and survive in the Belgrade environment.^[13-16] *Anopheles* mosquitoes, which may be carriers of malaria, have been reported to exist in the Belgrade district.^[15]

In 2012, West Nile virus was found in *Culex pipiens* mosquitoes on Belgrade city's territory.^[17] Permethrine, lambda-cyhalothrin, deltamethrin, and compositions based on malathion were used to inhibit adult mosquito forms in the past.^[18] The need to investigate the effects of alternative substances for the control and elimination of mosquito larvae was influenced by the long-term use of organophosphate larvicides in the Belgrade area, the lack of information regarding larvicide resistance, and their removal from the list of approved biocides.^[19-21]

Collection of mosquito larvae

Larvae collected from the habitats were categorized into 1st, 2nd, 3rd and 4th instars, and pupae numbers were recorded. The larval density per dip is calculated by dividing the total number of larvae collected by the number of dips taken (10dips). Percent reduction in different instars larvae and pupae were calculated using the Mulla, s formula as follows: % Reduction= $100 - \frac{C1 \times T2}{C2 \times T1}$ Where, C1= pretreatment immature density in control sites C2= post treatment immature density in control sites T1= pretreatment immature density in treated sites T2= post treatment immature density in treated sites Persistence of the larvicides in different breeding habitats of the target species was determined from the post treatment density.^[22]

Human landing catches (HLCs) are the gold standard method for collecting human-biting mosquito species, while there are other ways to gather entomological data.^[23] But only anthropophilic, host-seeking mosquito species are collected by HLCs. In order to take advantage of many facets of mosquito feeding and resting behavior, such as anthropophily, zoophily, endophily, exophily, endophagy, and exophagy, additional techniques for adult mosquito sampling can be employed both indoors and outdoors. However, considering the diversity of mosquito species' behaviors, comparison studies are required to ascertain the effectiveness of traps. Trap design, attractant use, and location are factors that can affect the collection's abundance, species composition, female physiological status (gravid, blood-fed, etc.), and infection prevalence.^[24,25,26]

To determine which trap is best suited for mosquito monitoring and surveillance goals in a certain area, it is crucial to take bias into account. To our knowledge, only a small number of studies have examined the effectiveness of mosquito traps in West Africa (for instance, in Ghana^[27] and Senegal^[26]), despite the fact that several traps in East Africa have been compared to HLCs.^[24] Both CDC light traps (LT) and HLCs conducted outside had higher captures rates for *An. Gambiae* in Western Kenya than HLCs conducted indoors, indicating that traps can be crucial for malaria entomological surveillance.^[24]

There are many methods for collection of mosquito larvae in which we explain some methods which are given below

Method for collection of flying adults' mosquito larvae

Among the traps used to collect mosquito samples during dispersal and foraging flights are malaise traps, truck traps, and electrocution traps. These traps can be positioned between mosquito breeding grounds and areas where they get their blood meals because they don't require attractants. They might be especially helpful in figuring out when mosquitoes fly and in determining when to implement adult mosquito control methods.

Over the past few decades, a number of innovative mosquito capturing or monitoring technologies have been invented in addition to the variety of traditional mosquito traps utilized by mosquito specialists. Some use new chemical lures, some have different airflow systems, and some power the fan with new technologies. Only sample protocols for the methods employed by the Vector Net project are provided in this paper. Additional information is available in two ECDC guideline documents^[29–31] as well as in Silver^[27] and Becker *et al.*^[28]

Trapping methods for collection of mosquito larvae^[32]

• HLC or human landing collection

The earliest and most straightforward technique for gathering female host-seeking mosquitoes is most likely HLC. HLC conducted during daily activity peaks can effectively gather anthropophilic organisms. It is best to collect day-biting mosquito samples in areas with shade. Although it is advised to gather the females before they bite, there are certain drawbacks, including labor expenses and the possibility of contracting the disease. It is advised to use a fixed sampling length (such as 15 minutes) for standard comparisons. If there are a lot of mosquitoes, it can be shortened to five minutes ($\geq 5/5$ min). The ability of the collectors and the attraction a person has on mosquitoes determine the results, which must be considered when comparing numbers to estimate abundance.

