

**GUIDELINES FOR
Aedes VECTOR SURVEILLANCE
AND
CONTROL IN SRI LANKA**



National Dengue Control Unit
Ministry of Health, Nutrition and Indigenous Medicine
Public Health Complex
555/5, Elvitigala Mawatha, Narahenpita, Colombo 05.

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GUIDELINES FOR *Aedes* VECTOR SURVEILLANCE AND CONTROL IN SRI LANKA

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This authoritative document is the essential resource on *Aedes* vector surveillance and control in Sri Lanka published by the National Dengue Control Unit in November 2016.

This guideline was developed based on the best available scientific evidence at the time of writing. The guideline will be reviewed periodically when new evidence becomes available. Please forward your comments and suggestions to the following address by post or email.

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FOREWORD

The transmission and magnitude of dengue epidemics have dramatically expanded in recent years. Introduction of integrated vector management based on strong real-time epidemiological and entomological surveillance, generate proper and regular information to reduce and prepare for outbreak mitigation. Hence, vector control interventions executed through vector surveillance is very important as a preventive measure of dengue fever. Emerging threat of various mosquito borne illnesses such as Zika, Chikungunya and Yellow Fever transmitted through *Aedes aegypti* mosquito requires wider attention.

The “National Guidelines on *Aedes* Vector Surveillance and Control for Sri Lanka” developed by the National Dengue Control Unit, is expected to further improve existing knowledge and practices on *Aedes* vector surveillance and control in Sri Lanka to execute activities more efficiently and effectively. Its guiding principle is to harmonize prevention via entomological surveillance within the existing health system ensuring this effort is coherent, sustainable and cost-effective.

I hope that this document will serve as a guide for synchronized and integrated action with partners within the health system and other stakeholders in strengthening and streamlining control and preventive activities to reduce the impact of the mosquito-borne disease burden in Sri Lanka.



Dr. Palitha Mahipala
Director General of Health Services

PREFACE

The mosquito –borne disease, Dengue and Dengue Haemorrhagic Fever is a major public health problem in Sri-Lanka. At present, more efforts have to be directed to control mosquito vectors, namely *Aedes aegypti* and *Aedes albopitcus* on a nationwide scale. The strategy adapted is an integrated programme incorporating, source reduction through environmental management, targeted vector control, health education and law enforcement. When applied in combination the strategy is expected to significantly reduce the *Aedes* mosquito population in vulnerable areas and prevent and control disease outbreaks early. The overall aim of this document is to adopt an integrated approach to eliminate Dengue as a public health problem, with adequate support and commitment from all stakeholders.

This new publication will be a useful reference document of the *Aedes* control programme in Sri Lanka developed by the National Dengue Control Unit. It is a useful document for programme managers in planning and rolling out a prevention and control programme on *Aedes* mosquitoes. It can also serve as a guide for training and research activities in Sri Lanka and other countries.

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THE NATIONAL DENGUE CONTROL UNIT

The National Dengue Control Unit (NDCU) is the focal point for the National Dengue Control Programme in the Ministry of Health, Sri Lanka. It was established in year 2005 through a policy decision taken by the Ministry of Health following the major dengue outbreak in year 2004. It is responsible to coordinate entomological surveillance, integrated vector control and inter-sectoral collaboration, social mobilization and capacity building, along with regular monitoring and evaluation of both national and subnational activities for control and prevention of dengue.



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01

INTRODUCTION

Thus Dengue has become the communicable disease of greatest public health importance in Sri Lanka. Although dengue was first reported in the Western Province, currently it is widely distributed throughout the island especially in urban areas.

INTRODUCTION

Dengue is an arboviral disease complex which includes Dengue fever (DF), more severe Dengue Haemorrhagic Fever (DHF) and life threatening Dengue Shock Syndrome (DSS). The disease is caused by four virus serotypes, which are designated as DENV-1, DENV-2, DENV-3 and DENV- 4.

Dengue is found in tropical and subtropical regions of the world. DF/ DHF is endemic in the Americas, South-East Asia, the Western Pacific, the Eastern Mediterranean and the tropical areas of Africa. Recent estimates suggest there are 3.6 billion people at risk and 390 million dengue infections occurring each year including 96 million symptomatic disease and 15,000 deaths.

In Sri Lanka, the first dengue patient was reported in 1962. Since year 2000, dengue has become endemic in the country with periodic epidemics. Thus Dengue has become the communicable disease of greatest public health importance in the country. Although, Dengue first reported in the Western Province, currently it is widely distributed throughout the island especially, in urban areas.

Transmission of the dengue virus depends on biotic and abiotic factors. Biotic factors include the virus, the vector and the host. DF /DHF is transmitted by female mosquitoes of *Aedes aegypti* (Linnaeus) and *Aedes albopictus* (Skuse). *Ae. aegypti* is the major vector of DF/DHF that causes epidemics; *Ae. albopictus* is an important vector of Dengue that may cause epidemics when its density is high. Abiotic factors include temperature, humidity and rainfall.

To date, vector control is the mainstay of dengue prevention and control. Application of appropriate vector control interventions depend on vector ecology, biology, behaviour (bionomics), efficacy and effectiveness of vector control methods which are generated through robust vector surveillance. This document provides practical guidelines on dengue vector surveillance and control in the Sri Lankan context.



02

BIOLOGY AND IDENTIFICATION OF DENGUE VECTORS

*This chapter discusses the life cycle and key external morphological characters for identification of different stages of the life cycle of dengue vector mosquitoes; *Ae. aegypti* and *Ae. albopictus*.*

BIOLOGY AND IDENTIFICATION OF DENGUE VECTORS

Vector biology, identification and study of the bionomics and ecology of dengue vectors are of paramount importance in targeting vector control interventions for dengue prevention and control. This chapter discusses the life cycle and key external morphological characters for identification of different stages of the life cycle of dengue vector mosquitoes; *Ae. aegypti* and *Ae. albopictus*.

2.1. Life cycle of *Ae. aegypti* and *Ae. albopictus*

The life cycle has 4 distinct stages, viz. egg, larva, pupa and adult (Fig 2.1). The first three stages are aquatic and the adult is terrestrial. The time taken to complete the life cycle is usually 7-10 days depending on the environmental factors.

Eggs

The female mosquito lays eggs (oviposit) singly on damp inner surface of wet containers above the water level, preferably with clear water. The eggs are smooth, long, ovoid shaped and about 1mm long. When first laid, the eggs appear white but within minutes they turn shiny black.

Within about 2 days, the eggs hatch to larvae. The eggs can withstand dry weather conditions and retain viability for up to six months or longer.

Larva

The larva undergoes 4 stages of development namely, 1st, 2nd, 3rd and 4th instar larvae (Fig 2.2). Developing larvae feed on organic matter contained in the water except late 4th instar larva which is a non feeding stage. All the larval instar stages are mobile with a characteristic “s” shape movement of the body. At the end of larval period of 4 - 5 days, the 4th instar larva develops into a pupa. The mosquito larval body consists of three major parts, viz., head, thorax and abdomen (Fig.2.3).

Pupa

The pupa is comma shaped and mobile. This stage is the final aquatic stage of the mosquito's life cycle and it develops into adult mosquito within 1-2 days. Pupa is a non feeding stage. Pupal body consists of two major parts, namely, cephalothorax and abdomen (Fig 2.4). The density of pupae is a crude proxy to the adult mosquito density.

CHAPTER 02

Adult

Adult *Aedes* mosquitoes are small to medium-sized (approximately 4 -7 mm), dark in colour with white markings/bands on the body (Fig. 2.5). The adult life span (longevity) can range from 02 - 04 weeks depending on environmental conditions such as temperature and humidity.

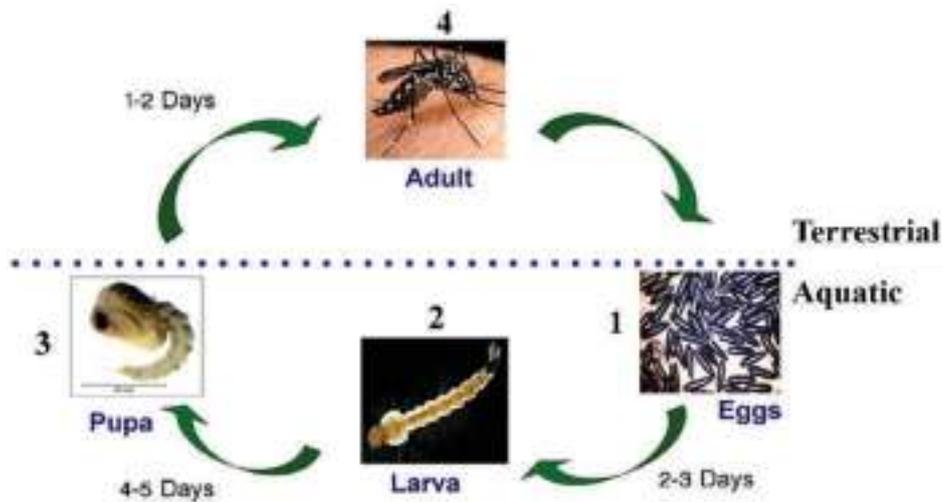


Fig. 2.1. Life cycle of the dengue vector (7 – 10 days)

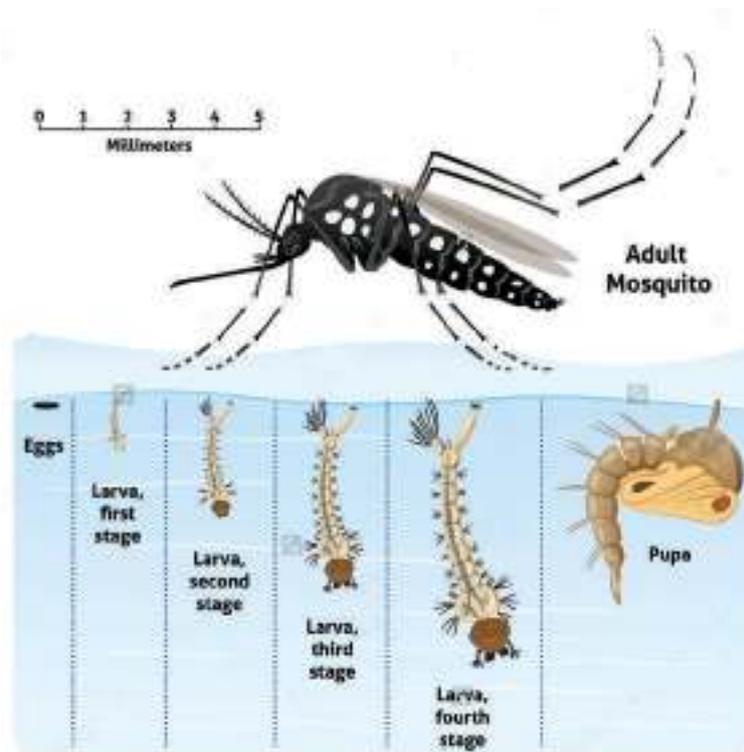


Fig. 2.2. Life cycle of *Aedes* showing different stages of larval instars

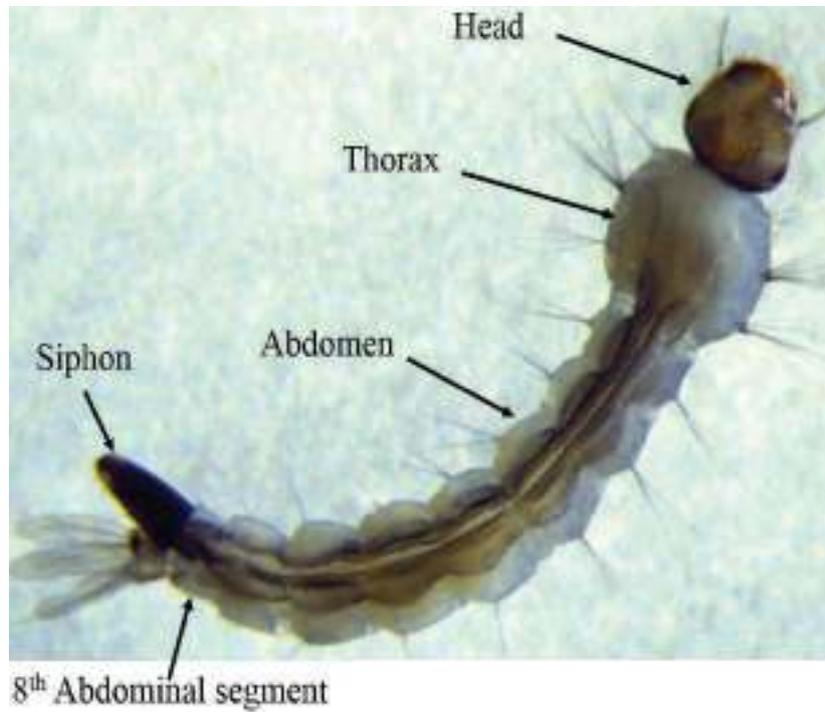


Fig. 2.3. Major body parts of the *Aedes* larvae
 Source: Florida Medical Entomology Laboratory, University of Florida



Fig. 2.4. Major body parts of the *Aedes* Pupa
 Source: Florida Medical Entomology Laboratory, University of Florida

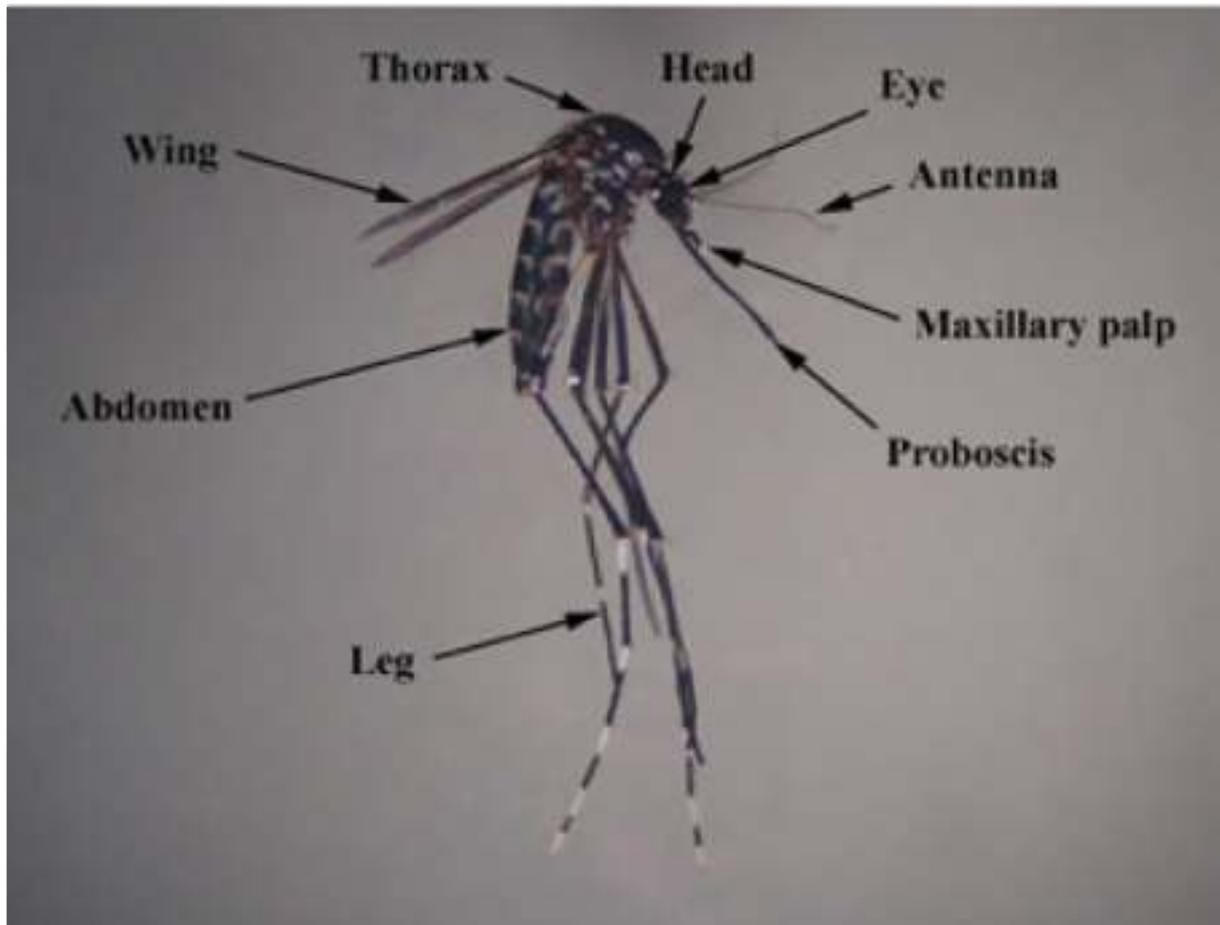


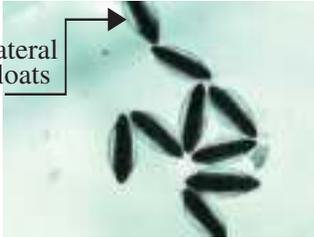
Fig. 2.5. Major body parts of the adult *Aedes* mosquito
 Source: Mr.Sampath Weerakoon, Entomologist/Badulla

2.2. Characteristic features of *Aedes*, *Anopheles* and *Culex* mosquitoes

The major differences between *Aedes* (that includes vectors of Dengue, Chikungunya and Zika), *Anopheles* (that includes vector of Malaria) and *Culex* (that includes vectors of Filariasis and Japanese encephalitis) are shown in Table 2.1.

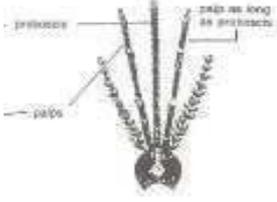
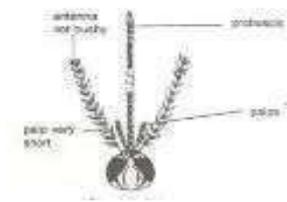
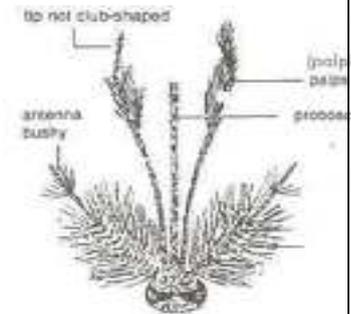
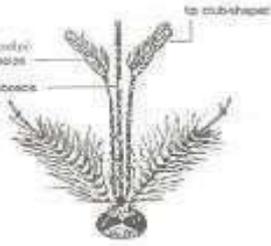
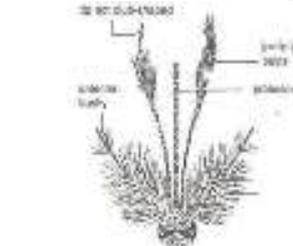
Aedes mosquitoes can be differentiated from *Anopheles* and *Culex* mosquitoes by the external morphological features, ornamentation and resting positions of this genus at different stages of the life cycle. Adult male mosquitoes of all three genera can be differentiated from females as the males have a pair of plumose (bushy) antennae and female mosquitoes bear pilose (less hairy) antennae (Table 2.1)

Table 2.1. Differentiation of *Aedes*, *Anopheles* and *Culex* mosquitoes at different stages of life cycle

Stage of the life cycle	Mosquito genera		
	<i>Aedes</i>	<i>Anopheles</i>	<i>Culex</i>
Eggs <ul style="list-style-type: none"> • Black in colour, Laid singly on damp surface of wet containers above the water level 	<ul style="list-style-type: none"> • Laid singly on the surface of water • Have lateral floats  <p>Lateral floats</p>	<ul style="list-style-type: none"> • Laid in the form of egg raft that floats on the surface of water • Never possess lateral floats 	
Larvae <ul style="list-style-type: none"> • Body has comparatively less hairs • Siphon present. The siphon is comparatively short, barrel shaped and more chitinous with a single pair of siphonal tufts • No palmate hairs • No tergal plates on the abdominal segments • Rest with an angle to the water surface. 	<ul style="list-style-type: none"> • Body has many hairs • Siphon absent • Has palmate hairs • Have tergal plates on the abdominal segments • Rest parallel to water surface.  <p>Tergal plates</p> 	<ul style="list-style-type: none"> • Body has many hairs • Siphon present and the siphon is comparatively long and slender with more than one pair of siphonal tufts. • No palmate hairs or tergal plates • Rest with an angle to the water surface. 	