• Traps baited with animals

Different cues from various hosts attract mosquitoes, and host preferences vary by species. For instance, *Culex territans* prefers to eat frogs, *Culex pipiens* can eat birds, other animals, and humans, and certain *Anopheles* species can eat both humans and animals. A wide range of animals, including cattle, horses, goats, pigs, rats, and different birds, are used in animal-baited traps. An aspirator is used to extract mosquitoes directly from a tethered animal, or they are gathered off the animal shed's walls. While they are less common in large-scale surveillance programs, animal baits are frequently employed in West Nile virus monitoring investigations.

• Suction traps baited with CO₂

The most widely used mosquito traps are suction traps baited with CO₂. They work with dry ice (which sublimates carbon dioxide) or a carbon dioxide tank and are a powerful lure for females of many species that are looking for hosts. These traps can be powered by a battery or a power source, and they may or may not contain a light source. They have the advantages of being easy to use, durable, portable, frequently powered by cheap 3 x 1.2 V batteries, and not being greatly affected by ambient light in metropolitan environments. The requirement to have access to dry ice or gaseous carbon dioxide is the primary drawback. When combined with light, they collect more male mosquitoes but this increases the sample pollution with other nocturnal insects, mainly Lepidoptera and non-hematophagous

Dipterans. This slows down identification by making separation time-consuming and frequently harming the mosquitoes that were sampled. While incandescent light typically attracts mosquitoes, some female mosquitoes may be repelled by it. Other hematophagous insects including Ceratopogonidae (biting midges), Simuliidae (blackflies), and Phlebotominae (sandflies) are also captured by carbon dioxide-baited traps. The CO₂ supply (maximum of 24 hours) or battery capacity (12V, 10A, maximum 48 hours) typically limit how long these traps may operate. When live females are required for mosquito-borne disease surveillance, CO₂-baited suction traps are an effective sampling method. Another benefit is that dry ice stored in insulated containers makes a great conservation medium while traveling to the lab.

- **Light traps**

Although they have also been used in arboviral monitoring, light traps are most frequently employed in malaria surveillance. Light traps are particularly effective at night when there is minimal light pollution. The light trap's benefits include portability and ease of use with a 6-volt battery (or three 1.2-volt batteries in CO₂-baited light traps). The drawbacks include their high by-catch of other insect species and their marginal or poor attractiveness to diurnal and crepuscular active mosquitoes. However, as previously mentioned, carbon dioxide can be introduced as the primary attractant. It is possible to screen live-trapped females for arboviruses carried by mosquitoes. A significant number of

technicians is required if light traps are to be utilized in large-scale surveys because their batteries only last one night. In nine hours, a technician can put up over fifty traps across a total distance of 450 km.

- **Gravid traps**

These traps are used to catch gravid females that have fed at least once and need to oviposit. They are made up of a black bucket filled with water, hay, or an infusion of dead leaves. In arbovirus surveys, gravid traps are employed because they have a higher chance of identifying viral infections in fed mosquitoes than in samples collected by CO₂-baited traps, which gather host-seeking females, the majority of whom are unfed. Although gravid traps are thought to be unappealing to *Aedes* mosquitoes, they can be made more appealing (for species that live in containers exclusively) by infusing them with dead oak leaves or grass.

- **Sticky traps**

Sticky traps draw gravid egg-laying females, just like ovitraps (for egg sampling) and gravid traps. Internal surfaces are where the mosquitoes land and adhere. The samples can be utilized for pathogen screening and species identification. Samples must be taken every day.