CHAPTER 02

Stage of the life cycle	Mosquito genera		
	<i>Aedes</i>	<i>Anopheles</i>	<i>Culex</i>
Pupae <ul style="list-style-type: none"> Breathing trumpet is long and slender with a narrow opening. No spines on abdominal segments 2-7  <p>Source:elp.tamu.edu</p>	<ul style="list-style-type: none"> Breathing trumpet is short with a wide opening apically. Short peg like abdominal spines on segments 2 or 3-7  <p>Source: medent.usyd.edu.au</p>	<ul style="list-style-type: none"> Breathing trumpet is long and slender with a narrow opening. No spines on abdominal segments 2-7  <p>Source:www.diptera.info</p>	
Adults (both sexes) <ul style="list-style-type: none"> Rests more or less parallel to the surface. Black and white scales on the body and legs arranged in different patterns  	<ul style="list-style-type: none"> Rests at an angle of between 50° and 90° to the surface. In most species dark and pale scales on wing veins arranged in distinct blocks  	<ul style="list-style-type: none"> Rests more or less parallel to the surface. Scales on wing veins are not arranged in blocks, scales frequently all brown or blackish  	

Stage of the life cycle	Mosquito genera		
	<i>Aedes</i>	<i>Anopheles</i>	<i>Culex</i>
Adult females <ul style="list-style-type: none"> • Palpi much shorter than proboscis 	<ul style="list-style-type: none"> • Palpi are as long as proboscis  	<ul style="list-style-type: none"> • Palpi much shorter than proboscis  	
Adult males <ul style="list-style-type: none"> • Palpi are longer than proboscis with tapered tips  	<ul style="list-style-type: none"> • Palpi are as long as proboscis and the tips of palpi are club-shaped (swollen)  	<ul style="list-style-type: none"> • Palpi are longer than proboscis with tapered tips  	

(Source:WRBU)

2.3. Identification of *Ae. aegypti* and *Ae. albopictus*

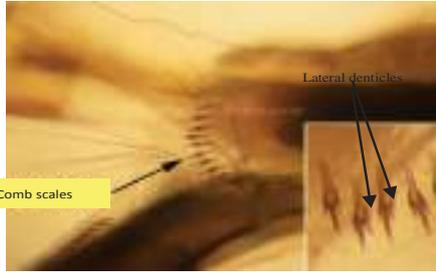
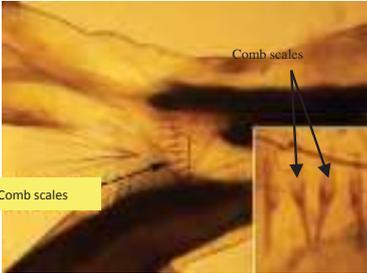
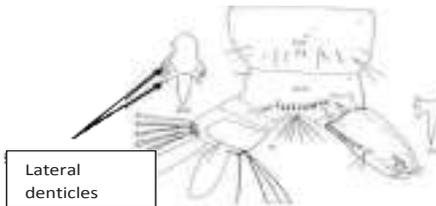
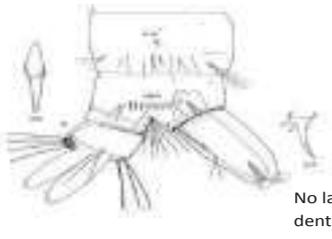
In Sri Lanka, 48 *Aedes* (Meigen) species belonging to 11 subgenera have been reported where as over 900 *Aedes* species are identified worldwide. *Ae. aegypti* and *Ae. albopictus* belong to the subgenus *Stegomyia* (Theobald) of the genus *Aedes*. In Sri Lanka, in addition to these two species, 4 other *Aedes* species belong to this subgenus as shown in Fig. 2.6.

Kingdom	: Animalia
Phylum	: Arthropoda
Class	: Insecta
Order	: Diptera
Family	: Culicidae
Genus	: <i>Aedes</i>
Subgenus	: <i>Stegomyia</i>
Species:	<i>Ae. aegypti</i> (Linneus) <i>Ae. albopictus</i> (Skuse) <i>Ae. novalbopictus</i> (Barraud), <i>Ae. krombeini</i> (Huang), <i>Ae. w-albus</i> (Theobald) and <i>Ae. mediopunctatus</i> (Theobald).

Fig.2.6. Taxonomical state of *Aedes* mosquitoes

Different *stegomyia* species can be identified by external morphological characters, both at the larval and adult stages, using available identification keys. However, *Ae. aegypti* and *Ae. albopictus* larvae can be identified by the shape of comb scales on the 8th abdominal segment of the body. The adult stages of these two species can be differentiated by the colour patterns (ornamentation) of white scales on the body (Table 2.2).

Table 2.2. Important characters for differentiation of larvae and adult stages of *Ae. aegypti* and *Ae. albopictus*

Stage of life cycle	<i>Ae. aegypti</i>	<i>Ae. albopictus</i>
Larva	<p>●Abdominal segment of <i>Ae. aegypti</i> larva</p> <p>Comb scales have lateral denticles</p>  <p>Source : Mr.Sampath Weerakoon, Entomologist/Badulla</p>	<p>●Abdominal segment of <i>Ae. albopictus</i> larva</p> <p>Comb scales have no lateral denticles</p>  <p>Source : Mr.Sampath Weerakoon, Entomologist/Badulla</p>
	<p>●Abdominal segment of <i>Ae. aegypti</i> larva</p> 	<p>●Abdominal segment of <i>Ae. albopictus</i> larva</p> 
Adult	<p>●Mesonotum has a pair of lateral curved (lyre-shaped) white markings and usually a pair of submedian yellowish lines.</p> 	<p>●Mesonotum has a median longitudinal white stripe of narrow scales extending from anterior margin to about level of wing root</p> 

2.4. Vector competency and Vectorial capacity of *Ae. aegypti* and *Ae. albopictus*

Ae. aegypti is the more efficient, and epidemic causing vector of DF/DHF. This is explained by the vector competency and vectorial capacity of the vectors.

2.4.1. Vector competency

Vector competency is the vector's capability to transmit a pathogen. It denotes high susceptibility of the vector to the virus, ability to replicate the virus within the vector and ability to transmit the virus to another host (human). Both *Ae. aegypti* and *Ae. albopictus* are susceptible to the dengue viruses, viruses are replicated within both species and both species can transmit the viruses to man. Thus, both *Ae. aegypti* and *Ae. albopictus* have high vector competency for dengue viruses.

2.4.2. Vectorial capacity

Vectorial capacity is a measurement of the efficiency of a vector for disease transmission. It is governed by environmental and biological characteristics of the vector species. There are a number of factors that contribute to the vectorial capacity of a mosquito towards an arbovirus including mosquito survival, density, proportion of infected mosquitoes that are feeding on human, longevity, vector susceptibility to the virus, and density of susceptible hosts.

Ae. aegypti is a highly domesticated, strong anthropophilic mosquito which may bite repeatedly to complete the blood meal (discordant species). This attributes to the high vectorial capacity of *Ae. aegypti* resulting in cross infections and clustering of dengue cases which makes this species a more epidemiologically important vector of DF/DHF.

Ae. albopictus feeds on both humans and animals, it usually takes one blood meal to complete the gonotrophic cycle (concordant species). Hence, *Ae. albopictus* has poor vectorial capacity and epidemiologically less important in DF/DHF.



"Aedes albopictus" adult



03

VECTOR BIONOMICS (BEHAVIOUR)

Knowledge on the bionomics (behaviour) of Ae. aegypti and Ae. albopictus is important for dengue vector control. This chapter describes oviposition (breeding) sites, resting and feeding habits, flight range of Ae. aegypti and Ae. albopictus



VECTOR BIONOMICS (BEHAVIOUR)

Knowledge on the bionomics (behaviour) of *Ae. aegypti* and *Ae. albopictus* is important for dengue vector control. This chapter describes oviposition (breeding) sites, resting and feeding habits, flight range of *Ae. aegypti* and *Ae. albopictus*.

3.1. Common oviposition sites (breeding habitats) of dengue vectors

The dengue vectors, are container breeders; they breed in a wide variety of artificial and natural wet containers/receptacles, preferably with dark coloured surfaces and holding clear (unpolluted) water.

Dengue vector breeding sites are found within and outside houses/premises, at ground level as well as above ground level in places such as roof gutters, overhead tanks and receptacles on slabs, and in small and large containers(e.g.old tyres place on roofs) These containers/are found at any type of premises including houses, offices, hospitals, garages, commercial sites, yards containing automobile parts, schools, religious places, cemeteries, bare lands, construction sites and plant nurseries. Furthermore, water holding discarded containers along river and stream banks, railway lines are also possible breeding sites of *Ae. aegypti* and *Ae. albopictus*. However, in some instances, the breeding sites are area specific(e.g.shallow cemented wells in Batticaloa and Jaffna).

Ae. aegypti prefers to oviposit in artificial containers. This species is a skip ovipositor, thus, a single female mosquito lays eggs in a number of containers in a single oviposition cycle. Although *Ae. albopictus* is considered as a sylvatic species and breeds in natural containers such as leaf axils and tree holes, it also breeds in artificial containers in urban and peri-urban areas. *Ae. aegypti* shares its habitat with other *Aedes* species including *Ae. albopictus*, non *Aedes* species and occasionally with some *Anopheles* species.

The most common breeding sites of *Ae. aegypti* and *Ae. albopictus* in Sri Lanka can be classified broadly into:

- a. Discarded receptacles (plastic containers, tins, clay pots, yoghurt and ice cream cups, bottles, cans, damaged ceramic items, coconut shells etc.)
- b. Water storage containers (water storage cement tanks, barrels and other containers)
- c. Automobile tyres and machinery parts
- d. Building structures (roof gutters, concrete slabs etc.)
- e. Household /institutional appliances (refrigerator trays, flower vases, ornamental ponds, non functional cisterns and squatting pans of wash rooms)
- f. Other artificial breeding sites (abandoned boats, cemeteries etc)
- g. Natural breeding sites (leaf axils, tree holes)

3.2. Indoor and outdoor breeding sites of dengue vectors

Dengue vectors breeding sites are found both indoors and outdoors. Common indoor and outdoor breeding sites of *Ae. aegypti* and *Ae. albopictus* are:

3.2.1. Common indoor breeding sites

- Refrigerator trays
- Flower vases
- Ornamental ponds
- Water storage tanks/ containers in the toilets, bathrooms, kitchens etc
- Non functional cisterns and squatting pans of toilets
- Ant traps
- Abandoned fish tanks
- Lift wells in partly constructed buildings

3.2.2. Common outdoor breeding sites

- Discarded containers (Ground level and on concrete slabs)
- Water storage tanks and barrels
- Shallow cemented wells and manhole service pits
- Ornamental ponds
- Tyres
- Flower pots
- Bamboo stumps
- Coconut shells
- Roof gutters
- Poles in fences
- Leaf axils and tree holes
- Cemented floors
- Unused items and damaged equipment
- Iron poles used to earth electricity
- Building materials in construction sites
- Temporarily removed items
- Covering items (plastic / polythene sheet).
- Pet feeder

(a) Discarded receptacles



Damaged ceramic items



Plastic containers and tins

(b) Water storage containers



Ground level water storage cement tank
(outside of premise)



Ground level water storage cement tank
(Inside of premise)



Overhead cement tank



Indoor water storage tank and barrel



Water storing plastic barrel

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Water storing plastic containers



Rainwater harvesting drums



Rainwater harvesting cement tanks

(C) Automobile tyres and machinery parts



Discarded used automobile tyres



Unused machinery parts in the open

(d) Building structures



Blocked roof gutters



Polythene roof covers



Concrete roof/ slab



Blocked cement lined drain



Fences with open poles / bamboo sticks

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(e) Household /institutional appliances



Neglected flower pots



Saucer of a flower pot



Ornamental ponds of a house



Damaged structures



Refrigerator trays



Temporarily removed items eg. Boots



Animal feeding trays



Bird baths

(f) Other artificial breeding sites



Abandoned boats



Unused sea saw in a play ground



Cemeteries

(g) Natural breeding sites



Cut Bamboo stumps



Tree holes



Leaf axils of plants (e.g. Bromeliads)



Fig. 3.1. Common breeding sites of *Ae. aegypti* and *Ae. albopictus* in Sri Lanka

Any other containers which can hold water more than 5-7 days can serve as breeding sites for dengue vectors.

3.3. Important dengue vector breeding sites in Sri Lanka in 2013, 2014 and 2015

Major Breeding sites of dengue vectors detected in the years 2013, 2014 and 2015 are shown in table 3.1.

Table. 3.1. Dengue vector breeding sites in 2013, 2014 and 2015.

Type of breeding sites	% positivity for <i>Aedes</i> in 2013	% positivity for <i>Aedes</i> in 2014	% positivity for <i>Aedes</i> in 2015	Average % for 3 years
Discarded receptacles	48	40	36	41
Water storage containers	15	18	22	18
Cement tanks	07	06	06	06
Natural	05	07	04	05
A/C or Refrigerator trays	03	03	02	03
Ponds and ornamentals	02	03	04	03
Wells	01	01	01	01
Concrete slabs	01	01	01	01
Tyres	07	06	08	07
Gutters	02	01	01	01
Other *	19	14	15	16

* Other : Temporarily removed items, Non use cisterns/commode/squatting pans, wet cement floors, pet feeding cups, earth pipes

Ae. aegypti and *Ae. albopictus* breed in a wide variety of containers. However, the productivity (the number of larvae/ pupae/ adults produced) of vectors in different containers can vary.

e.g. 1. In the rainy season, roof gutters and discarded containers become significantly positive for *Aedes* larvae, thus contributing to seasonal outbreaks; in the dry season, water storage containers are important breeding sites of *Ae. aegypti* and *Ae. albopictus*.

e.g. 2. In an area with irregular water supply, water storage tanks are the major breeding sites of dengue vectors while discarded containers are the most productive breeding site in an area where solid waste management is poor. This shows that the most productive containers are area specific and seasonal specific.

3.4. Vector behaviour

In this section, resting and feeding behaviour and the flight range of dengue vector are discussed.

3.4.1. Resting

Ae. aegypti primarily rests inside houses or buildings in dark and humid surroundings on secluded hanging objects including curtains, clothes, shoes, bed nets, underside of furniture and empty containers. Less often this species is found outdoors in vegetation or other hidden places. *Ae. albopictus* generally rests outdoors in vegetation, empty containers such as pots and tyres and in other hidden places.

3.4.2. Feeding

Female *Ae. aegypti* is highly anthropophilic and it tends to feed on more than one person for a full blood meal (multiple feeder). This species shows gonotrophic discordance (takes more than one blood meal to complete the gonotrophic cycle). This multiple feeding habit and gonotrophic discordant behaviour increases the human-biting rate and thereby greatly increases the epidemic transmission efficiency. *Ae. albopictus* is an aggressive feeder and takes a full blood meal in one go. It is a concordant (takes one blood meal to complete the gonotrophic cycle) species and therefore, considered a less efficient vector.

Dengue vectors are primarily day time biters with two peaks of biting activity. i.e. one in the morning for few hours after day break (dawn) and the other in the afternoon for few hours before dark (dusk). The morning peak of biting activity falls between 0600 - 1100 hours and the afternoon peak falls between 1500 - 1900 hours. However, the actual peaks of biting activity may vary slightly, within these ranges, depending on the location and season of the year. These vector species may bite humans in between these peaks, especially in shady places and when the light and dark conditions are conducive for their activity. These species generally do not bite at night, although some may feed at night in well lit rooms.

3.4.3. Egg laying pattern

Female *Ae. aegypti* mosquito produces on average 100 to 200 eggs per batch. The females can produce up to five batches of eggs during its lifetime. Eggs are laid singly. The female mosquito will not lay the entire clutch at once at a single site, but rather spread out the eggs over many sites over several hours depending on the availability of suitable surfaces. Eggs will most often be placed at varying distances above the water line.

3.4.4. Flight range and dispersal of adult

Dispersal of adult female depends on the availability of oviposition sites and human hosts to feed on. Generally, the dispersal is short, the horizontal dispersal usually being about 100 - 200m from the breeding site. However, when oviposition sites and/ or human hosts are not available, dispersal may be wider, as much as 400m. Vertically, these species are found up to many floors in multi storied buildings including condominiums.



04

DENGUE VECTOR
SURVEILLANCE

Vector Surveillance is the analysis and interpretation of systematically collected entomological data for facilitating appropriate decisions on vector control

DENGUE VECTOR SURVEILLANCE

Vector Surveillance is the analysis and interpretation of systematically collected entomological data for facilitating appropriate decisions on vector control. Vector surveillance is an important and essential component of the dengue control programme, as the information generated from vector surveillance guides the vector control programme and helps for early warning and epidemic forecasting.

4.1. Objectives of dengue vector surveillance

- To determine the breeding sites of dengue vector mosquitoes
- To describe the temporal and spatial distribution of vector mosquitoes
- To describe seasonal fluctuations of vector population
- To determine feeding and resting habits of vector mosquitoes (vector bionomics/ behaviour)
- To forecast outbreaks and early warning
- To determine the effectiveness of vector control interventions used for dengue vector control

4.2 Types of dengue vector surveillance

Dengue vector surveillance is carried out at regularly at sentinel sites and or routine sites. Surveillance at spots (spot checks) is carried out in special localities and circumstances based on environmental and epidemiological information

4.2.1. Sentinel site surveillance

Sentinel site dengue vector surveillance is a surveillance system in which regular entomological surveys are carried out in pre-arranged and designated areas to collect entomological data that are useful to make trend observations on vector density, dynamics of vector breeding sites, changes in vector behaviour and monitoring vector susceptibility/ resistance status to insecticides that are used in dengue vector control. Sentinel site vector surveillance facilitates early warning and forecasting of dengue outbreaks.

Criteria for selection of sentinel sites

- a. Sentinel sites are identified at district level and monitored monthly. It is recommended that a minimum of 02 (one urban, one semi urban or rural) sentinel sites per district to be established and monitored.
- b. Sentinel site should be (i) an area where dengue transmission/ high-risk of transmission is present over a period of time or (ii) an epidemic prone area (areas experiencing/potential for periodic or seasonal outbreaks (it may be a cluster of Municipal wards/ Grama Niladari areas or a Public Health Inspector area).
- c. In a sentinel site adjacent Municipal wards/ Grama Niladari areas having more or less homogeneous prevalence of *Ae. aegypti* and reported dengue cases for the past 3-5 years should be selected for entomological surveillance

Entomological techniques to be carried out in sentinel site surveillance

- Larval surveys
- Pupal surveys
- Indoor and outdoor adult mosquito resting collections
- Human bait collections (using the double net method)
- Insecticide susceptibility/ resistance tests
- Bio-efficacy tests for larvae and adult *Ae. aegypti* and *Ae. albopictus*.

4.2.2. Routine site surveillance

Routine vector surveillance is a surveillance method in which regular entomological surveys are carried out in high dengue transmission/ transmission risk areas (localities) to collect entomological data that are useful to guide dengue vector control activities and for epidemic prevention.

Larval (larvae and pupae) surveys are the commonly used entomological surveillance method in routine vector surveillance. Monitoring of larval density helps to identify (i) potential dengue transmission areas and seasons well ahead of the outbreak period and (ii) area and time specific vector breeding sites that facilitate application of most appropriate and cost-effective vector control interventions.

Criteria for selection of routine surveillance sites

- a. Routine surveillance sites are identified at the district or sub district (Medical Officer of Health MOH) level and monitored regularly (ideal if monitored fortnightly; otherwise at least monthly).
- b. Routine surveillance site is (i) an area where dengue transmission/ high risk of transmission is present over a period of 3 years or (ii) an epidemic prone area. Epidemic prone areas include:
 - the areas that are subjected to frequent or seasonal outbreaks/ epidemics
 - areas with increased vector breeding sites due to development activities, urbanization, interruptions of regular water supply etc.

Entomological techniques to be performed in routine surveillance site

- Larval surveys –fortnightly/monthly
- Insecticide susceptibility/ resistance tests – once in 6 months and
- Bio-efficacy tests for larvae and adult *Ae. aegypti* and *Ae. albopictus*. – once in 6 months

4.2.3. Spot checks

Spot check is a surveillance method that is carried out to generate entomological information for a particular locality for guiding the vector control activities in that locality/site. Spot checks are carried out:

- in an areas where there are outbreaks of dengue in spite of regular vector control interventions
- in contact places of reported dengue cases
- in high-risk institutions such as schools, bus depots, public places, hospitals, religious places, areas where development projects are carried out and construction sites etc.
- in new areas where dengue cases are reported.
- in an area where there is an increase in the reporting of fever /suspected dengue cases
- When environment changes occur favouring vector breeding (eg. flooding, development projects etc)
- When there is a need to evaluate the impact of control measures (e.g. cleaning programmes, fogging etc.)
- to identify the new establishment of *Aedes aegypti* or *Aedes albopictus* in areas where there were no reports of the vector previously

Entomological techniques to be performed during spot checks

- Larval surveys
- Indoor and outdoor adult mosquito resting collection
- Bio-efficacy tests for larvae and adult *Ae. aegypti* and *Ae. albopictus*

In order to carry out dengue vector surveillance more efficiently, it is important to define priority areas for surveillance.

4.3. Priority areas for dengue vector surveillance

Dengue is a focal and local disease; intensity, season and localities of dengue transmission/ epidemics depends on the temporal and spatial prevalence and density of the vectors, primarily, *Ae. aegypti*. Therefore, knowledge on the temporal and spatial distribution of dengue vectors is important for prevention and control of DF/DHF transmission. However, to work with the limited manpower and the logistics of the dengue control programme, priority areas need to be identified for vector surveillance. The following criteria are used for setting priorities (priority 1, 2, and 3) for vector surveillance (Table 4.1).