- CDC light traps and gravid traps were used to gather mosquitoes indoors. (Hoto 1, 2, 3)

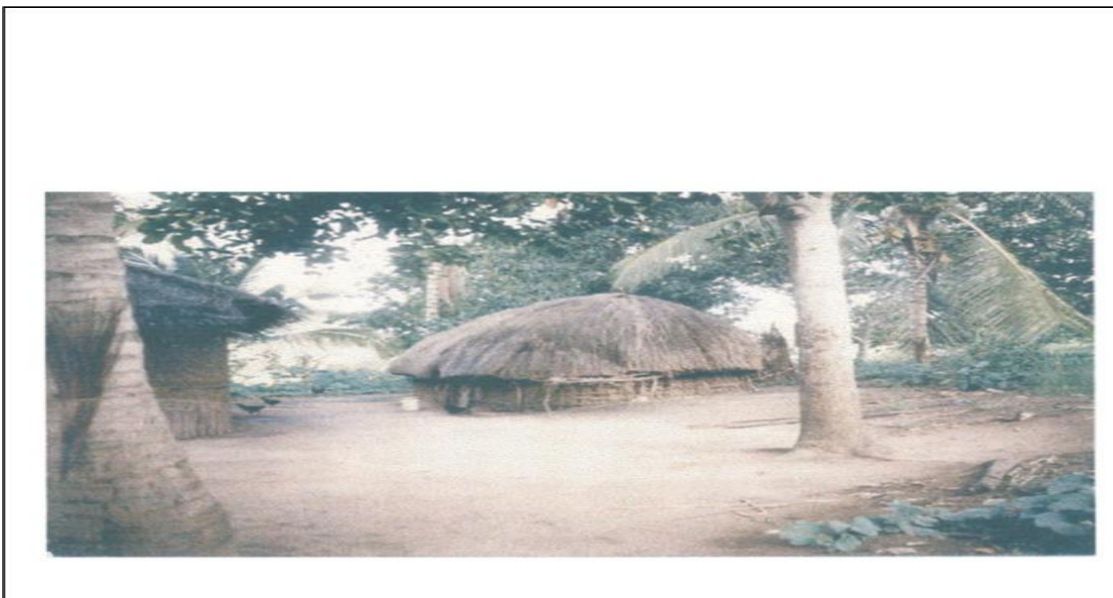


Photo 1: A typical home in the research area with a high mosquito contact. The house has a grass-thatched roof and stick walls.



Photo -2: A CDC light trap is placed inside a home to catch mosquitoes that are looking for an indoor host.



Photo-3: A CDC gravid trap is placed inside a home to catch mosquitoes that are looking for an indoor host.

Mosquito breeding site

Every year, mosquitoes spawn, and if you don't stop them from doing so, you won't be able to develop an effective mosquito control strategy. To get rid of them and reduce the risk of mosquito-borne illnesses, you must first be informed of the typical mosquito breeding sites. Here are five typical locations for mosquitoes to breed:^[33]

1. **Clogged Gutters and Drains:** When gutters and drains are clogged, water can be readily trapped and provide the perfect environment for mosquitoes of all kinds to reproduce. Therefore, be sure to clean your drains and gutters on a regular basis to maintain them clear and avoid water buildup, which will discourage mosquitoes from breeding.
2. **Pooled Water and Puddles:** Because these species need a lot of puddles to spawn quickly, many people believe that the rainy season is the busiest time of year for mosquitoes. Therefore, to reduce the possibility of mosquito breeding, be sure to remove any standing water from inside or outside your home.
3. **Ponds and Bird Baths:** Although you may think they're lovely, did you realize that a number of mosquitoes breed in neglected ponds and birdbaths each year? Therefore, to prevent mosquito breeding, it is essential to install pumps in ponds and change the water in birdbaths on a regular basis.
4. **Plant saucers and flower pots:** Since they frequently contain standing water, mosquitoes will not spare these items. Therefore, make sure to drain these pots

and saucers on a regular basis to stop mosquitoes from reproducing.

5. **Water Storage Containers:** If you don't remember to cover those barrels, drums, and water tanks with a tight lid, they can quickly become mosquito breeding grounds. Although these are meant to hold water, mosquitoes will seize the opportunity to lay their eggs in them if you are careless. As a result, when not in use, empty them or keep them well covered with lids.

Rearing of mosquito

Larval stage

According to Balestrino et al. (2012, 2014), larvae are raised in a steel rack with thermoformed ABS (acrylonitrile butadiene styrene) plastic trays (Glimberger Kunststoffe Ges.m.b.H., Austria).^[34] Two long ridges, each measuring 32 cm in length and 2.5 cm in height, run down the main axis of the 100 × 60 × 3 cm trays' flat surface. Fifty large plastic trays can fit in a fully loaded rack, but before rearing, each tray needs to be carefully inspected to ensure it is positioned properly horizontally. However, the rack may be made to accommodate fewer than fifty trays, depending on the height of the rearing room. 5L of water with a depth of 1.2 cm can be added to each tray, and 18,000 first instar larvae (L1), or 3.6 larvae/cm², can be sown into each tray (Zhang et al. 2017).^[35] Thus, 900,000 larvae can be housed at a time in each rearing rack. Although the larval rearing process is now handled and maintained manually (e.g., by adding food and water), a system that uses a dosing gun to aliquot a predetermined portion of feed into each tray is currently being developed. There is an automated tray-rack prototype that enables electrical tilting of the entire rack.

An artificial liquid diet (4% w/v) was created at the IPCL in Seibersdorf, Austria, and is fed to larvae (Puggioli et al. 2013, FAO/IAEA, 2017).^[36] All of the nutrients required for larval growth, such as vitamins, proteins, and fatty acids, are sufficiently supplied by the diet. It is made up of 60% powdered tuna meal, 36% bovine liver powder, and 14% yeast. The hatching solution (nutrient broth and yeast) and brushed eggs, or boiling and cooled osmosis water for hatching, are placed in an airtight-lidded jar.

Before being moved to each rearing tray in the rack, freshly hatched first instar larvae are sieved. Locally accessible and least expensive diets are utilized since the cost and availability of larval food may vary from one nation to another. Before utilizing it for mass-rearing, it is advised to make sure that its production is standardized and free of contamination and to thoroughly evaluate the impact of each food composition on the life history features of mosquitoes. For instance, certain research and release programs in Brazil (*Ae. aegypti*), Mexico (*Ae. aegypti*) (Bond et al. 2017).^[37] and Italy (*Ae. albopictus*) (Puggioli et al. 2013)^[36] use diet mixtures that include Friskies dry adult cat food (Nestle

S.A., Vevey, Switzerland) and rodent diet (PMI Nutrition International LCC, St. Louis, MO).

Pupal stage

It is necessary to separate pupae from the remaining larvae since pupation occurs gradually. A mechanical system made specifically for the separation of mosquito sexes and developmental stages can be used for larger-scale rearing (Focks, 1980).^[38] A roughly horizontal platen holds glass panes, creating an adjustable, downward-pointing wedge-shaped opening between them that is used to pour the contents of an aquatic insect culture. Thus, by adjusting the thickness and angle of the wedge-shaped area using control knobs, the different forms (sexes, developmental phases) can be divided according to size.^[37] The smaller forms (male pupae and larvae) flow through the lower hole into a receiving container below, while the larger organisms are kept in the narrowing area between the glass panes. The larger organisms (female pupae) are flushed into a second receiving container once the wedge is opened to complete the procedure. By altering the platen's angle, one can change the force with which the insects move between the plates.^[34]

Adult stage

In a separate room, adults are kept at 26± 2 °C, 70± 10 RH%, and 14:12 hours of light to dark, which includes one hour of dawn and one hour of nightfall. A 100-liter (100 × 100 × 10 cm) edes mass-rearing cage (MRC) has been created for colonies of *Aedes albopictus* and *Aedes aegypti* brood stock. The entire cage may be managed from the outside, reducing the possibility of escape and making adult rearing colony maintenance easier. Devices for collecting eggs, giving sugar and blood, introducing pupae (drainage system), and cleaning the cage are outlined.^[39]

Hanging the cage up

The adult rearing cages are made to hang from the ceiling in order to reduce ant infestation and make the most use of insectary space. Two carabineers can be fastened to the two metal eyelets on the upper side of the cage. The cage (17 kg) may be suspended from the ceiling at a comfortable height by hooking the carabineers to two chains that are the right length and location.