Table 4.1. Criteria for setting priorities for dengue vector surveillance

Priority	Locality/Area	Type of survey	Frequency of surveys
Priority I	1. Dengue endemic areas (endemicity is defined on case data). eg. > “n” number of indigenous cases reported per week in the MOH area during the past 3 years.	Sentinel surveillance and Routine surveillance	Monthly Monthly

Priority	Locality/Area	Type of survey	Frequency of surveys
	<p>2.a. Receptive/ vulnerable areas for dengue transmission such as:</p> <p>a. In an area where there is an increase in the reporting number of fever/suspected dengue cases, in areas where there are out breaks of dengue in spite of regular vector control interventions.</p> <p>b.Premises such as hospitals, schools, construction sites, bus depots, hostels, condominiums, camps, public places, religious places, new developmental projects and areas with migrant population, large population gatherings (perahera/procession, religious events)</p>	Spot checks	On requirement
Priority II	Localities with current and past outbreaks within the immediate past 3 years	Routine surveillance	Monthly
Priority III	Localities where there are no indigenous dengue cases but have perceived transmission (areas with imported dengue cases and areas with low <i>Aedes</i> indices).	Spot checks	Biannually

4.4. Sampling procedures and sample size in dengue vector surveillance

The number of premises/ sampling units to be inspected in a locality depends on the level of precision required, the total number of houses in the area and the surveillance method. Use of a proper sampling method would minimize the bias in the selection of premises/houses. Following sampling methods can be used in selection of houses. In dengue vector larval surveys at least 100 premises should be surveyed to calculate the vector indices.

4.4.1. Simple random sampling:

In simple random sampling, houses (premises) to be surveyed are selected using a list of random numbers (either from the random number tables or from the computer-generated number). This method would require detailed house lists or location maps for identifying selected houses.

4.4.2. Systematic sampling

Systematic sampling of every 'nth house throughout a community or along a street: For example, in an area of 400 premises if a sample of 25% of the house of 400 houses to be inspected, every 4th house (=400/4) would be inspected.

4.4.3. Stratified random sampling:

The area intended to be surveyed is stratified to a few strata; each is more or less epidemiologically homogeneous. Houses (survey units) are selected from each stratum randomly for the survey.

4.4.4. Cluster sampling:

The survey area is divided into several clusters of a fixed number of houses (e.g. each cluster of 100 houses). A set of clusters are randomly selected and a randomly selected sample of houses from each cluster is surveyed.

Note: Frequent and regular visit to a sentinel surveillance site would result in low vector density indices over time, thus, the houses in the sample should be changed in subsequent visits.

4.5. Commonly used entomological techniques for dengue vector surveillance

Larval surveys (both larvae and pupae) are the most commonly used survey technique in dengue vector surveillance. Pupal and adult surveys, and oviposition traps are used in special studies, and for research purposes.

4.5.1. Larval surveys

In larval surveys, the basic sampling unit is the house or premise. During the larval survey, all potential dengue vector breeding sites that are in and around the selected houses/ premises (100 m around the reported case or designated number of houses) should be examined for *Aedes* larvae.

Following equipment should be carried for the larval survey (Fig.4.1)

- Dippers (Plastic and aluminium)
- Pipettes
- Vials and labels
- Torch (to use when dipping storage tanks inside the houses)
- Siphoning pipette
- Strainer
- Relevant format



Strainers to dip water storage tanks

Fig. 4.1. Larval collections using dipping and pipetting techniques

From each *Aedes* larvae/ pupae positive container, a minimum of 10 larvae and 10 pupae (if present) are collected by dipping, pipetting and/or siphoning (Fig. 4.1). In instances where 10 larvae/ pupae are not present, all larvae/ pupae are collected (since mixed breeding/ habitat sharing of *Aedes* and non *Aedes* species is common in Sri Lanka, collection of only one larvae results in underestimating of the vector density). The collected larvae are placed in a labelled plastic vial labelled with date, location and code number and the species are identified in the laboratory using standard identification keys. Details of the larval collection should be recorded in the larval survey forms (Annexure I -Daily Dengue Entomological survey, Details of positive /potential premises and containers). Type of breeding places are recorded in Annexure II (Breeding places -check list).

It is important to note that if there are multiple breeding sites of the same type in one place (e.g. heaps of tyres, coconut shells), larvae should be collected at least from 10 randomly selected such containers.

Daily summary report should be given to the relevant MOH areas according to the Annexure (III) format (Daily Dengue Entomological Survey Summary Report).



Fig. 4.2. Checking roof gutters for water collections using a mirror



Fig. 4.3. Larval collection in a roof gutter by pipetting

Larval collection by dipping (suggested to use a strainer rather than the dipper for larval survey of water storage tanks and barrels as the larvae (specially *Aedes*) immerse and rest in the bottom of the tank for a long time without coming to the surface after first dip is taken)



Fig. 4.4. Dipping

4.6. Larval identification

Third and fourth instar larvae can be identified at the laboratory immediately after the field survey. However, 1st and 2nd instar larvae should be allowed to develop to 3rd and 4th instar larvae and identified. Pupae is allowed to develop to adults and identified.

4.7. Calculation of larval density indices

The relevant format should be used for reporting (Annexure IV; Dengue Entomological Surveillance Report). Once identification of larvae/ pupae is completed, larval density indices are calculated for *Ae. aegypti* alone, *Ae. albopictus* alone, and for *Ae. aegypti* and *Ae. albopictus* combined, in order to determine the vector density in the area. If a particular container is positive for both *Ae. aegypti* and *Ae. albopictus* (mixed breeding), this particular container is considered as two containers (01 for *Ae. aegypti* and 01 for *Ae. albopictus*) when calculating vector density indices separately for *Ae. aegypti* and *Ae. albopictus*.

4.7.1. Larval density indices

Three indices, viz. Container index (CI), House/premise index (HI or PI Index) and Breteau index (BI) are used to give vector larval density. The method of calculation of these indices with examples is given below.

Container Index (CI) : Percentage of water-holding containers infested with *Ae. aegypti*/*Ae. albopictus* larvae and/ or pupae.

$$CI = \frac{\text{Number of positive containers for } Ae. aegypti/ Ae. albopictus \text{ larvae and/ or pupae}}{\text{Number of wet containers inspected}} \times 100$$

Premise Index (PI) : Percentage of premises infested with *Ae. aegypti*/*Ae. albopictus* larvae and/ or pupae.

$$PI = \frac{\text{Number of positive premises for } Ae. aegypti/ Ae. albopictus \text{ larvae and/ or pupae}}{\text{Number of premises inspected}} \times 100$$

Breteau Index (BI): Number of positive containers for *Ae. aegypti*/*Ae. albopictus* larvae and / or pupae per 100 houses inspected

$$BI = \frac{\text{Number of positive containers for } Ae. aegypti/ Ae. albopictus \text{ larvae and/ or pupae}}{\text{Number of houses inspected}} \times 100$$

By definition, BI is the number of positive containers for *Ae. aegypti*/*Ae. albopictus* larvae and/ or pupae per 100 houses/ premises inspected. Thus, inspection of at least 100 houses is necessary to calculate BI in a particular area.

An example for calculating the larval density indices using hypothetical data are shown in Tables 4.2 and 4.3

Table 4.2. Results of a hypothetical larval survey

No. of houses Examined	No. of houses/premises positive for			No. of wet containers examined	No. of containers positive for		
	<i>Ae. aegypti</i> alone	<i>Ae. albopictus</i> alone	Both <i>Ae. aegypti</i> and <i>Ae. albopictus</i>		<i>Ae. aegypti</i> alone	<i>Ae. albopictus</i> alone	Both <i>Ae. aegypti</i> and <i>Ae. albopictus</i>
100	5	3	2	85	6	5	3

A-*Ae.aegypti* B-*Ae.albopictus*

Table 4.3. Calculation of larval density indices using data in Table 4.2.

Name of the index	Formula	Use of data in the formula	Index
CI (A)	$\frac{\text{Number of containers positive for (A alone+both A and B)}}{\text{Number of wet containers examined}} \times 100$	$= \frac{(6 + 3)}{85} \times 100$	10.6%
CI (B)	$\frac{\text{Number of containers positive for (B alone+both A and B)}}{\text{Number of wet containers examined}} \times 100$	$= \frac{(5 + 3)}{85} \times 100$	9.4%
Combined CI	$\frac{\text{Number of containers positive for (A alone +B alone+both A and B)}}{\text{Number of wet containers examined}} \times 100$	$= \frac{(6 + 5 + 3)}{85} \times 100$	16.4%
HI (A)	$\frac{\text{Number of houses/premise positive for (A alone+ both A and B)}}{\text{Number of premises examined}} \times 100$	$= \frac{(5 + 2)}{100} \times 100$	9.4%
HI (B)	$\frac{\text{Number of houses/premise positive for (B alone+both A and B)}}{\text{Number of premises examined}} \times 100$	$= \frac{(3 + 2)}{100} \times 100$	5.0%
Combined HI	$\frac{\text{Number of premises positive for A alone + B alone + both A and B}}{\text{Number of premises examined}} \times 100$	$= \frac{(5 + 3 + 2)}{100} \times 100$	10.0%
BI (A)	$\frac{\text{Number of positive containers for A alone + both A and B}}{\text{Number of premises examined}} \times 100$	$= \frac{(6 + 3)}{100} \times 100$	9.0
BI (B)	$\frac{\text{Number of positive containers for B alone + both A and B}}{\text{Number of premises examined}} \times 100$	$= \frac{(5 + 3)}{100} \times 100$	8.0
Combined BI	$\frac{\text{Number of containers positive for (A alone + B alone + both A and B)}}{\text{Number of premises examined}} \times 100$	$= \frac{(6 + 5 + 3)}{100} \times 100$ $= \left(\frac{14}{100}\right) \times 100$	14.0

4.7.2. Importance and interpretation of larval indices

In dengue prevention and control, all three larval density indices, especially of *Ae. aegypti*, need to be considered. High container index indicates the important container types a dengue vector breeding sites and the vector control interventions need to be directed to eliminate such types of containers immediately. These larval indices are important for decision making on elimination of the most common habitats, developing educational messages and orientation of community-based initiatives. However, it is difficult to give a single country-wide cut-off point of larval density indices as the larval productivity depends on the area, container types and the most productive container types. In Sri Lanka, HI and BI (*Ae. aegypti*) >3 is an indication for dengue outbreaks and for application of appropriate dengue vector prevention/ control interventions.

4.7.3. Limitations of larval indices

Container index provides information on the percentage of wet containers that are positive for dengue vectors and the most important container types. House index provides information on the percentage of houses that are positive for vector species, and the Breteau index provides information on the number of positive containers per 100 houses. The larval density indices are a crude indication of adult production. For example, the larval productivity of a water storage tank is likely to differ markedly from that of a discarded coconut shell. However, in the calculation of CI, HI or BI, productivity of these containers is not considered. Instead, it is taken as positive or negative for vector breeding. Thus, the transmission potential of dengue may be quite different in localities with similar larval indices but with different container profiles.

4.7.4. Reporting and communication of larval survey results

Standard entomological surveillance formats for daily and weekly reporting of surveillance data are given in annexures (I) and (IV) respectively. Format for reporting of surveillance at the institutions/ construction sites etc. is given in annexure (V) The daily report is an interim report which has to be submitted to the respective Medical Officer of Health (MOH) after the day's work. (Annexure (III). Breeding places check list mentioned in Annexure (II). Although format (IV) is for weekly reporting of surveillance data, separate sheets of format (IV) should be used if more than one survey/ locality is surveyed within a week. The weekly report/s should be submitted to the MOH, Regional Malaria Officer (RMO), MOIC-AFU, Entomologist and Regional Epidemiologist (RE) of the district and the National Dengue Control Unit in Colombo.

4.8. Pupal surveys

Since adult mosquito production from different types of containers may vary, an estimate of relative adult production can be made based on the pupal density (Pupal index = number of pupae per 100 houses/premises).

$$\text{Pupal index} = \frac{\text{Number of pupae in all containers}}{\text{Number of houses/ premises inspected}} \times 100$$

Pupal index can be determined by pupal counts in different types of containers separately, giving the relative importance of different types (e.g. tyres, water storage tanks etc.). This information is useful for targeted elimination of the most productive containers.

Making pupal counts is labour intensive and has practical difficulties, hence this method is not used for sentinel/routine surveys but may be reserved for special studies and research.

4.9. Adult Surveys

Adult vector surveys provide information on the seasonal trends of mosquito density, peaks of biting activity, resting places, potential dengue transmission areas, transmission risk, and the effectiveness of vector control interventions.

Objectives of adult vector surveys

- To estimate the adult vector populations in a locality
- To determine seasonal fluctuations of vector density
- To study vector behaviour such as biting times and resting places
- To determine the effectiveness of vector control interventions including evaluation of larvicidal and adulticiding interventions

Adult vector survey methods include:

- Human landing collections,
- Human bait collections using double nets,
- Indoor and outdoor resting collections.

4.9.1. Humans landing collections

Landing collections on humans are a sensitive means of detecting the number of infestations of *Ae. aegypti* and *Ae. albopictus* in an area. Presence of males in the collection indicates the presence of larval habitats in close by areas. In landing collections, the adult mosquitoes landing on the bare legs below the knees are collected using test tubes or a sucking tube. The data are summarized to determine the number of female *Ae. aegypti*/*Ae. albopictus* mosquitoes landing per human bait per hour (number of mosquitoes collected per man hour). Human landing collections are conducted both indoors and outdoors between 0600-1800hrs as dengue vector mosquitoes are day time biters.

In the absence of prophylaxis for dengue, every effort should be made to collect female mosquitoes before they begin to bite. Furthermore, this method is done only in dengue non endemic periods. During endemic periods, human bait collections using double net traps method is more suitable.

$$\text{Human landing rate} = \frac{\text{Number of female } Ae.aegypti/Ae. albopictus \text{ mosquitoes}}{\text{Number of man hours}} \times 100$$



Fig.4.5 Human Landing catch

Source: Dr. S. Aryaprema, Entomologist/Colombo

4.9.2. Human bait collections using double net traps

Human bait collection using a double net method can be used for adult vector surveys. In this method, a human bait is allowed to rest on a folding bed inside the inner mosquito proof net and another net is arranged around this net. The outer net is arranged in such a way to keep a space of about 6 inches between the lower edge of the net and the ground and with sufficient space between the two nets for moving a mosquito collector between the two nets. The mosquito collector collects the mosquitoes attracted to the bait (the adult mosquitoes trapped between the two nets) using a sucking tube or a battery powered aspirator. Mosquito collections are made hourly during the collection period and the mosquito density is given as the (a) number of adult *Ae. aegypti* and *Ae. albopictus* mosquitoes per trap and (b) number of adult mosquitoes per bait per hour.



Fig.4.6 Indirect catch using double trap method

Source: Ms. B.S.L Peiris, RMO/Hambantota

4.9.3. Indoor resting collections of dengue vectors

Adult dengue vector mosquitoes rest indoors on hanging objects such as clothes, closets and other sheltered places such as inside empty containers and domiciliary objects. In resting collections, sites are checked for adult mosquitoes with the aid of a flashlight, mosquitoes are collected using mouth or battery powered aspirators (Fig. 4.7). The flying mosquitoes are collected using sweep nets (Fig. 4.8).



Fig.4.7. Collection using sucking tube



Fig. 4.8. sweep net

Battery operated aspirators and back-pack aspirators are also used in collections of indoor and outdoor resting mosquitoes (Fig. 4.9).

Outdoors

Indoors



Fig. 4.9 Adult resting mosquito collections using a back pack aspirator

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In resting collections, mosquito density is given as the number of adult mosquitoes collected per man hour of collection.

$$\text{Adult mosquito density} = \frac{\text{No. of vector mosquitoes collected}}{\text{No. of man hours spent}} \times 100$$

Surveys of adult mosquitoes are time consuming and labour intensive, less practical in routine surveillance. When the collection technique involves human baits, extreme care should be taken to prevent him from mosquito bites.

4.9.4. Oviposition traps

Oviposition traps (commonly referred to as ovitraps) are used to detect the presence of *Ae. aegypti* and *Ae. albopictus* when and where the other vector density indices (larval and adult densities) are low.

Objectives and uses of ovitrap collections:

- To assess dengue vector density when and where larval surveys produce low vector indices (e.g. when the BI (*Ae. aegypti*) is < 3)
- To detect new areas of infestations of dengue vectors early
- To determine the effectiveness/ efficacy of vector control interventions
- To collect eggs of dengue mosquitoes for susceptibility and bio-efficacy tests
- To assess vector population fluctuation over long-term

The standard ovitrap is a wide-mouthed plastic cup of approximately 250 ml which is painted in black on the outside to attract the *Ae. aegypti*/ *Ae. albopictus* females to oviposit. A piece of hardboard or a wooden paddle is placed diagonally inside the cup as an oviposition substrate and the cup is partially filled with clean water to provide the right ovipositing medium for the female mosquito (hay infusion attracts *Culex* and *Armigeres* mosquitoes, thus tap water is preferred for the ovitraps). Instead of the wooden paddle, white towelling or filter paper strips can be placed inside the cup and attached by paper clips (Fig. 4.10).



Fig. 4.10. An ovitrap

The ovitraps are placed appropriately in a suspected mosquito frequenting place, both indoors and outdoors. Collection of ovitraps is made once in 3 -5 days, and the paddles/ paper strips are examined under a dissecting microscope for the presence of eggs of *Aedes*. The numbers of eggs are counted to give the egg density as (a) the number of eggs per ovitrap, and (b) the percentage of positive ovitraps. In areas where both *Ae. aegypti* and *Ae. albopictus* are present, eggs should be allowed to hatch and then the larvae or adults should be identified since the eggs of both species cannot be reliably distinguished from each other.

- a. Number of eggs per trap = $\frac{\text{Total number of eggs}}{\text{Total number of ovitraps installed}}$
- b. Percentage of positive ovitraps [Ovitrap Index (OI)] = $\frac{\text{no.of positive ovitraps}}{\text{no.of ovitraps placed}} \times 100$

standard ovitrap surveillance format is given in Annexure (VI)

4.9.5. The BG sentinel traps

BG Sentinel Traps use a combination of attractive visual and olfactory cues for collection adult *Ae aegypti* and *Ae. albopictus* female mosquitoes. This trap is comparatively light and the female mosquitoes are trap in it. BG-Sentinel traps are more effective in capturing *Ae. aegypti*, and also collect adult females in all physiological states. Thus it is more advantageous as compared to the traditional ovipositional trap. The efficiency of BG traps can be increased by baiting them with lures (e.g., CO₂, BG-Lure®).



Fig.4.11. BG Sentinel trap

4.10. Planning of vector surveillance activities

Dengue prevention and control is a decentralized programme in Sri Lanka. The National Dengue Control Unit (NDCU) provides technical guidance and necessary logistic support while planning is done at the Provincial, District and Medical Officer of Health (MOH) area levels. When planning entomological surveys for the forthcoming month, the advance programme of the Entomological Assistant/team should be prepared by the Entomological Assistant in consultation with the Regional Epidemiologist, Entomologist, Regional Officers/Medical Officers of the Anti-Malaria Campaign, Anti Filariasis Campaign and the Medical officer of Health of the respective MOH area.



05

MANAGEMENT AND CONTROL OF DENGUE VECTORS

Integrated vector management is a rational decision making process for the optimal use of resources for vector control

MANAGEMENT AND CONTROL OF DENGUE VECTORS

Dengue vector control measures are aimed at both immature and adult stages of *Ae. aegypti* and *Ae. albopictus*. These measures are included in the integrated vector management (IVM) strategy which incorporates environmental, biological and chemical methods.

5.1. Integrated vector management (IVM)

Integrated vector management is a rational decision making process for the optimal use of resources for vector control. IVM uses the available resources, existing health system and evidence based appropriate vector control interventions singly or in combination to suppress the vector population. Key elements of IVM strategy are shown in Table 5.1.

5.1.1. Operationalising of IVM to local situation

a. Selection of vector control methods based on knowledge of local vector biology, bionomics and ecology and the epidemiology of dengue (Evidence based decision making)

In Sri Lanka, there are two peaks of dengue transmission each year in association with the South- West and North-East monsoonal rains. The major peak falls in June/ July with the South- West monsoon while the other peak falls in November/ January (Fig 5.1).