Drainage system

As a moving spout, elbow plumbing fixtures are screwed to the stopcocks, being careful to keep them loose enough to swivel up or down. To prevent unintentional water spills, pupae, and/or floating eggs, it is advised to make sure the outlet stopcocks are in the "closed" position when not in use. When the cage is ready, water is added to the bottom along with the pupae. Drainage is accomplished by simultaneously flushing water from the inlet valve and opening the bottom valve.

A funnel can be used to pour water into the input valve. When all of the pupae have emerged, which is typically 48 hours following pupal introduction, the first draining should be carried out. To prevent the dead adult that dropped into the water from fermenting, the water must also be changed once or twice after the egg papers are collected. Drops of sucrose solution from a sugar device can also aid in fermentation.

Internal sugar feeding device

This apparatus is composed of a cylindrical, 6 cm-diameter stainless steel tube that is sealed on both ends. An entrance line with a watertight stopper is attached to one side of the tube, 5 cm from the end, so that sugar solution can be added from outside the cage. Holders fastened to the cage sidewalls hold this gadget in place throughout the lower half of the cage. On the same side of the tube as the entrance pipe are three sizable cuts that are encased in 20-micrometer stainless steel mesh. The adults can feed through the mesh if it is filled with sugar solution (1.2L maximum capacity) and rotated 180 degrees.

Net fitting

Rectangles of netting must be cut to size and lined with Velcro® on all four edges to form the cage's side panels. This is attached by pressing the Velcro® against the matching Velcro® trim that surrounds the cage's metal frame.

Velcro strips are sewed along the screen's edges and can be secured to the cage frame using Velcro® strips that have already been glued (Henkel Central Eastern Europe GmbH, Austria). Make sure there are no gaps and that the Velcro® is securely fastened to the cage frame. After that, time should be set out on a regular basis to check the netting for damage. It is also necessary to examine the Velcro®'s adherence to the metal frame, which can be fixed with contact adhesive.

Blood feeding ports

Two circular, three to five centimeter-diameter holes on the top of the cage are used as blood feeding ports. For blood feeding, two mesh socks, each measuring 17 cm and hanging vertically from these ports, enable the insertion of sausage casings loaded with blood. To improve access to the blood meal, the mesh socks can alternatively be placed at either end or in the center of the cage.^[40]

Significance of the study

The study of rearing and collecting mosquito larvae from natural breeding points for laboratory bioassays holds significant importance in understanding mosquito ecology, vector control, and public health. By examining larvae from natural habitats, researchers gain insights into the environmental factors influencing mosquito populations, such as water quality and breeding preferences, which are crucial for designing targeted interventions. Laboratory bioassays using these larvae

allow for the evaluation of larvicides' efficacy and the monitoring of insecticide resistance, providing critical data for improving vector management strategies. Additionally, studying mosquito larvae contributes to understanding the dynamics of disease transmission, particularly for diseases like malaria, dengue, and Zika, by identifying species-specific behaviors and adaptations. This research supports advancements in vector control methodologies, aids in community education about breeding site management, and informs evidence-based public health policies aimed at reducing the impact of mosquito-borne diseases.

Future outcomes

The future outcomes of studying the rearing and collection of mosquito larvae from natural breeding points for laboratory bioassays include the development of more effective and targeted mosquito control strategies, reducing the prevalence of mosquito-borne diseases. It will enhance our understanding of mosquito breeding patterns and ecological preferences, leading to innovations in habitat management and larvicide application. Additionally, the study will support the identification and monitoring of insecticide resistance trends, enabling the formulation of sustainable resistance management practices. This research may also guide the creation of novel control tools, such as genetically modified mosquitoes or biological control agents. Ultimately, the findings will contribute to improved public health outcomes by reducing vector populations and disease transmission while informing policies for integrated vector management and community engagement programs.

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