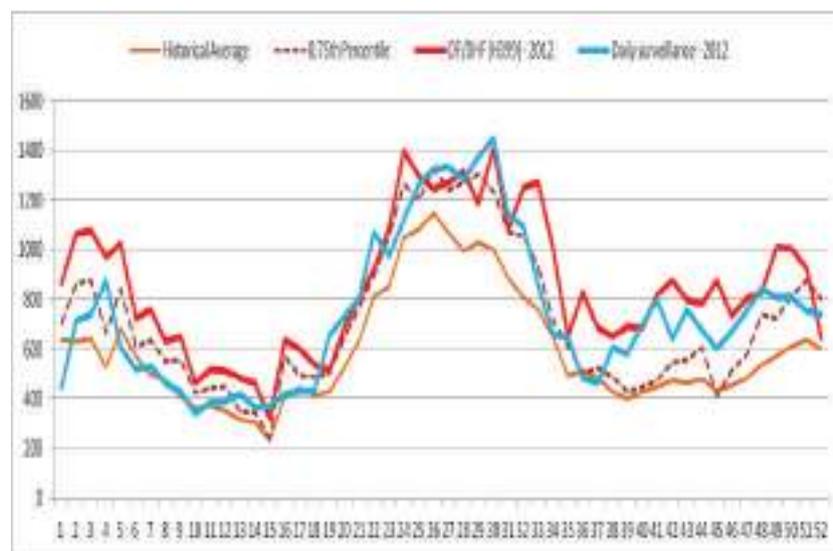


Fig. 5.1. 5 year average showing the two seasonal peaks of dengue transmission

Table 5.1. Key elements of IVM strategy

No.	Key Element	Description
1.	Advocacy, social mobilization and legislation	<ul style="list-style-type: none"> ● Promotion and embedding of IVM principles in designing policies in all relevant agencies, organizations and civil society. ● establishment or strengthening of regulatory and legislative controls for public health. ● empowerment of communities.
2.	Collaboration within the health sector and with other sectors (inter and intra- sectoral collaboration)	<ul style="list-style-type: none"> ● Consideration of collaboration within and between public and private sectors including the engagement with local communities and other stakeholders; application of the principles of subsidiarity in planning and decision-making; strengthening channels of communication among policy-makers, vector-borne disease control programme managers and other IVM partners.
3.	Integrated approach	<ul style="list-style-type: none"> ● Ensure rational use of available resources by addressing several diseases, integrating non-chemical and chemical vector control methods and integrating with other disease control methods; use of a range of interventions, often in combination and synergistically.
4.	Evidence-based decision making	<ul style="list-style-type: none"> ● Adaptation of vector control strategies and interventions guided by the knowledge of local vector biology, bionomics and ecology, disease epidemiology and resources, and subject to routine monitoring and evaluation.
5.	Capacity-building	<ul style="list-style-type: none"> ● Provision of the essential material infrastructure, financial resources and human resources at national and local level to manage IVM strategies on the basis of situational analysis.

The current dengue disease surveillance system is primarily based on hospital notifications (H 544) to the relevant MOH. The Epidemiology unit and Regional Epidemiologists receive the aggregated data (H 399) from all MOOH every week. Simultaneously suspected dengue patients are notified by a web based system (DenSys) from sentinel hospitals and data are shared among relevant stakeholders. All reported patients are investigated by Public Health Inspectors to identify the source of infection and patient locations are mapped. The distribution of patients are summarized to identify "high risk clusters " based on Municipal area/Grama Niladari (GN) area wise distribution.

The entomological surveillance data are summarized to identify the GN area wise distribution of *Ae. aegypti* and *Ae. albopictus*. Since *Ae. aegypti* is the principal vector of dengue in Sri Lanka, information on the distribution of this species is of immense importance. Entomological surveillance data provides information on the vector breeding sites and dynamics of vector density over time. Regular monitoring of such data would help identify the fluctuations of vector density (Fig 5.2).

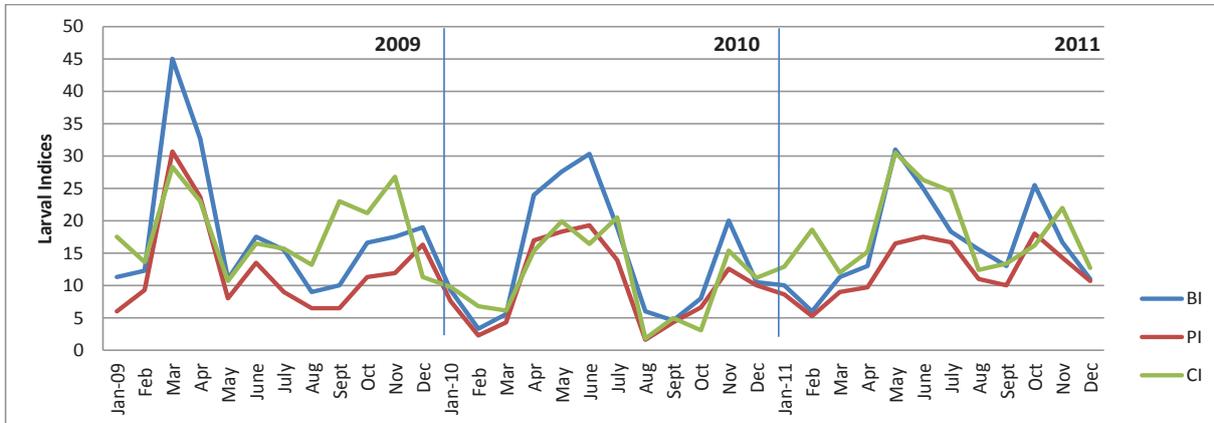


Fig. 5.2. Seasonal fluctuation of larval indices (MOH area Kaduwela, Colombo District)

PI=Premise index, BI=Breteau index, CI=Container index

Source: Dr. Subhashinie Aryaprema, Entomologist/Colombo district

It is observed that high larval indices precede increase in the number of dengue patients reported with a lag period of 1-2 months (Fig. 5.3). Monitoring and mapping such data is helpful in identifying outbreak prone areas, potential risk levels (Fig. 5.4).

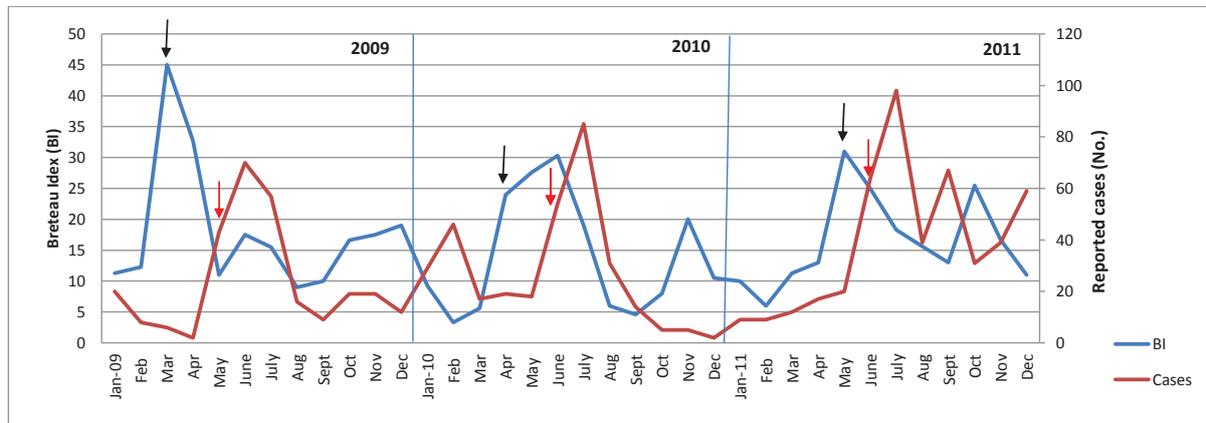


Fig.5.3. Relationship between BI and dengue cases (MOH area Kaduwela, Colombo District)

Source: Dr. Subhashinie Aryaprema, Entomologist/Colombo district

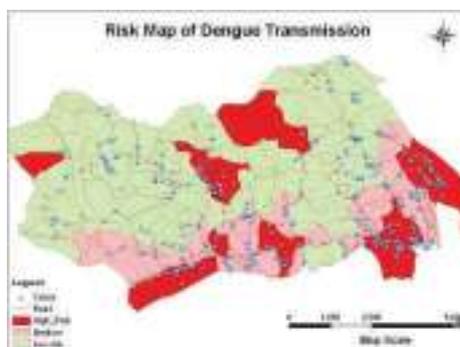


Fig. 5.4. Finer scale (GN level) risk map of dengue transmission generated using entomological and epidemiological data using GIS mapping

Source: WHO Dengue Bulletin (2009)

Entomological surveillance provides information on vector breeding sites and vector behavior. Biting behavior of *Ae. aegypti* is shown in Fig. 5.5. This information is useful in performing space spraying during peak biting hours.

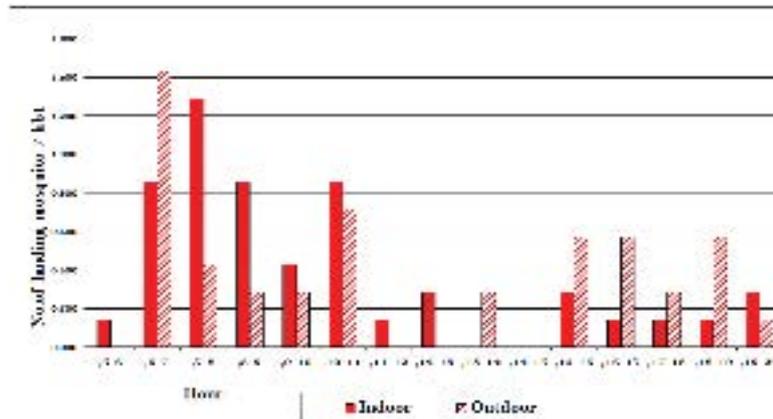


Fig. 5.5. Biting Behaviour of *Aedes aegypti* in Tangalle area

Source: Ms. B.S.L.Peiris, RMO/Hambantota

b. Utilization of a range of interventions, often in combination and synergistically – (integrated vector control)

Source reduction by cleaning campaigns is carried out routinely during inter epidemic periods and before the expected dengue transmission period in order to prevent outbreaks. Cleaning can be accompanied by space spraying of insecticide in vulnerable and application of temephos 1% SG for water storage tanks and in combination.

Single or multiple vector control interventions can be used to control two or more diseases. For example, a source reduction programme could prevent dengue and chikungunya.

c. Collaboration within the health sector, researchers and with other agencies in the public and private sectors that impact on vector breeding – (intra and inter sectoral collaboration/ participation)

Intersectoral collaboration is of utmost importance for dengue vector control. All relevant sectors such as Ministry of Health, Education, Local Government, Environment, Public Administration, Construction, NGOs, CBOs and other relevant sectors should cooperate for prevention and control of dengue vector breeding sites with the technical guidance that is provided.

d. Engagement with local communities and other stakeholders – (Community participation)

Active community participation and community mobilization are important activities for sustainable elimination of vector breeding sites. Communities can be involved in small scale and mass cleaning campaigns, for desirable behaviour change and larvivorous fish programmes etc.

e. A public health regulatory and legislative framework

The households, and owners of premises that are positive for mosquito breeding are warned and prosecuted (5.7).

f. Rational use of insecticides

As the number of insecticides for vector control is limited and the indiscriminate use of insecticides results in vector resistance, extreme care should be taken when using insecticides ensuring the use of insecticides in areas where such interventions are extremely necessary.

5.2. Dengue vector control methods in Sri Lanka

Dengue vector control programme should be planned on scientific evidence and implemented by trained personnel including community groups. The programme should be well supervised, monitored and reviewed monthly in inter-epidemic periods and weekly in the epidemic periods.

Considering the socio economic, cultural and religious diversity of the human society, breeding sites in different types of premises and vector control intervention methods described in this chapter can be integrated appropriately.

Environmental, biological and chemical methods are used for dengue vector control in Sri Lanka. However, source reduction through environmental management is the most cost effective activity. Major approaches of dengue vector management and control are shown in Fig. 5.6.

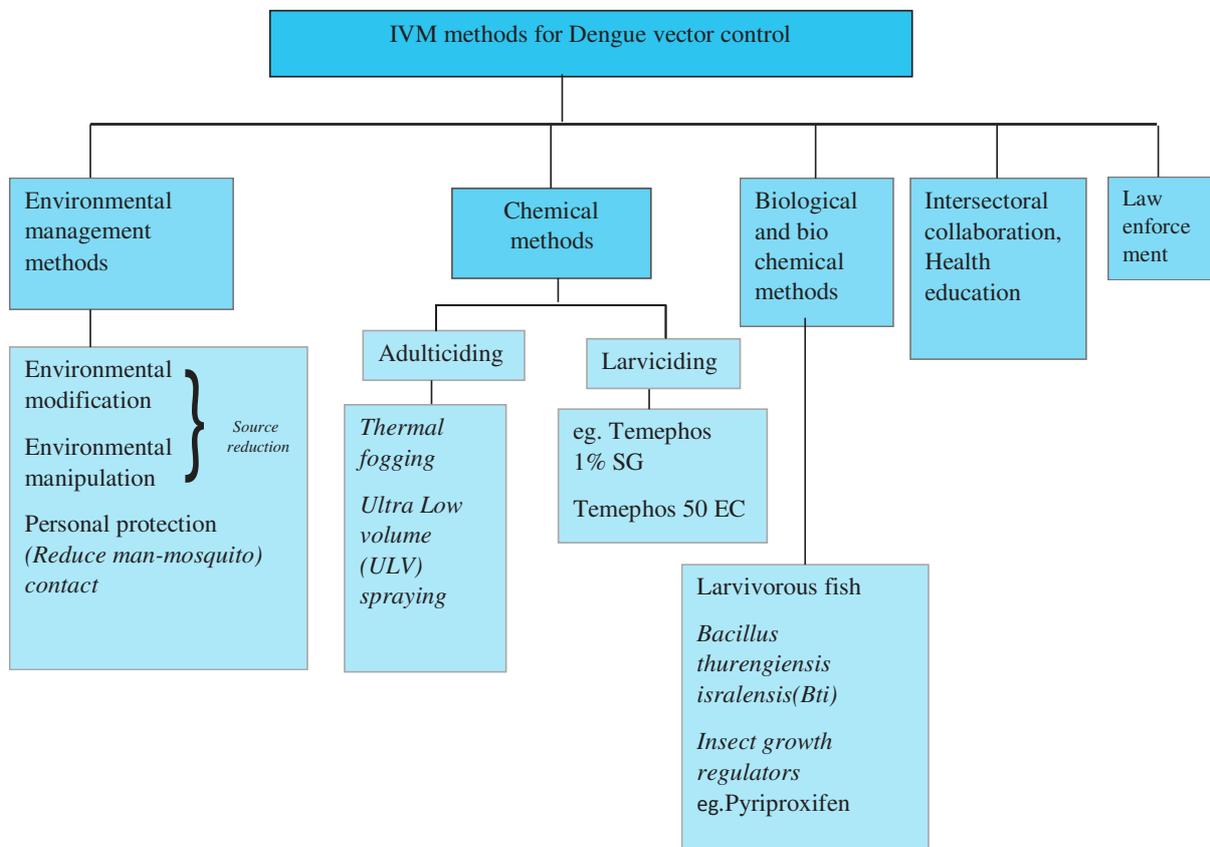


Fig. 5.6. Dengue vector control methods used in Sri Lanka

5.2.1.Environmental management methods

Environmental management seeks to change the environment in order to prevent or minimize vector propagation and human contact with the vector-pathogen by destroying, altering, removing or recycling non-essential containers that provide larval habitats. Such actions should be the mainstay of dengue vector control. Three types of environmental management are defined:

a. Environmental modification: Environmental modification includes long-lasting physical transformation of vector breeding habitats to eliminate and/ or prevent mosquito breeding.

For example,

- Providing continuous water supply to minimize storage of water in cement tanks, barrels, and other containers in areas with irregular water supply
- Mosquito-proofing of water storage cement tanks, domestic wells and overhead tanks/cisterns permanently
- Construction of buildings without roof gutters
- Removal of unserviceable roof gutters
- Removal of unwanted cement tanks
- Use of mosquito proof plastic overhead tanks instead of open tanks



Fig.5.7 a. Mosquito-proofing of water storage cement tanks

Source:<http://www.appropedia.org/>
Original:Rainwater_harvesting



Fig.5.7 b. Removal of unserviceable roof gutters

b. Environmental manipulation: Environmental manipulation involves temporary changes in vector habitats to prevent or reduce mosquito breeding. The effects of environmental manipulation is short term, thus, such type of activities needs repeated application.

For example,

- Regular cleaning with scrubbing of water storage tanks, flower pots, flower vases, ant-traps, refrigerator trays etc.
- Application of larvivorous fish, Bti and temephos 1% sand granules to the non-potable water storage containers and application of salt or oil to ant traps
- Proper draining of cement lined drains, water collections on cement floors and concrete slabs and periodic cleaning of roof gutters
- Proper disposal of solid waste by reducing, reusing and recycling of discarded receptacles
- Proper storage of used tyres, household and garden utensils under a shade
- End capping or filling the cavities of iron/ galvanized poles by cement or soil
- Cutting bamboo at the nodes or filling bamboo stumps and tree holes with sand or cement



Fig 5.8.a. Regular cleaning with scrubbing of flower pots, flower vases

Source: <https://blogs.extension.org/mastergardener/emg/plants-2/page/6/>



Fig 5.8.b. Proper disposal of solid waste by reducing, reusing and recycling of discarded receptacles

Source: <https://www.flickr.com/photos/janiquegoff/8309063703>

Potential environmental measures that can be taken for the control of *Ae. aegypti* and *Ae. albopictus* larvae in different types of breeding sites are shown in Table 5.2

Table 5.2. Potential environmental measures that can be taken for the control of *Ae. aegypti* and *Ae. albopictus* larvae in different types of breeding sites

	Breeding site	Scrub empty, clean weekly	Mosquito proof cover	Store under roof	Modify design/remove	Fill sand/soil/cement	Reduce, reuse recycle Collect and dispose	Puncture or drain
1	Water storage cement tanks and barrels	+	+					
2	Discarded receptacles						+	
3	Used tyres			+		+	+	+
4	Inventory items in the institutions			+				
5	Flower pots with saucers	+						
6	Flower vases	+						
7	Refrigerator trays	+						+
8	Ant traps	+						
9	Water collection on cement floors and roofs				+			+

	Breeding site	Scrub empty, clean weekly	Mosquito proof cover	Store under roof	Modify design/remove	Fill sand/ soil/ cement	Reduce, reuse recycle Collect and dispose	Puncture or drain
10	Roof gutters				+			+
11	Bamboo stumps, tree holes					+		
12	Plants with water holding leaf axils				+			

c. Changes to human habitation or behavior (Personal Protection)

Actions to reduce human–vector contact, such as installing mosquito screening on windows, doors and other entry points, and using mosquito nets while sleeping during daytime.

For example,

- Personal Protection/Physical barriers
- Screening doors and windows of houses/ premises using mosquito proof mesh
- Protective clothes to cover the body
- Curtains/ insecticide treated curtains
- Use of repellents - Application of natural and chemical repellents such as citronella oil, lemon grass oil, neem oil and chemical repellents containing DEET (N, N-Diethyl-m-Toluamide). However, repellents have short term effect(½ - 10 hours).

Different premises/ institutions can have different mechanisms for dengue vector prevention and control. Potential dengue vector control measures for different types of premises are shown in Table 5.3.

Table 5.3. Potential dengue vector control measures for different types of premises

	Type of premise	Recommended activity	Stakeholders
1	Living premises	Regular examination and elimination of breeding sites (e.g. cleaning on every Sunday) and ensure no breeding sites	Households
2	Construction site	Inspections by the PHII/ MOH to ensure mosquito free environment e.g. Cleaning, application of larvicides	Contractors (appointing inspection team), safety officers etc.

	Type of premise	Recommended activity	Stakeholders
3	Schools	Establishing committees for cleaning environment Weekly cleaning (half an hour on Friday)	School principal/ Responsible teacher/ staff member, School Development Committees, Parents associations
4	Condominium	Weekly cleaning (Half an hour cleaning on every Sunday) Proper waste disposal	Condominium authorities, Cleaning services/ Municipal councils
5	Institutions/ Offices	Weekly cleaning (half an hour on Friday)	Head of the institutions, dengue committee members
6	Hospitals	Establish an active committee to look after the hospital environment Weekly cleaning (half an hour on Friday)	Director/ MS/ DMO/ MOIC and PHI, Dengue committees
7	Bare lands/ abandoned lands	To trace owner and issue notices and ensure no vector breeding sites	Land owner, Local authority
8	Public places	Display notices and ensure no vector breeding sites	Local authority
9	Religious places	Clean weekly (half an hour on Sunday)	Chief priest, Dayaka sabha,

5.2. 2. Biological and bio-chemical methods for dengue vector control

Biological vector control agents prey upon, parasitize and/ or compete with the target vector species and bio-chemical methods interfere with the development process of the vector species, thus, eliminating/ reducing vector populations. Use of biological and bio-chemical agents for vector control avoids chemical contamination of the environment and reduces/averts development of resistance of vectors against insecticides. Larvivorous fish (biological), *Bacillus thurengiensis isralensis* (Bti) and insect growth regulators (bio-chemical) are used for dengue vector larval control in non potable water-storage tanks and barrels, ornamental ponds and fountains.

a. Larvivorous fish

Poecilia reticulata (guppy), *Aplocheilus* spp (nalahandaya) *Rasbora daniconius* (dandi), juvenile stages of *Tilapia* spp (*Oreochromis mossambicus* and *O. niloticus*) (Fig. 5.9) feed on mosquito larvae. These fish species can be used for dengue vector control in water storage tanks, barrels, ornamental ponds, fountains, cement lined drains and wells. (Technical specifications for preparation of larvivorous fish-annexure VII).

Poecilia reticulata



Rasbora daniconius



Tilapia species (juvenile stages)



Aplocheilus dayi (Nalahandaya)



Fig.5.9. Larvivorious fish species suitable for dengue vector control

Limitations of use of larvivorious fish

- the fish die in the absence of food in the breeding site and due to changes in the pH and salinity in the breeding sites
- The fish die when the domestic water storage containers are refilled with chlorinated water.
- Fish cannot tolerate high water temperatures

Messages for the community in stocking larvivorious fish for dengue vector control

- Remove fish when refilling the tanks and place the fish back after 6 hours of refilling

b. *Bacillus thurengiensis isralensis* (Bti)

Bacillus thurengiensis isralensis (Bti) is a spore forming bacterium that produces highly specific insecticidal proteins, called δ -endotoxins during sporulation (Fig. 5.10). The endotoxin is degranulated solely in an alkaline medium. When the spores containing endotoxin are swallowed by mosquito larvae, the endotoxin is degranulated in the midgut (alkaline medium) of the mosquito larvae, midgut wall is destroyed resulting in the death of the larvae.

Bti has an extremely low-level of mammalian toxicity and does not affect non-target organisms.

In Sri Lanka, liquid and briquette (a slow release formulation) formulation of Bti are used. Liquid formulation diluted product of Bt (Bactivec) is applied in the form of drops or using hand compression sprayers / and mist blowers.

Bti is recommended for *Ae. aegypti* breeding sites that cannot be eliminated by source reduction methods, e.g. water storage tanks, artificial and ornamental ponds, fountains and roof gutters.

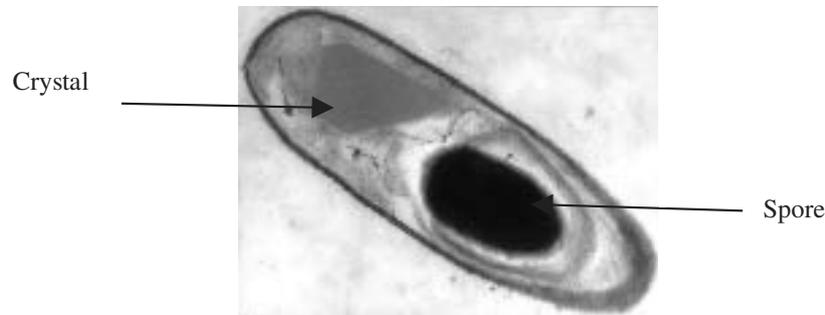


Fig. 5.10. *Bacillus thurengiensis* bacterium containing a spore and the crystal

Limitations of the use of Bti for dengue vector control

- Bti is not recommended for water containers where the water is likely to be used for human consumption (drinking) due to the possibility of the presence of other microbial contaminations in the product.
- Bti formulations tend to rapidly settle at the bottom of water containers, thus, frequent applications or mixing is required.
- Liquid formulation of Bt H-14 is effective for 10-12 days and the briquette formulation is effective for 30 days or more in the treated water. Thus, fortnightly application of liquid Bti and monthly application of Bti briquette is required for effective dengue vector larval control
- the late 4th instar larvae and pupae are not killed by Bti as they are in the non feeding stages.
- Types of breeding sites suitable for application of Bti is limited

Dosages for application of Bti (liquid) and Bti (Briquette) formulations are shown in Table 5.4.

Table 5.4 . Dosages for application of Bti (liquid) and Bti (Briquette) formulations

Product	Formulation	Application methods	Dosage/ Locality	Dilution factor	Frequency of application
<i>Bti</i> (e.g. Bactivec)	Liquid	Drops	04 drops of <i>Bti</i> per 10 liters of water.	Not applicable	Fortnightly intervals
		Using hand compression sprayers,		540ml of <i>Bti</i> to 09 liters of water.	Fortnightly intervals
		Mist blowers	For urban area	3.5l of Bti to 6.5l of water	Fortnightly intervals
		Mist blowers	For semi urban area	1.8 l of Bti to 8.2 l of water	Fortnightly intervals
<i>Bti</i>	Briquette	Briquette	01 dunk/100 sq ft of water.		Monthly intervals

These dosages give 100% mortality of 1st – 3rd instar larvae of both *Ae. aegypti* and *Ae. albopictus* larvae within 48 hours.



Figure 5.11: Bti applied using mist blowers

c. Insect growth regulators (IGR)

Insect growth regulators (IGRs) interfere with the development of the immature stages of the mosquito by :

- Interfering with chitin synthesis during the molting process in larvae or
- Disrupting the pupal and adult transformation processes.

IGR is recommended for dengue vector control in water storage tanks and containers. In waters that have been treated with the recommended dosage (0.01 mg/litre) of IGR, the larvae do not become pupae, instead, the larval period is extended and causes death.

The disadvantage of application of IGR for dengue vector control is that larvae and pupae remain visible in the IGR treated water. This may impact on legislative actions. IGR too is not recommended for application in drinking water sources.

5.2.3. Chemical methods for dengue vector control

Chemical methods of vector control include use of insecticides for larval and adult mosquito control. Classification and major groups of chemical insecticides that are used for adult and larval control are shown in Fig 5.12

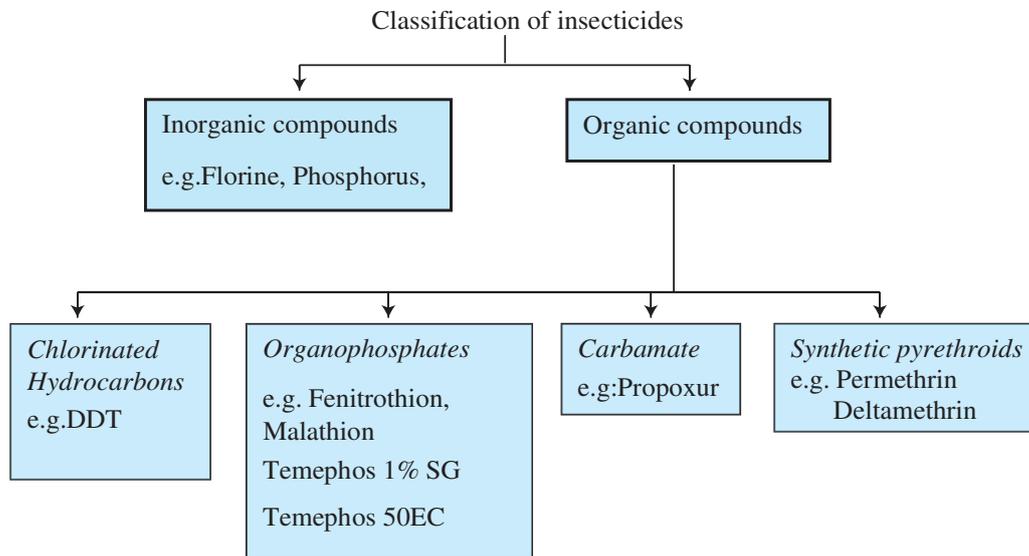


Fig. 5.12. Classification of major groups of insecticides that are used for adult mosquito and larval control

The NDCU in Sri Lanka, currently uses Temephos 1% SG and Temephos 50 EC for dengue vector larval control. Malathion, Deltacide and Pesguard are used for thermal fogging.

5.2.3.1. Chemical larviciding

Use of Temephos 1% sand granules (SG)

Temephos 1% SG is a slow releasing formulation of larvicide that is recommended for *Ae. aegypti* and *Ae. albopictus* control in domestic water storage containers such as water storage tanks, barrels and other containers that cannot be destroyed, eliminated or otherwise managed. However, this insecticide is not recommended for use in potable water.

Dosage, application methods and frequency of application of Temephos 1% SG are shown in Table 5.5.

Table 5.5. Dosage, application methods and frequency of application of Temephos 1%SG

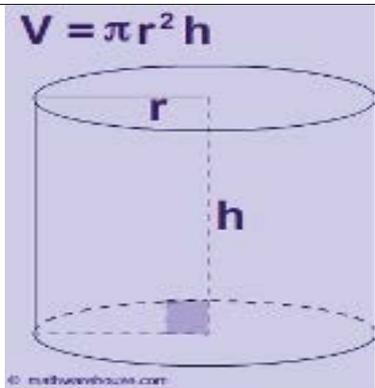
Product	Formulation	Application methods	Dosage (active ingredient) Container breeding (mg/l)	Frequency of application
Temephos	1% SG (GR)	In cotton cloth pouches (put the required amount of granules in the pouch and keep it suspended in the water)	1ppm dosage (1g of the product in 10 liters of water)	03 month intervals

Indications for use of Temephos 1% SG

- in situations of dengue epidemics
- Prior to peak transmission of dengue in order to prevent an epidemic when and where epidemiological factors (disease incidence, entomological indices and environmental factors) indicate potential dengue outbreaks/ epidemics.

Application of temephos 1% SG should be carried out assuming that the storage volume of water in the tanks is maximal (or the usual storage volume), even if the tanks are not full of water. Method of calculation of the volume of the container are shown in table 5.6

Table 5.6. Method of calculation of the volume of the container

Shape of the container	Method of calculation of the volume	
Square	Length x width x height of the tank	
Cylindrical (pictures)	$22/7 \times \text{radius}^2 \times \text{height}$ of the container	

Quantities of temephos 1% SG for containers of different sizes are given in Table 5.7.

Table 5.7. Quantities of temephos 1% SG required to treat water containers of different sizes to kill mosquito larvae

Capacity of the container in liters	Grams of Temephos 1% SG required	Number of teaspoons required (one teaspoon contains approximately 5 grams)
< 25	Less than 5	Pinch (small amount held between thumb and finger)
50	5	1
100	10	2
200	20	4
250	25	5
500	50	10
1000	100	20

Use of temephos 50% EC

In situations, when other larval control measures are not practical/ feasible, Temephos 50%EC can be used for dengue vector larval control.

eg., during dengue epidemics, when vector breeds in water collections in abandoned/ unused boats, concrete slabs, in yards with machinery parts, construction sites, etc.).

Application of Temephos 50 EC can be done using hand compression sprayers or mist blowers. The dosage, application methods and frequency of application of Temephos 50 EC is shown in Table 5.8.

Table 5.8. Dosage, application methods and frequency of application of Temephos 50 EC

Product	Formulation	Application methods	Dosage (active ingredient) Container breeding (mg/l)	Dilution factor	Frequency of application
Temephos	EC	By hand compression sprayers,	01	20 ml in 09 liters of water	Fortnightly intervals

Susceptibility/ resistance level of *Ae. aegypti* and *Ae. albopictus* larvae should be monitored at regular intervals (once in 3 months) according to WHO guidelines in order to ensure effective use of insecticide.

5.2.3.2. Adult Vector Control

5.2.3.2.a Space spraying to control adult mosquitoes using insecticides

Space spraying includes application of small droplets of insecticide into the air for rapid knock -down and eventual death of adult vectors. This method is used in emergency vector control to suppress the vector population and thereby to interrupt ongoing dengue outbreaks/epidemics or to prevent an impending dengue outbreak from occurring. Space spraying can be done using 2 methods viz., thermal fogging and ultra low volume(ULV)spraying.

Thermal fogging

Generally, thermal fogging machine employs the resonant pulse principle to generate hot gas (over 200 °C) at high velocity.

These hot gases atomize the insecticide solution (mixture of the insecticide and the diluents) instantly so that it is vaporized. As soon as this vapour encounters with the cooler ambient air, the vapour gets condensed rapidly into tiny droplets forming a dense fog.

Each of the micro droplets of the fog contains the appropriate dose of the active ingredient, and has the ability to float long distances. These droplets can penetrate and access sites with dense vegetation and reach corners in open buildings and houses.

Once the droplets of insecticide come in contact with the target mosquito species in sufficient dosage, the mosquito is rapidly knocked down and eventually killed.

Thermal fogging is carried out by hand carried (portable) and vehicle mounted thermal foggers (Fig 5.13 and 5.14).



Fig 5.13 Thermal fogging using a hand carried fogging machine



Fig. 5.14 Thermal fogging using vehicle mounted fogging machine

Hand carried machines are meant for restricted outdoor use and for enclosed spaces (building). This type of machine is used for fogging in congested housing areas, multi storied buildings, or in areas that are inaccessible by a vehicle.

In epidemic situations, especially in urban areas, when a large area is to be covered, fogging is carried out by using vehicle mounted fogging machines

In some areas, especially in the hilly areas, the fog of a vehicle mounted machine may not reach some parts of the target areas. In such situations, hand carried fogging machines are used to cover these areas simultaneously with the vehicle mounted machine ensuring full coverage of the target area.

Ultra-low volume (ULV) applications

Ultra-low volume applications are mechanically generated by ULV generators. ULV application involves the application of a minimum volume of insecticide liquid concentrate over a wide area.

ULV applications are made by backpack sprayers with ULV attachments or nozzles as well as by vehicle mounted aerosol generators. Backpack sprayers are used for house to house spraying when the area to be treated is not very large or in areas where vehicle-mounted machines cannot reach.

The weight of the machine and the vibrations caused by the engine make it necessary to allow the operators to rest adequately and hence two or three operators are required per machine.

The vehicle mounted aerosol generators have an engine driven air compressor system to produce a flow of air into which insecticide formulation is released and sheared into fine aerosol droplets.

Effectiveness of space spraying heavily depends on the environmental conditions under which spraying is carried out.

Similarities and differences between thermal fogging and ULV are shown in Table 5.9

Table 5.9. Similarities and differences between Thermal fogging and ULV

Thermal fogging	ULV
<i>Similarities</i>	
Both create a dry fog where the user can determine the droplet size and amount of product being dispensed.	
Both can be mounted on trucks or other vehicles for ease of application.	
<i>Differences</i>	
Thermal foggers use heat to produce a very visible fog	ULV machines use a high volume of air to create a fog without heat
Thermal Fogging reaches deep into obstructed areas and overgrown thickets	ULV machines apply more on surfaces and may not penetrate obstructed areas and dense foliage completely
Thermal fogger produces a range of droplet sizes including a large number of very small droplets.	ULV sprayer generates fog droplets of a more precise size
Presence of very small droplets enables the fog to reach spaces in areas highly obstructed by vegetation, or other physical obstructions in buildings.	The absence of a large number of very small droplets will limit penetration of the fog into highly obstructed areas.
Presence of a large number of very small droplets in the fog makes the fog highly visible.	Fog is comparatively less visible
Visibility of fog helps the operator to monitor the fog and ensure thoroughness of application.	
Comparatively more diluent is required (type of diluent)	ULV sprayers can dispense formulations in a more concentrated form and less diluent is required.
	Able to calibrate the machine to produce droplets of the optimum size for the type of chemical being used
Thermal foggers make much noise	ULV machines create comparatively less noise

5.2.3.2.b. Insecticides that are used for space spraying

Organophosphate and synthetic pyrethroid insecticides are used for adult vector control. WHO recommended insecticides for space spraying against mosquitoes are shown in Table 5.10.

Table 5.10: WHO recommended insecticides for space spraying against mosquitoes

Compound and formulation	Indoor		Outdoor	
	(g AI/1000 m ³)		(g AI/ha)	
	Cold	Thermal	Cold	Thermal
	fog	fog	fog	fog
Deltamethrin UL	0.5	0.05	0.5	0.5-1.0
Delatamethrin EW	-	0.05	1	-
Lambda-cyhalothrin EC	-	-	1-2	2
Malathion EW and UL	-	-	112-600	112-600
Permethrin(25 cis: 75 trans;10.35%w/w)+s-bioallethrin(0.14w/w)+ piperonyl butoxide(9.85% w/w)EW	0.55	0.73	-	-
d-d, trans-cyphenothrin EC	0.1-0.2	0.2	3.5-4.00	3.5-4.0

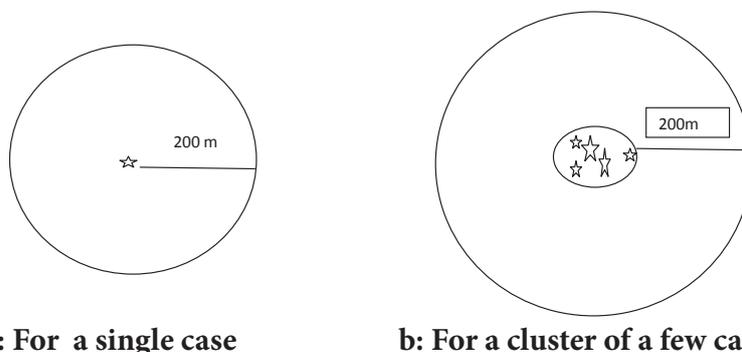
5.2.3.2.c Technical details of space spraying

I. Area to be covered by space spraying

For a single case of dengue, fogging should be carried out to cover an area of about 200m radius of the patient’s house/ source of infection as shown in Fig 5.15.a

When there is a cluster of dengue cases in an area (2 or more cases within at < 200m distance from each other within 2 weeks), fogging should cover within and 200m distance of the perimeter of the cluster as shown in Fig. 5.15.b

Fig. 5.15.a. and . 5.15.b. Area to be covered by space spraying for a single dengue case and a cluster of cases



a: For a single case

b: For a cluster of a few cases

Fig. 5.15: Area to be covered by space spraying for a single dengue case and a cluster of cases

For fogging a single dengue case, 02 hand carried fogging machines are required. To cover the 200m radius of a case 2-3 fillings (10 -15 liters) of the insecticide solution is sufficient.

In the case of a cluster of cases, two or more machines needs to be used and the number of machines to be used depends on the extent of the cluster.

Use of vehicle mounted fogging machines for space spraying

Vehicle mounted fogging machines are used to cover large areas when there are scattered cases in a village or when there are cases along the same or adjacent streets in an urban area within the past 2 weeks. When space spraying is carried out using vehicle mounted fogging machines, the route of the vehicle should be planned prior to the fogging operation in order to employ hand operated fogging machines to reach the rest of the target area (to make sure the entire target area is covered). Possible movement of a vehicle mounted fogging machine is shown in Fig. 5.16. The speed of the vehicle should be 6-8 Km per hour.

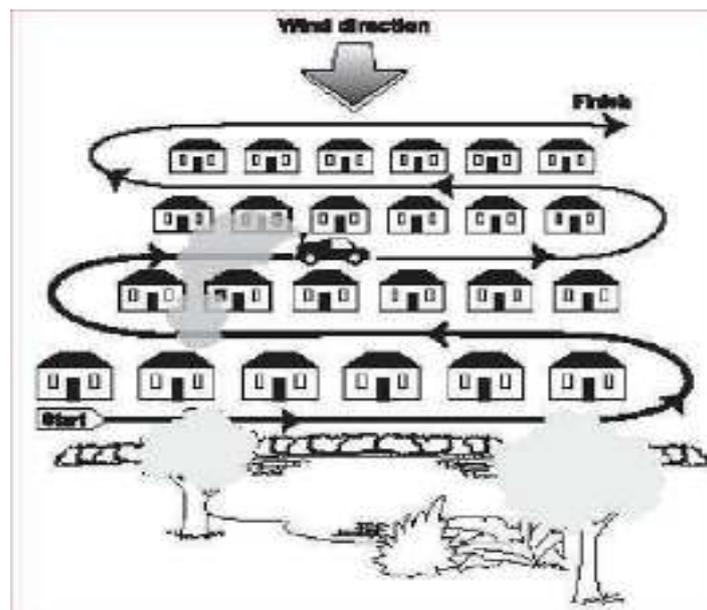


Fig.5.16. Possible movement of a vehicle mounted thermal fogging machine

II. Frequency of space spraying

In the case of an indigenous case, minimum two rounds of fogging should be carried out. The first round of fogging should be carried out as early as possible of the notification of the case (at least within 48 hours of the notification) and the second round within 5-7 days after the first round of fogging.

In the case of an exogenous case in receptive areas, one round of fogging may be sufficient. If the patient contracted dengue illness outside his/her residence area and returned to the residence after the viraemic phase, fogging in the residence area may not be necessary. However, fogging should be carried out in the area where source of infection occurred.

III. Activities to be followed prior to , during and after space spraying

III.a Steps to be followed prior to space spraying

- Demarcate the target area to be covered by space spraying by studying a sketch map of the area. When vehicle mounted machine is used for fogging, study the street map and determine the route of the vehicle to cover the target area.
- Make the community aware about fogging through health education and using public addressing system. Specific instruction should be given to keep the doors and windows of their houses, including those of all bedrooms open, cover their food and water and food items kept for sale by vendors and in all instances to extinguish fire sources while fogging operations are in progress.
- Advice on moving out of houses when fogging is in progress.
- Advise the community to close all doors and windows of premises for half an hour immediately after the fog dispersed in the interior of the house, then to open the doors and windows and enter the house
- Study wind direction and decide the path of sprayman to ensure full coverage of the target area. The sprayman should move from downwind to upwind, i.e. against the wind direction.
- Ensure proper traffic control especially when using vehicle mounted fogging machines since fog can pose a traffic hazard to motorists and pedestrians.

III.b Steps to be followed during space spraying

When fogging is done using hand operated (Portable) thermal fogging machines

- Spraymen should move from house to house
- Fog in and out of the premise (peridomestic fogging)
- Fog the interior of the premise by directing the fog to the interior of the premise through an open door or a window for 10 – 15 seconds with the nozzle of the machine at a distance of about 3m to the door/ window. Stepping into a premise/house with the fogging machine should be avoided due to potential fire hazard with thermal fogging.
- In single-storey houses, fogging can be done from the front door or through an open window without having to enter every room of the house. All bedroom doors should be left open to allow dispersal of the fog throughout the house.
- After spraying, all doors and windows should be shut for half an hour to ensure good penetration of the fog within the house.
- When fogging is done in a multi-storied building, fogging should be begin from the uppermost floor and the sprayman move from upstairs to downstairs and from the back of the building to the front ensuring full coverage of the target area. This motion gives good visibility of the path of the sprayman.
- Fogging should essentially cover all possible mosquito resting sites including hedges, covered drains, bushes, and tree-shaded areas based on the evidence and depending on the local situations.
- The most effective type of thermal fog for mosquito control is a medium/dry fog, i.e. it should just moisten the hand when the hand is passed quickly through the fog at a distance of about 2.5-3.0 meters in front of the fog tube.
- Adjust the fog setting so that oily deposits on the floor and furniture are reduced.

III.c Special steps to be followed when fogging is carried out using vehicle mounted thermal foggers

- Study the street map of the target area and determine the route for the vehicle
- Ensure proper traffic control since it can pose a traffic hazard to motorists and pedestrians
- In areas where the roads are narrow and the houses are close to the roadside, vehicle should move against the wind direction with the spray head pointing backwards. In areas with wider roads, move the vehicle in zigzag manner across the wind direction ensuring coverage of the target area
- Drive the vehicle at a steady speed of 6-8 Km/hr (3.5 – 4.5 miles/hr)
- Turn off the fogging machine just before the vehicle stop
- When some parts of the target area are inaccessible for the vehicle and not covered by vehicle mounted fogging machine, use hand operated fogging machines to cover such area.
- In case of roads having dead-ends, the spray production should be started only when the vehicle is coming out of the dead-end, and not while going in.
- The spray head should be pointed at a 45° angle to the horizontal to achieve maximum throw of droplets.

Inside the house fogging

Ae. aegypti and *Ae. albopictus* rest and bite indoors as well as outdoors. Thus, indoor fogging is recommended in epidemic situations and in areas with impending epidemics. However, special precautions are necessary in indoor fogging in order to prevent potential fire hazards and to protect the spray men, house occupants, poultry and household pets. Following precautions are needed to be taken in indoor fogging.

- Shut off all electricity at the master switch
- Turn off all heating and cooking equipment and allow some time to cool down before spraying.
- Ensure all occupants and animals remain outside the house during spraying and stay outside for 30 minutes after spraying.
- Ensure that the building is ventilated before reoccupation
- Cover all water containers and foodstuffs
- Cover fish tanks
- Close all doors and windows before spraying and keep them closed for 30 minutes after spraying to ensure maximum efficacy.
- Spray operators should work backwards and away from the fog to minimize exposure.

eg. In single room houses and small single-storey houses, the spray can be delivered from the front door or through an open window without entering every room of the house, provided that adequate dispersal of the insecticide droplets can be achieved.

In large single-storey buildings, it may be necessary to apply the spray room by room, beginning at the back of the building and working towards the front or vice versa (Fig 17 and 18).

In multi-storey buildings, spraying is started from top floor to the ground floor and from the back of the building to the front. This ensures that the operator has good visibility at all times.

- A fog must be “dry” before being directed into a building. Test the fog by placing the machine on the ground and checking that the area immediately in front of the nozzle is not wet due to the fog.
- To reduce the production of large wetting droplets, obtain the correct balance between flow rate and combustion temperature. This is usually done by reducing the flow rate.
- Water-diluted products are recommended in indoor fogging in order to minimize fire hazards.

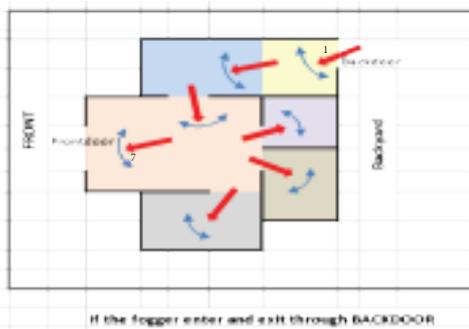


Fig 5.17 pathway for the fogger when do fogging indoor start with 1 and end with 7

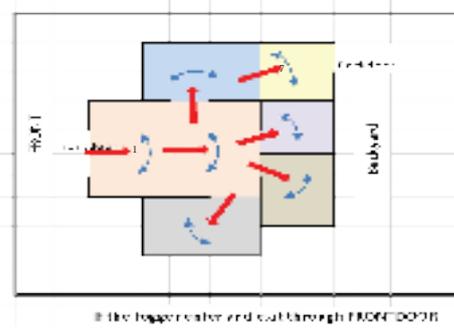


Fig 5.18 pathway for the fogger when do fogging indoor start with 1 and end with 7

IV. Optimum conditions for space spraying for dengue vector control

Space spraying should be carried out during periods of peak biting activity of *Ae. aegypti* and *Ae. albopictus* and when the right weather conditions are present for its optimum effectiveness. Time of the day, ambient temperature, wind speed and precipitation are important environmental conditions that affect the effectiveness of space spraying.

IV.a Timing

Ae. aegypti and *Ae. albopictus* are mostly active in the morning for a few hours after sun rise hours and for a few hours before sunset Thus, space spraying is carried out within these peak periods of activity.

IV.b Temperature

In the middle of the day, when temperature is high, convection currents from the ground will prevent concentration of the fog close to the ground where adult mosquitoes are flying or resting, thus rendering the space spraying ineffective. Thus, the most appropriate periods for fogging are the mornings and the afternoons when the ambient temperature is cooler and favours optimal dispersion of droplets targeting the vector.

IV.c Wind speed and wind direction

Air movements of less than 3 km/hr may result in vertical mixing, while winds greater than 13km/hr disperse the spray too quickly. Thus, optimum wind speed for fogging is between 3 – 13 km/hour.

The sprayman should be moving opposite the wind direction with the nozzle holding backwards so that the spray would move with the wind in the same direction. However, the spraymen (with the hand carried machine) or the vehicle (with the vehicle mounted machine) may move perpendicular to the wind direction. Such movements take the fog with the wind to one direction, so that the sprayman/ vehicle should make the next movement to cover the other part of the target area in such instances.

IV.d Precipitation

In heavy rain, the spray loses its consistency and does not disperse in the air to the target area. Therefore fogging should not be done during heavy rains. However, fogging is permissible during light showers.

Fogging is carried out only when the right weather conditions are present and usually only at the prescribed time. These conditions are summarized in table 5.11

Table 5.11. Most favorable, average and unfavourable weather conditions for space spraying

	Most favourable conditions	Average conditions	Unfavourable conditions
Time	Early morning (0630 -0830) or late evening (1600- 1800)	Early to mid-morning (0830 – 1030)	Mid-morning to mid afternoon
Wind	Steady, between 3-13 km/h	0-3km/hr	Medium to strong over 13km/h
Rain	No rain	Light showers	Heavy rain
Temperature	Cool or mild	Mild	Hot

IV.e Droplet size

Space spraying is only effective while the droplets remain airborne. Droplets larger than 30µm in diameter are less effective as they do not remain airborne for a sufficient time. Droplets smaller than 5µm in diameter do not readily come in contact with flying insects, as the movement of the smallest droplets is affected by the air turbulence created by the insect’s flight. The optimum droplet size for space spraying against mosquitoes is 10–20 µm. The droplet size of a thermal fog is usually less than 15 microns in diameter and the optimum droplet size for ULV spray usually falls within the range of 10 – 25 microns in diameter

V. Types of insecticides used for space spraying in Sri lanka

Dilution factors of different insecticides that are used for dengue vector control in Sri Lanka by thermal fogging and ULV are shown in Table 5.12 and 5.13.

Table 5.12 Dilution factors of different insecticides that are used for dengue vector control in Sri Lanka by thermal fogging

Insecticide	Mixing proportion		Required insecticide amount for 5 litre capacity hand held fogging machine	Required insecticide amount for 50 litre capacity vehicle mounted fogging machine
	Insecticide	Kerosine oil/Diesel		
Technical malathion	1	19	250 ml	2.5litre
Pesguard	1	159	30 ml	300ml

Table 5.13 Dilution factors of different insecticides that are used for dengue vector control in Sri Lanka by ULV application

Insecticide	Mixing proportion		Required insecticide amount for 5 litre capacity hand held fogging machine
	Insecticide	Water	
Pesguard	1	15	~330 ml

5.2.3.2.d. Supply of insecticides and logistics for space spraying

Insecticides are issued by the NDCU based on the local requirements. Kerosene oil and other logistics are to be obtained from the respective local government authorities on the request by the MOH/RMO/MOIC-AFU/Entomologist/RE of the area.

When using insecticides for vector control, the safety of the spray personnel, public and their domestic pets and animals and the environment should be considered. The precautions that are to be taken to ensure safety are discussed in Chapter 6

5.3. Use of residual insecticides for dengue vector control

Residual insecticide spraying for adult mosquito vector control is the application of a long lasting residual insecticide to potential resting surfaces of vector mosquitoes. When a vector come into contact with a sprayed surface and absorbs a lethal dose of insecticide, it reduces the lifespan of the vector mosquito. This results in a progressive decline in vector density and longevity and a reduction in overall vectorial capacity thereby contributing to a reduction in disease transmission.

According to the available knowledge, the Aedes vector rests on hanging objects, under surfaces of furniture and inside containers both indoors and outdoors such as tires, pots etc. This resting habit limits the use of residual insecticides for dengue vector control. Up to now there is little evidence on the use of residual insecticide for dengue vector control. However, residual insecticide spraying could

not be totally ruled out against dengue vector control. Thus, before deciding use of residual insecticide for dengue vector control, it is necessary to study the vector resting sites and the susceptibility of vector.

5.4. What is WHOPEs

The WHO Pesticide Evaluation Scheme (WHOPEs) was set up in 1960. WHOPEs promotes and coordinates the testing and evaluation of pesticides for public health. It functions through the participation of representatives of governments, manufacturers of pesticides and pesticide application equipment, WHO Collaborating Centres and research institutions, as well as other WHO programmes, notably the International Programme on Chemical Safety.

In its present form, WHOPEs comprises a four-phase evaluation and testing programme, studying the safety, efficacy and operational acceptability of public health pesticides and developing specifications for quality control and international trade.

WHOPEs collects, consolidates, evaluates and disseminates information on the use of pesticides for public health. Its recommendations facilitate the registration of pesticides by Member States.

WHOPEs' objectives

- To facilitate the search for alternative pesticides and application methods that are safe and cost-effective; and
- to develop and promote policies, strategies and guidelines for the selective and judicious application of pesticides for public health use, and assist and monitor their implementation by Member States.

(source: <http://www.who.int/whopes/en/>)

5.5. Insecticide resistance monitoring

Insecticide resistance is “the development of ability in a strain of insects to tolerate doses of toxicants which would prove lethal to the majority of individual in a normal population of the same species”. Development of resistance is a complex and dynamic process and it depends upon genetic, biological and operational factors. Currently dengue vector population in Sri Lanka have showed resistance and possible resistance level for currently use insecticides. Therefore, resistance monitoring should be an integral part of dengue vector control programme Sri Lanka.

Objective:

The purpose of the susceptibility test is to detect the presence of resistant individuals in an insect population early to preserve the effectiveness of available insecticides. If use of insecticide continues without knowing the presence of resistance, percentage of selected survivors will increase and the susceptibility of the population will decline to a point that the insecticide no longer provides an acceptable level of control. Therefore the susceptibility monitoring will help to plan alternative control strategies to deal with the situation when the insecticide in question is no longer having the desired effect.

Test procedures for insecticide monitoring mention in annexure (VIII)

5.6 Outbreak Management Plan

Definition of an outbreak

“Outbreak” (used synonymously with “epidemic”) is defined as a “sudden unexpected increase of cases” or as ‘the occurrence in a community or region of cases clearly in excess of expectancy’. A “sudden and unexpected increase” (outbreak) may differ from the seasonal peak, which is an “expected increase of cases” that usually occurs during the wet season (monsoons). However, the response should be swift in both these situations.

In Sri Lanka for practical purposes, country has been divided into 3 risk categories (Priority High-risk, High-risk and Low-risk). This risk categorization is determined according to the disease surveillance data (both historical average of cases and reported cases during current year), vector indices, demographics and population density and other parameters such as case fatality rate etc.

- **Priority High-risk Area:** Constant number of cases reported throughout the year, during both monsoonal and non-monsoonal seasons
- **High-risk Area:** Higher number of cases reported particularly during monsoonal seasons
- **Low-risk Area:** Only few sporadic cases reported any time during the year

This section briefly outlines the sum of measures that should be taken during the period of outbreak preparedness, during the outbreak and the post outbreak period based on risk level.

Objectives of controlling an outbreak are to reduce the case fatality rates, patient numbers and entomological parameters.

As a Public Health Manager, one should detect early stage of outbreak by doing:

- Regularly monitoring of entomological data
- Conducting active and passive surveillance of dengue cases
- Prompt reporting and communication with relevant stakeholders

5.6.1. Outbreak preparedness

Outbreak preparedness and control measures should be conducted according to the risk level. The risk categorization for a particular location or division is dynamic and will vary both temporally and spatially.

As a part of outbreak preparedness, following steps are to be taken:

Establishing dengue action committees at different levels

Both national and sub-national level (Provincial and District) should have an outbreak response committee with multi-sectoral representation for emergency response. The committee must have sub

committees at the sub national level that can take quick decisions, mobilize resources and respond quickly during the outbreaks.

The committee will have following responsibilities:

- Capacity building of curative and preventive health staff, and other relevant personnel
- Improve facilities and establish “fever corners” for screening suspected fever patients in hospitals
- Ensure the supply of necessary logistics without interruption to all treatment facilities and make available patient transport facilities at all treatment centres
- Prompt notification of suspected cases from the health care facilities to identify vulnerable areas
- Capacity building of all relevant staff on proper communication and health education
- Ensure availability of necessary facilities for communication and health education in all hospitals
- Ensure communication within Division (MOH), District, Provincial and National level units as well as with other stakeholders including media
- Conducting regular entomological surveys and analysis of entomological and disease data
- Establishing operation centres (or a communication channel) at different level depending on the extent of the outbreak

5.6.2. During the outbreak

Establishing an Operation Centre

During an outbreak, the Operations Centre must be open throughout, even on weekends and public holidays (preferably 24/7). The setting up of an operations centre at different levels (such as District/ Provincial/National level) is based on the following criteria:

- a. District level; when, at any one time, an outbreak occurs in two separate MOH areas, or there are five or more new cases in any one priority high-risk locality.
- b. Provincial level; when, at any one time, there are 2 or more Districts having outbreaks
- c. National level; if, at any one time, there are three or more Provinces experiencing an outbreak.

All Divisions/Districts/Provinces must have a contingency plan to increase the man power needed and equipment for immediate action when an outbreak detects.

Following measures should be initiated as early as possible once you detect an outbreak:

- **Adult and larval vector control**
 1. Plot reported cases on the area map to identify clusters.
 2. Determine the number of hand held fogging machines, vehicle mounted fogging machines and back pack sprayers available and determine the size of the area to be sprayed
 3. Immediately commence adult space spray operations (thermal Fogging or ULV aerosols) in the event of notification of a dengue case (ideally within 24 hours). Targeting both indoor and outdoor areas, space spraying should be done within the radius of 200 meters of the index case house.

4. All houses within 200m radius of the 'dengue case' house and 'dengue case contact points' must be totally surveyed for *Aedes* breeding and the quality of the surveys should be ensured by the MOH and the Entomological Teams.
5. Special attention should be given to hospitals to prevent virus transmission. Fogging should be continued at least once a week till the virus load reduces.
6. Continue space spray operations in localities with the notified cases and in nearby crowded areas such as schools and towns. Repeat applications after 5 to 7 days till no more cases are reported.
7. Intensify long term larval control measures in permanent water storage containers that are difficult to clean and empty. These measures should include using larvicides and deposition of fish.
8. Monitor larval and adult mosquito densities using relevant techniques

• Source Reduction

1. Activate the Divisional and District Committees. Members of the committees should communicate regularly with their stakeholders including Local Government, Healthcare Providers in curative and Preventive sectors, Vector control personnel, Laboratory Scientists and Community Leaders
2. Intensify source reduction campaigns especially in priority high-risk localities involving the local community and members of the Presidential Task Force
3. Encourage local authorities to intensify solid waste separation and collection and environmental clean-up.

• Health Education

1. Encourage patients to seek medical attention if they have any of the following symptoms with sudden onset of fever; headache & retro-orbital pain, nausea or vomiting, arthralgia, myalgia or bleeding manifestations. Those diagnosed with dengue (including inward patients) are encouraged to use mosquito nets when sleeping/ resting and applying insect repellent to break the dengue transmission chain.
2. Enhance public awareness through mass media, newspapers and local communication systems. Reinforce the importance of seeking medical attention if they have dengue symptoms
3. Deploy a mobile health education team during fogging operations to obtain more corporation from public.

• Legal Action

1. Legal actions to be initiated if continuous breeding places are found in same locality (premises) or any major breeding places found (e.g. construction sites, factories)

During the outbreak, the relevant health authorities should;

- Monitor the situation daily and act accordingly
- When needed solicit support of higher levels within health sector and other stakeholders.

5.6.3. Post outbreak activities

- A detailed report should be developed once the outbreak is curtailed highlighting the lessons learnt for long term prevention of further outbreak.
- Larval and adult surveys should be continued according to the risk levels.
- Closing up of operation room.

5.7. Law enforcement

Though majority of the people adhere to guidance given by health authorities some do not, either due to negligence or lack knowledge or confidence on this strategy. Therefore, legal action against such persons/ organizations is important, with a view to mitigating the threat to public health.

Legal action could be taken against an individual if he/she owns/occupies premises with potential and actual mosquito breeding sites which would be a threat to public health under different provisions of following acts, ordinances and statutes by the authorities depending on the situation.

- Quarantine and prevention of disease ordinance, No.3 of 1897
- Nuisances Ordinance , No.15 of 1862
- Prevention of Mosquito Breeding Act, No. 11 of 2007
- Statute of preventing public health nuisances of the Western Province Council no. 03 of 2012
- Section 262 of CHAPTER XIV of the penal code

Quarantine and prevention of disease ordinance, No.3 of 1897 had been enacted more than a century ago in order to prevent spread of contagious diseases. Under this ordinance, provisions are there to destroy the goods that are capable to spreading of infectious disease. Also under this ordinance if a person is found guilty of any offence he/she would be punished with either a fine or imprisonment or both (Can access in http://www.commonlii.org/lk/legis/consol_act/qapod553410.pdf)

Under Nuisance ordinance, an occupant or owner of any house, building or land who maintains them in an unsatisfactory manner creating a nuisance or injurious to health of any person as stipulated in this ordinance would be considered to cause an offence. Whoever would commit this offence shall be liable to a fine. (Can access in http://www.commonlii.org/lk/legis/consol_act/n562160.pdf)

In order to pay special attention for prevention of spreading of dengue; Prevention of Mosquito Breeding Act was enacted in the parliament in year 2007. Director General of Health Services is the competent authority as per this act and his powers are delegated to the Medical Officers of Health and the Public

CHAPTER 05

Health Inspectors.

This act includes prohibition against creating conditions favourable to breeding of mosquitoes, directions to owner or occupent to take certain measures, action to be taken by competent authority in case of a failure to adhere to guidelines stipulated in the act.

Where an offence is found to have been committed under this Act by an owner or occupier, prior to a prosecution being instituted, a Public Health Inspector is required to serve a written notice with required corrective measures to be taken with a time frame.

If the alleged offender does not take necessary corrective actions, he/she shall be guilty of an offence under this Act, and on conviction after summary trial before a Magistrate, be liable to a fine or to a term of imprisonment or both. In case of repeated offence the intensity of the punishment increases if found guilty.

(Can access in <http://www.documents.gov.lk/Acts/2007/Prevention%20of%20Mosquito%20Breeding%20Act%20No%2011/Act%20No%2011%20E.pdf>)

Statute of preventing public health nuisances of the Western Province Council no. 03 of 2012 enables Public Health Staff to impose spot fines on those who breach the law within the Western Province (Can access in <http://www.healthmin.wpc.gov.lk/en/wp-content/uploads/2012/08/PHH-ENGLISH3.pdf>).

Under the Section 262 of CHAPTER XIV of penal code, if an individual does any act negligently or unlawfully which he/she knows or has reasons to believe to be, likely to be spread the infection of any disease dangerous to life would be punished with imprisonment or fine or both following a summary trial before a magistrate. Police officer is the law enforcement officer under the provisions of this ordinance.

(Can access in http://www1.umn.edu/humanrts/research/srilanka/statutes/Penal_Code.pdf)

Flow chart of Dengue risk levels and responses





06

**SAFETY PRECAUTIONS
TO BE FOLLOWED TO
MINIMIZE HEALTH AND
ENVIRONMENTAL HAZARDS**

Safety measures in insecticide use are necessary to protect the health and lives of spraymen, spray team supervisors, the public and their pets and domestic animals, and to minimize hazard to the environment



SAFETY PRECAUTIONS TO BE FOLLOWED TO MINIMIZE HEALTH AND ENVIRONMENTAL HAZARDS

Safety measures in insecticide use are necessary to protect the health and lives of spraymen, spray team supervisors, the public and their pets and domestic animals, and to minimize hazard to the environment. To minimize routine and accidental exposure to insecticides, safety precautions must be taken at all stages of insecticide use such as while storage, transport, preparation of insecticide solutions, and during and after spraying of insecticides.

6.1. Safety precautions to be taken when storing insecticides

- Store the insecticide in the container with the original label. The labels should identify the contents, nature of the material and precaution methods.
- Do not transfer insecticides to other containers, or to containers used for food or beverages.
- Keep all insecticide containers sealed or properly closed.
- Store/ keep insecticides in a properly-designated place, away from direct sunlight, food, medicine, clothing, children and animals, and protected from rain and flooding.
- Store the insecticide in a locked room with posted warning signs such as “Dangerous insecticides, children and unauthorized persons are not allowed”.
- Use the first received insecticides first and this avoids prolonged storage of any batch of insecticides.

6.2. Safety precautions to be taken before use of insecticides

- Read the label carefully and understand the directions for the preparation and application of the insecticide as well as the precautions listed, follow the directions and precautions exactly as recommended.
- Know the first aid measures and antidotes for insecticides being used (mentioned).

6.3. Safety precautions to be taken during mixing and fogging

Wear personal protective equipments (masks, cap/ helmet, overalls, gloves, boots, goggles, etc during mixing of insecticides and fogging.

- Wash clothes daily after working
- Mix insecticides in a properly ventilated area, preferably outdoors
- Stand with the wind blowing from behind when mixing insecticides
- Make sure that the spray equipment does not leak
- Keep all unnecessary people away from where insecticides are being mixed
- Always, keep a fire extinguisher with the spray team
- Do not blow with the mouth to clear blocked spray nozzles
- Do not wear unwashed protective clothing
- Do not drink, eat or smoke while fogging.
- Do not smell or inhale insecticides



Figure 6.1 Personal protective equipment wearing machine operator

6.4. Safety precautions to be taken after fogging

- Wash all spray equipments thoroughly and keep in the storeroom
- All workers must wash themselves thoroughly with soap and water to remove insecticide deposits on the skin
- All protective clothing should be washed daily after use
- Properly discard the empty insecticide containers. Do not store food or drinking water in these containers
- Make records on the daily use of insecticides
- Before consuming food, wash hands thoroughly with soap and water.
- Do not consume food while fogging
- Do not fog too close to an object (<2m)
- Do not use the fogging machine if it is not working properly, especially when leaking.

6.5. Authorities responsible for vector control and insecticide management in districts

RMO/MOIC - AFU and Entomologist are the responsible officers for distribution and maintenance of a three months stock of insecticides in their respective districts for smooth functioning of vector control activities in the district (Format annexed IX).



07

NOVEL METHODS
FOR DENGUE VECTOR
CONTROL

*Ae.aegypti and Ae. albopictus their potential for
integration with other chemical or biological
vector control interventions*

NOVEL METHODS FOR DENGUE VECTOR CONTROL

Novel vector control methods improve to disrupt different stages of the mosquito life cycle.

7.1. Egg stage

Lethal ovitraps

The ovitrap (oviposition trap) was first recommended by WHO for surveillance of *Aedes* vectors, then modified to render it lethal to adults or larvae of *Ae. aegypti* (Fig 7.1). On principle, ovitraps could kill adult mosquitoes if the ovistrip was treated with insecticide or destroy progeny by using fine nylon netting for trapping the larvae. In Singapore, lethal ovitraps were used to eradicate *Aedes* vectors from Singapore International Airport and the autocidal ovitrap was designed and developed for the control of *Aedes* vectors in urban areas with a high density of *Aedes* and a high incidence of dengue haemorrhagic fever. In Brazil, lethal ovitraps with deltamethrin-treated ovistrips killed 89% of *Ae. aegypti* adults and produced more than 99% larval mortality during one-month field trials. The advantages of lethal ovitraps for controlling *Aedes* vectors include their simplicity, their specificity for and effectiveness against container breeders like *Ae. aegypti* and their potential for integration with other chemical or biological vector control interventions.



Fig.7.1. Lethal oviposition traps

7.2. Larval stage

7.2.1 Predatory copepods

Mesocyclops spp. (Crustacea; Eudecapoda) (Fig 7.2) have been studied for their potential to control mosquito larvae. Two species in particular, *M. thermocycloides* and *M. aspericornis*, have proven effective against dengue vectors. In Vietnam, a large-scale vector control programme using copepods and clean-up campaigns successfully controlled dengue transmission for a number of years.

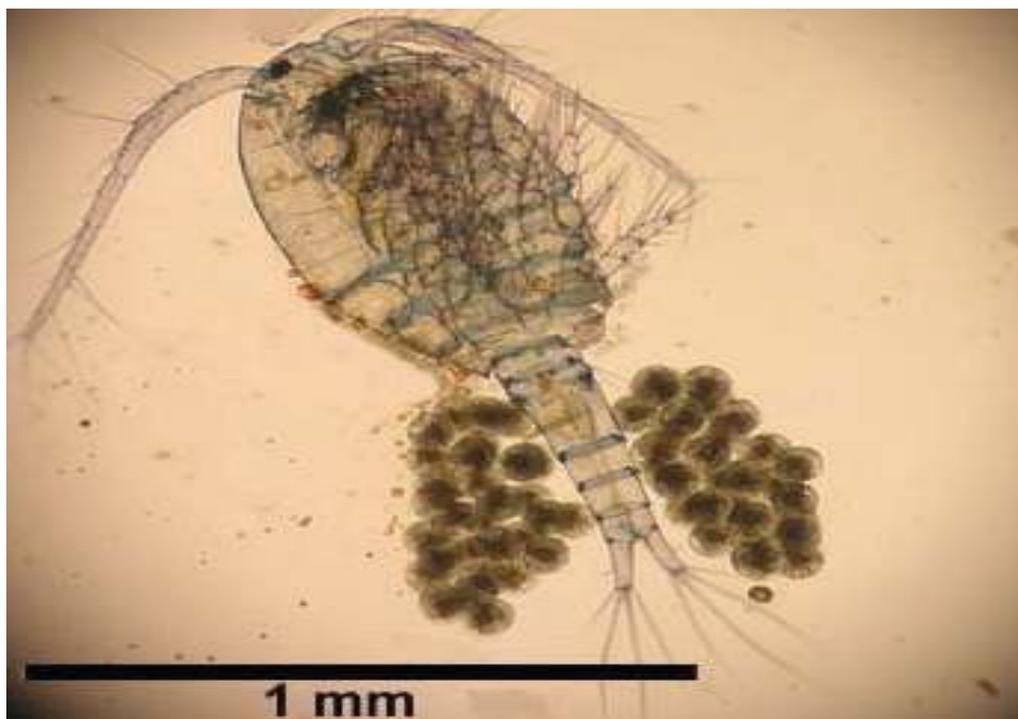


Fig 7.2. *Mesocyclops*

Copepods can survive up to 6 months in containers, however, they are often lost when water is removed, containers are cleaned or (copepod) food is limited. Thus, reintroduction of *Mesocyclops* is necessary for sustainable use of this species for dengue vector control.

7.2.2 Mosquito densovirus

Densovirus or densovirus (DNVs) are viruses in the genus *Brevitendovirus* of the family *Parvoviridae*. Five strains of *Aedes* densovirus have been identified to date. These viruses are either lethal to the treated populations, or it shortens the lifespan of adult mosquito. Current experiments show that the efficiency of densovirus in vector control could vary greatly on both viral strains and geographic origins of the mosquito vectors.

7.3. Adult stage

7.3.1. Irradiated, or Genetically Modified(GM) Adult mosquito releasing technique

Scientists have proposed two distinct strategies involving the release of irradiated, or GM insects: population suppression and population replacement. Population suppression; is a method in which insects are engineered to ensure that when they mate with wild individuals no viable offspring are produced. This is achieved by creating GM insects carrying a lethal gene and when they mate with the wild insects, the lethal gene is passed to the offspring causing them to die. If enough of the GM males were to be released to inundate the wild females this would result in the elimination of the insect population from the area. Most suppression strategies are self-limiting because the lethal genes are designed to kill successive generations, eventually removing all the GM individuals from the wild.

7.3.1.1 Suppression (males releases)

- Irradiation
- Transgenic mosquitoes
- *Wolbachia*-carrying mosquitoes

7.3.1.2 Replacement (males & females releases)

- *Wolbachia*-carrying mosquitoes

Irradiation

Sterile insect technology (SIT)

This method is introduced by Edward F Knipling. In Sterile Insect Technique (SIT), laboratory-reared male insects are sterilized by radiation and released over an area in large numbers. These sterilized males compete with fertile wild males to mate with wild females in a form of area-wide birth-control. If a female mates with a sterile male then it will have no offspring, thus reducing the next generation's population. Repeated release of insects can diminish small populations, though it could be impossible to eradicate it and is not efficient against dense insect populations.

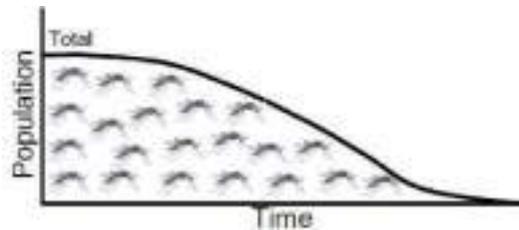


Fig.7.3. Eradication of insect population after repeated release of insects

Transgenic mosquitoes

Genetic Modification of Insects

Genetically modified (GM) insects are produced by inserting new genes into their DNA. Many genes that can alter the behaviour and biology of insects have been identified. When these genes are inserted into an insect's genome they are called transgenes and the insect is described as transgenic or genetically modified. By injecting DNA containing the desired genes into the eggs of insects, genetically modified strains can be created (Fig. 7.3).

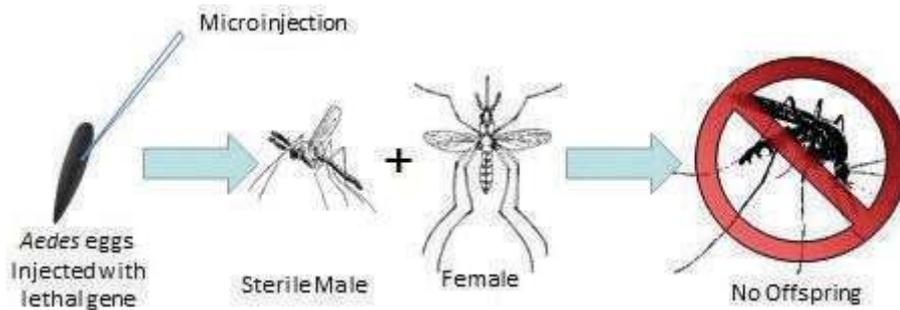


Fig. 7.4. Genetically sterile mosquito technique

source: WHO Dengue vector management workshop 11-15 March 2013

GM *Aedes aegypti* (strain OX513A) was developed in UK and evaluated at the Institute for Medical Research Malaysia (IMR) in 2006, both under laboratory and semi-field conditions, and Institute Pasteur in Paris, France. According to these studies, so far, the results are very encouraging.

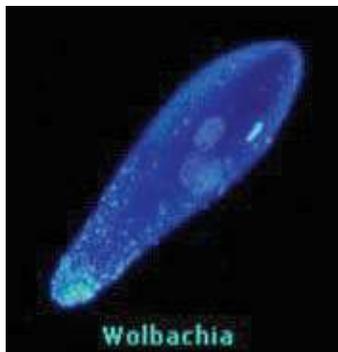
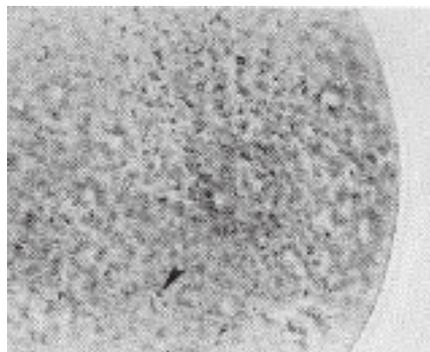
Release of insects carrying dominant lethal mutations (RIDL) technology

Release of insects carrying dominant lethal mutations (RIDL) are designed to reduce or eliminate mosquito populations, especially those that are infected with dengue viruses. A method using recombinant DNA technology to create genetically modified insects called RIDL ("release of insects carrying a dominant lethal") is under development by a company called Oxford Insect Technologies (Oxitec), UK. The method works by introducing a repressible "dominant lethal" gene into the insects. This gene kills the insects but it can be repressed by an external additive (tetracycline), which allows the insects to be reared in manufacturing facilities. This external additive is commonly administered orally, and so can be an additive to the insect food.

The RIDL males are released in large numbers into the affected region. The released males are not sterile, but any female offspring their mates produce will have the dominant lethal gene expressed and so will have a high probability to die.

Wolbachia*-carrying mosquitoes**Wolbachia* for dengue vector control**

Wolbachia are bacteria (Fig 7.5) that live within reproductive tissues of the insect hosts, manipulate the reproduction in the hosts and passed from one generation to the next through the insect's eggs. This bacteria is symbiotic rather than parasitic and present in up to 60% of all the different species of insects including some human biting mosquitoes, but, not the major mosquito species involved in the transmission of diseases such as dengue. Scientists are now engaged in studying *Wolbachia* to see its potential as a new tool for the control of dengue vectors.

*Wolbachia* bacteriumInsect egg containing *Wolbachia***Fig. 7.5. *Wolbachia* bacterium and an insect egg containing *Wolbachia***

source: WHO Dengue vector management workshop 11-15 March 2013

The bacterial obligatory endosymbionts are passed vertically in the cytoplasm of the eggs of their hosts. It causes male killing and sterility in males and also induces parthenogenesis (unfertilized eggs produce offspring). There is cytoplasmic incompatibility (conflict between cytoplasmic and nuclear components) that impacts on the offspring as follows:

- a) When male mosquitoes with *Wolbachia* mate with female wild mosquitoes that don't have *Wolbachia*, those females will lay eggs but they won't hatch. -Population suppression
- b) When male mosquitoes with *Wolbachia* mate with females that are already having *Wolbachia* all offspring will have *Wolbachia*. – Population replacement
- c) When female mosquitoes with *Wolbachia* mate with males without *Wolbachia* all her offspring will have *Wolbachia* (Fig.7.7) - Population replacement

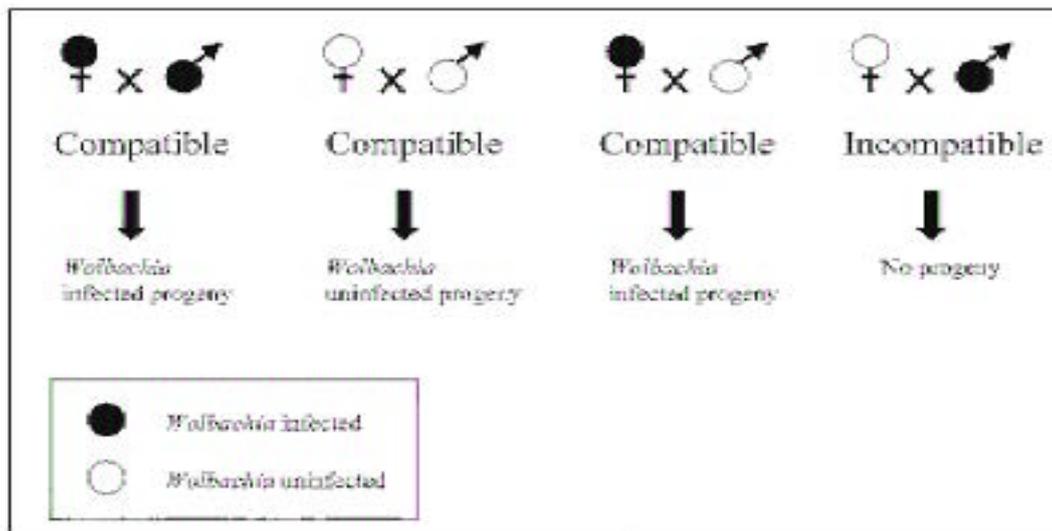


Fig. 7.6. Cytoplasmic Incompatibility and vertical transmission
 source: WHO Dengue vector management workshop 11-15 March 2013

Releasing a limited number of mosquitoes with *Wolbachia* to breed with female wild type mosquitoes, over a small number of generations, will result in all the mosquitoes having *Wolbachia* (Fig. 7.7).

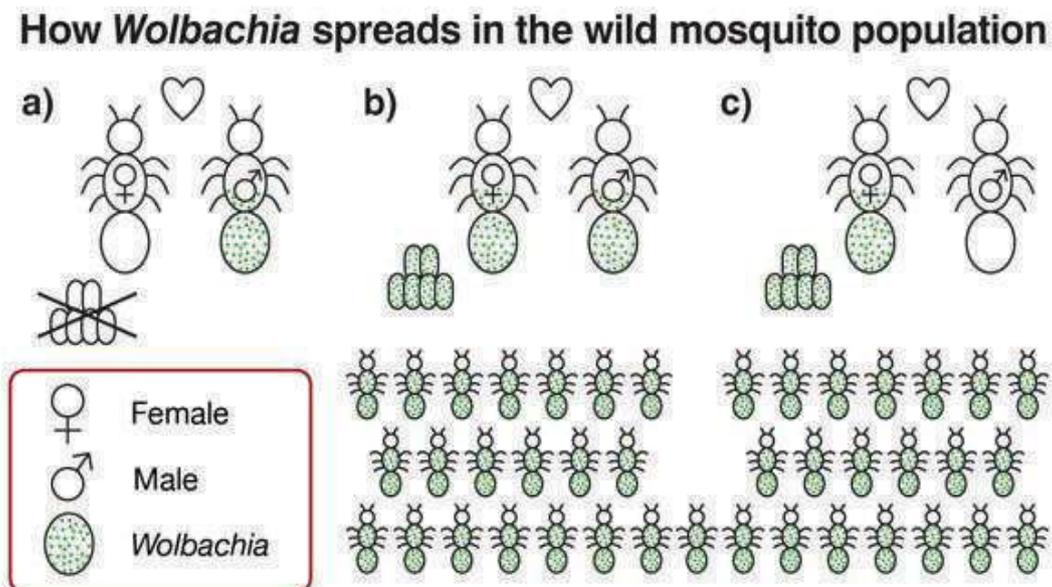


Fig. 7.7. Spread of *Wolbachia* in the wild mosquito population
 source: WHO Dengue vector management workshop 11-15 March 2013

7.3.3. Insecticide-treated materials and insecticide treated bed nets

Efficacy of insecticide-treated materials (ITMs) and insecticide-treated bednets (ITNs) (Fig. 7.8) in controlling diurnally-active *Ae. aegypti* is now being evaluated. In the case of long-lasting insecticide-treated nettings (LLIN), the netting is loaded with appropriate dosage of insecticide during manufacture to avoid the need for re-impregnation. Presumably, during the frequent visits of vector mosquitoes to houses for host-seeking, it contacts with the ITM, thereby, reduces the life expectancy of the mosquito so that the mosquito would not live long enough to transmit dengue. Presence of insecticide in treated curtains reduce entry into houses by repelling incoming mosquitoes or, the ITNs function in the as 'residually-treated resting surfaces' thus, result in the death of the mosquitoes resting on the curtain/net.



Fig. 7.8. Insecticide treated material and insecticide treated bed net

Low-level *Ae. aegypti* biting was reduced to zero after introduction of ITNs in a village in the Democratic Republic of the Congo. A cluster-randomized trial in Latin America has demonstrated that insecticide-treated window curtains and/or insecticide-treated domestic-water container covers can reduce dengue vector densities to low levels. Results of a pilot study in Haiti indicated that bednets reduced peridomestic dengue vector breeding and may have helped to reduce sero-conversion rates over 12 months.

List of Annexures

- I Daily Dengue Entomological Survey Form-
Details of positive /potential premises and
containers
- II Breeding places -check list
- III Daily Dengue Entomological Survey Summary
Report
- IV Dengue Entomological Surveillance Report
(Final Summary)
- V Entomological survey format- Institutions/
construction sites
- VI Ovitrap Surveillance Format
- VII Technical specifications for larvivorous fish tanks
(Wild Guppy)
- VIII Guidelines for susceptibility test of insecticide on
dengue vector adult mosquitoes and larvae
- IX Quarterly Stock Return of Insecticide

ANNEXURES

ANNEXURE - I

Daily entomological survey - details of positive / potential premises and Containers

MOH area :
 PHI area :
 GN Division :

Locality :
 Date of inv :
 Number c :

S · N o	Name and address of the house holder/public site	Type of premises	1			2			3			4			5			6			7			8			
			po.	wet	dry																						
1			Id	od																							
2			Id	od																							
3			Id	od																							
4			Id	od																							
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31			Id	od																							
32			Id	od																							
33			Id	od																							
34			Id	od																							
	Total																										

Observations:
 Name of the EO :

Signature of the EO : Date :
 ** Refer to the sheet containing the details
 G- Houses H: Commercial Sites I: Industrial Sites

ANNEXURES

ANNEXURE -II

Breeding Places - check list

1. Water storage barrels

- a. water storage metal barrels(indoor/outdoor)
- b. water storage plastic barrels(indoor/outdoor)

2. Water storage cement tanks

- a. water storage cement tanks (indoor/outdoor)

3. Water storage other

- a. plastic cans(indoor/outdoor)
- b. clay/plastic pots(indoor/outdoor)
- c. water storage plastic tanks(indoor/outdoor)
- d. other water collecting utensils(indoor/outdoor)

4. concrete slabs

- a. half constructed buildings(out door)
- b.....

5. Roof gutters

6. Tyres

Re usable tyres

7. Ornamental and horticulture items

- a. Flower pots
- b. Flower pots with hardened soil
- c. Flower vases
- d. Tanks without fish
- e. bird baths

8. Natural

- a. Leaf axils
- b. Tree holes
- c. Bamboo stumps

9. Ponds

ponds without larvivorous fish

10. Wells

shallow cemented well

11. Tube wells

non use/functional or motor connected tube wells

12. A/C refrigerators

A/C with water collecting tray

refrigerator with water collecting tray

13. Temporary removed items

- a. temporary removed wheelbarrows
- b. temporary removed metalware
- c. temporary removed glassware
- d. temporary removed plasticware
- d. temporary removed other

14 Covering items

- a. polythene
- b. other

15. Discarded (degradable)

16. Discarded (non degradable)

17. Discarded (Reuse)

18. pet feeding cups

19. Nonuse cisterns/commode

20. Other

- a. wet cement wet cement floors
- b. earth pipes
- c. non use squatting pans
- d. concrete holes
- e. barrel lid

ANNEXURE -III
Daily Summary Report



Dengue Entomological Survey
RMO/AFU/MOH..... Entomology team
Daily summary report



Locality:
No. of EO visited:.....
No. of labour visited:
Total no. of premises inspected:.....
No. of positive premises:.....
No. of positive containers:.....
No. of wet containers:
No. of dry containers :
Comments and suggestions:.....

Name of the report received officer :.....

Signature:.....

Name of the EO
.....
.....

Signature:.....
Signature:.....
Signature:.....



Ovitrap Surveillance Format

National Dengue Control Unit

Public Health Complex , 555/5, Elvitigala Mawatha, Narahenpita, Colombo 05
 Telephone: (0094) 011 2368416, 2368417 Fax: (0094) 011 2369893 Email: ndcu2010@yahoo.com



Climatic condition: (Weather) Bright sun shine Gloomy Raining heavily Drizzling

Date: / /
 MOH area:
 PHI area:
 GN Div:
 Nature of Locality: Residential Town
 Public site Other

S.No.	Name & address of the house holder/public site	Ovitrap place area		Positive /Negative (On the collection day.)		Indoor				Outdoor				Total
		Indoor	Outdoor			No of eggs	<i>Ae.aegypti</i>	<i>Ae.albopictus</i>	Mix	No of eggs	<i>Ae.aegypti</i>	<i>Ae.albopictus</i>	Mix	
				Od	Id									

A-*Aedes aegypti* B-*Aedes albopictus*
 G- Houses H: Commercial Sites I: 1.Government Inst.2. private Inst. J: Construction Sites K: Open Areas L: 1.schools2.other Education centres M:Religious N:Factories O: Other places

.....
 Name of the Entomological Assistant

ANNEXURE VII

Technical specifications for larvivorous fish tanks (Wild Guppy) Importance of rearing guppy for dengue control activities

A variety of fish species feed on mosquito larvae and thus is recommended for eliminating mosquitoes from some habitats. Larvivorous fish are those that feed on immature stages of mosquitoes. Guppy eats mosquito larvae and thus are recommended for eliminating mosquitoes from some containers such as those that are used to store potable water (water storage tanks, barrels), open fresh water wells (Batticaloa district, Kalmunai) in Sri Lanka (Fig. 1.1) As this is a biological control activity it is more environmental friendly and economically beneficial.

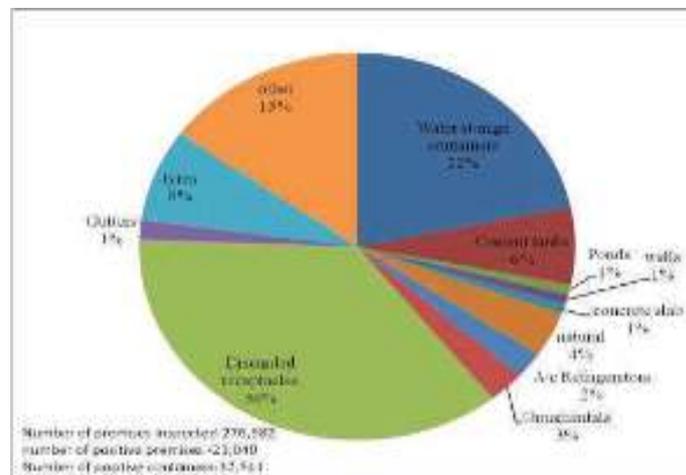


Figure. 1.1: Type of containers positive for *Aedes* larvae, 2015

Essential features of larvivorous fish,

1. small, hardy and capable of moving about easily in shallow waters among thick weeds where mosquitoes find suitable breeding grounds.
2. drought resistant and capable of flourishing in both deep and shallow waters as well as living in drinking water tanks and pools without contaminating the water.
3. have the ability to withstand rough handling and transportation for long distances.
4. be prolific breeders having shorter span of life cycle.
5. breed freely and successfully in confined waters.
6. be surface/column feeders and carnivorous in habitat
7. have a predilection for mosquito larvae even in the presence of food materials.
8. compatible with the existing fish life in that environment.

Advantages of use of larvivorous fish

- These fish are self-perpetuating after its establishment and continue to reduce mosquito larvae for long time.
- The cost of introducing larvivorous fish is relatively lower than that of chemical control.

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- Use of fish is an environment friendly method of mosquito control.
- Larvivorous fish such as *Poecilia* prefer shallow water where mosquito larvae also breed.

Some other important larvivorous fish species that can be used for dengue mosquito larval control are; Following description is focused on rearing *Poecilia reticulata* as a larvivorous fish species.

1. “Nalahandaya” (*Aplocheilus sp.*) – most efficient in clear water
2. Guppy (*Poecilia reticulata*) – can be used successfully even in organically polluted water
3. Juvenile stages of Tilapia (*Oreochromis mossambicus/ Oreochromis niloticus*) – hardy fish
4. *Rasbora daniconius* (Dandi)

Following description is focused on rearing *Poecilia reticulata* as a larvivorous fish species.

Guppy (*Poecilia reticulata*)

Topics mentioned below are discussed under *Poecilia reticulata*.

- § Habitat, size and longevity
- § Breeding Habitat
- § Breeding Season
- § Larvivorous Efficiency
- § Fish Hatchery
- § Transportation of fish
- § Collection of fish
- § Precaution during Transportation
- § Release of fish
- § Indications to use fish
- § Monitoring

1.1 Habitat, size and longevity

It is a very hardy fish and survives in all types of water bodies. It tolerates high degree of pollution with organic matter. The temperature range suitable for breeding is from 24°C to 34°C. It can survive in water with pH ranging from 6.5 to 9 (However, it cannot survive in cold water often below 10°C) and stock may need replenishment if the temperature falls below 10°C. The Guppy lives for 4 + 1 years. The male is 3 cm long, whereas the female is up to 6 cm in length.



Figure 1.2: Male Guppy
Length: 3cm

Figure 1.3 : Female Guppy
Length: 6cm

1.2 Breeding Habitat

The guppy takes about 90 days to mature. Each ovary contains 100 to 160 eggs. The female gives birth to young ones in broods of 5 to 7 at a time. About 50 to 200 young ones are released by the female every four weeks.

1.3 Breeding season

Reported to breed throughout the year at about four weeks interval after maturity. However breeding season will depend on climatic conditions. In warmer climate it may breed from April to November.

1.4 Larvivorous efficiency

The larvivorous efficiency of *Poecilia* is due to following characters:

- § A single fish eats about 80 to 100 mosquito larvae in 24 hours.
- § It is a surface feeder.
- § Tolerates handling and transportation very well.
- § Does not require specialized equipment for transportation.
- § Survives and reproduces when introduced into new water bodies. Once well established, it can be found in the habitat even after many years.

(Note: It is highly carnivorous and parents or older brood may eat up their own young ones. Therefore, a fair amount of weeds is required in the water so that young ones can hide and survive.)

1.5 Fish hatchery

In order to have continuous supply of fish, it is necessary to establish a hatchery where the fish may survive and multiply before contemplating their use in the local waters for the control of mosquito breeding.

The hatchery for larvivorous fish can be established in:

- a Natural water body
- a special hatchery

1.5.1 The Natural water body

Criteria for selecting a water body for a fish hatchery are:

- § It should be a permanent water body.
- § Depth of water should be at least 1.5m or more.
- § Water should be confined and without big natural outlet.

ANNEXURES

- § The minimum size of water body should be at least 5 m x 4 m. The water body of 10 mx 5 m can support 50000 fish.
- § It should be free from other carnivorous fish.
- § Water should not be contaminated by chemical or other harmful substances.
- § Easily accessible for daily or periodic inspection and for collection of fish.
- § De-weeding in ponds and shallow water bodies and cleaning of margins should be carried out periodically.

1.5.2 Special hatchery

Following points may be kept in view, while constructing the special hatcheries for the rapid reproduction of the fish.

- § There should be a constant supply of fresh dechlorinated water so that the required level of water in the tank does not drop.
- § Submerged vegetation such as hydrilla, vallisneria should be available in the tanks.
- § Salinity of water should not exceed 20 grams per litre. These fish may survive salinity up to 52 gms. per litre. But it cannot reproduce at high salinity level.
- § Hatchery should not be subjected to strong water current and should be protected from heavy rains and floods.
- § Brick made tanks, lined with good quality of cement plaster, thickness of wall about 50cm.
- § The tank should be divided into two portions of equal size of 5 m x 4 m with central separator of 0.5 m thick.
- § Area, sufficiently big for construction of 3 tanks of 5mx4 m (one for laying young Ones, one for holding mature full grown fish and the other for stocking fish when cleaning one of the present tanks).
- § Depth of water in the hatchery should be 1.5 m.
- § Proper outlet at the bottom of tank should be provided.
- § Overflow outlet about 5cm below inlet protected with proper wire mesh to prevent escape of fish.
- § Floor of tank 0.5 m thick with slope from the partition towards sides.
- § Proper inlet at 1.25 m height.
- § Bottom of tank covered with uniform thickness of sand for about 10 cm.
- § The bottom should be seeded with organic matter about 2 kg/m².
- § The tank should be allowed to mature for 10-15 days.
- § Minimum 25% of water should be replaced once a week.
- § The fish should be transferred from the tank to avoid over population.
- § In case of scarcity of natural food, artificial food may be given.
- § Chlorination of water beyond the tolerance levels, or presence of insecticides can be lethal to the fish.

1.6 Collection of fish for transportation

- Fishes are collected with help of netting, which is fitted on a circular iron ring of 60 to 90 cm diameter with a wooden handle

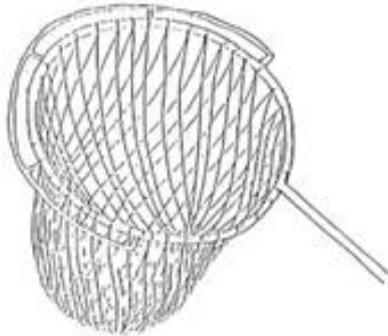


Figure 1.4 : Fish hand net

- § Sufficient quantity is collected by repeated dips.
- § Collection in bucket or drum till they are packed for transportation.

1.7 Transportation of fish

- § The fish are best transported in small containers of up to 40 litres, such as plastic buckets cans, or in strong plastic bags, half filled with water from the rearing pond.
- § Fish should be transported in water at ambient temperatures and should not be exposed to direct sunlight. The containers should have sufficient openings to allow flow of air .
If fish are transported in polythene bags,
- § Take polythene bag of 3 -5 litre capacity.
- § Fill it with 1.5 liter of water.
- § Introduce the fish in the bag till the total volume of water + fish is two litres.
- § Bubble the oxygen in bag from O₂ cylinder or from air pump.
- § Close the mouth of bag with a string leaving sufficient space at the top.
- § Put the bag in a thermo cool container and close the mouth of container.
- § The container can be transported for a period of 24 hours without further filling oxygen.
If the period of transport is more than 24 hours then arrange for change of water and oxygenate.

1.8 Precaution during Transportation

- § Fish do not tolerate sudden temperature changes.
- § Preferably the fish should not be given any food for 10-12 hours period prior to packing for transportation.

1.9 Release of fish

- § To release the fish in a water body, measure the perimeter of water body.
- § Release the fish at the rate of 5 to 10 fish per linear meter.
- § If the mosquito larval density is high more fish up to 20 can be released.

1.10 Indications to use fish

- § Fish should be preferably introduced in all unused wells in the rural and peri-urban areas before the high mosquito breeding season to maximize impact. However, the fish may be used in such wells even if the seeding has been delayed.
- § Fresh water bodies in rural areas such as stagnant ponds, slow moving streams quarry pits, large borrow pits, margins of ponds should be targeted apart from wells. Such places should be surveyed and numbered to facilitate subsequent monitoring of impact.
- § In open mosquito breeding sites or rice fields, the fishes need to be protected from pesticides applied to crops, when used in rice fields

1.11 Monitoring

- § Supervisors should check the fish hatcheries at least once a month during the high

transmission season.

- § At least 10% of the sites where fish have been introduced should be checked for:
 - Whether fish have been introduced or not
 - Whether the fish are surviving or not
 - Identification of possible reasons, in case the introduced fish are not surviving

Guppy production tanks

There are 3 types of small scale production tanks. These are:

1. Larval rearing tanks/Primary nursery
2. Grow out tanks/Secondary nursery
3. Brood stock rearing tanks

Example:

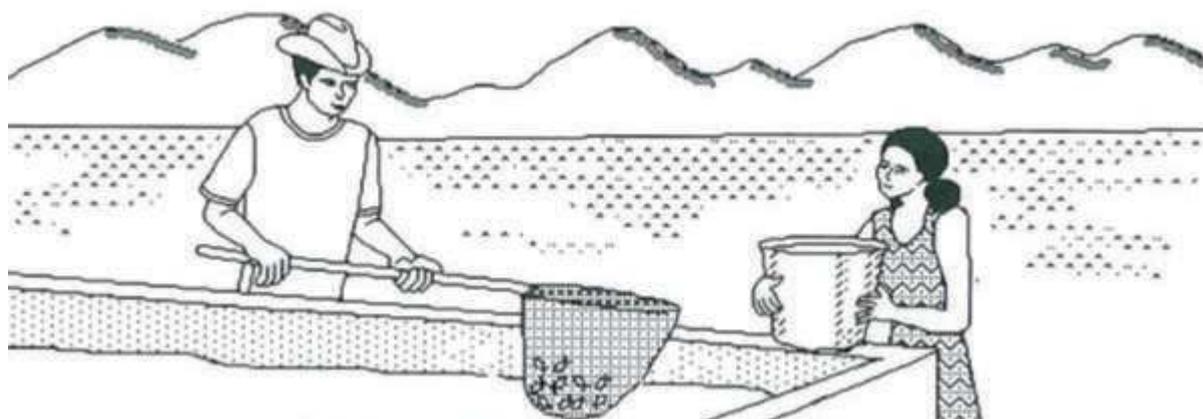


Figure 1.5: Collecting fry in a fine-mesh dip net

Example:

If a particular MOH area/District needs 2500 fingerlings per month, calculations are as follows.

Total no of fingerlings needed to be distributed to the district/MOH area = 2,500

Grow out tanks

- § Tank shape: circular
- § Material: concrete tanks

Tank No 02: Grow out tank

Number of fingerlings obtained from
larval rearing tank = X
Grow out stocking density = 15ft²
Grow out mortality = 10%
Grow out production = X * (90/100) = 2,500

$X \times 90/100 = 2,500$ $X = 2,778$

Total surface area of the tank = X/15
= 2,778/15
= 185ft²

Larval rearing tanks

Tank shape: circular/rectangular
Material: concrete tanks
Stocking density = 25ft²
Nursery mortality = 20%
Nursery production = Total production * 0.8
= 2,778
Nursery growing period = 1 month
Total production = 3,472
Therefore "One day old" guppy requirement (monthly) = 3,472 3475 for district/MOH

Total area for tank = 3,475/25
= 139 ft²

ANNEXURES

3. Brood stock tanks

$$\begin{aligned}
 &\text{Monthly production for a female} &&= 60 \\
 &\text{Breeding interval: 21days} && \\
 &\text{Female brood stock requirement} &&= 3,475/60 = Y \\
 &\text{Brood stock ratio} && \\
 &\quad \text{Female: male} && \\
 &\quad \quad 3:1 && \\
 &\quad Y/60 : (Y/60) * 1/3 && \\
 \\
 &\text{Total no of brood fish} &&= (3,475/60) + (3,475/60)*1/3 \\
 & &&= 58+20 \\
 & &&= 78 \\
 &\text{Stocking density} &&= 3\text{ft}^{-2} \\
 &\text{Total surface area of brood stock tank} &&= 78/3 \\
 & &&= 26\text{ft}^2
 \end{aligned}$$

Summary

Production of 2,500 of fingerling guppy per month,

Tank type	Stocking density	Surface area of the tank(ft ²)	Circular tank size (Tank diameter/ft)
1. Larval rearing tank	25ft ⁻²	139	6.7
2. Grow out tank	15ft ⁻²	185	7.7
3. Brood stock rearing tank	3ft ⁻²	26	2.9

(Source: NAQDA)

ANNEXURE VIII

Guidelines for susceptibility test of insecticide on dengue vector adult mosquitoes and larvae

Objective:

The purpose of the susceptibility test is to detect the presence of resistant individuals in an insect population early to preserve the effectiveness of available insecticides. If use of insecticide continues without knowing the presence of resistance, percentage of selected survivors will increase and the susceptibility of the population will decline to a point that the insecticide no longer provides an acceptable level of control. Therefore, the susceptibility monitoring will help to plan alternative control strategies to deal with the situation when the insecticide in question is no longer having the desired effect.

Source

National Dengue Control unit has purchased Insecticide impregnated papers, solutions and test kits, from the University Sains, Malaysia which prepared them on behalf of WHO.

This guideline prepared according to the Monitoring and managing insecticide resistance in Aedes mosquito populations interim guidance for entomologists (2106), Global plan for insecticide resistance management in malaria vectors, global malaria programme(2014) and vector resistance to pesticides, WHO/TRS/818, fifteenth report of the WHO expert committee on vector biology and control(1992).

1. The WHO susceptibility test for adult mosquitoes

Composition of standard diagnostic test kit

1. Twelve (12) plastic tubes (125 mm in length and 44 mm in diameter). Each tube fitted at one end with 16-mesh screen. The 12 tubes are,
 - a. Four (4) tubes marked with a red dot will be used as exposure tubes, i.e. for exposing mosquitoes to the insecticide impregnated papers.
 - b. Two(2) marked with a **yellow** dot for use as control tubes, for exposure of mosquitoes to the oil-treated control papers (i.e. without insecticide);
 - c. Six(6) marked with a **green** dot for use as holding tubes for pre-test sorting and post-exposure observation.
2. Six(6) slide units, each fitted with a screw-cap on both sides and a 15 mm filling hole.
3. Twelve (12) spring wire clips, 6 steel and 6 copper, to hold the paper in position against the walls of the tubes; the 6 steel clips are to be used with the green-dotted holding tubes and 6 copper clips are to be used with the 4 red-dotted exposure and the two-yellow-dotted control tubes.
4. Two (2) glass or plastic aspirator tubes of 12 mm internal diameter, together with 60 cm of tubing and mouthpieces.

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5. Fourty(40) sheets of clean paper (12 x 15 cm) for lining the holding tubes.
6. One (1) roll of self-adhesive plastic tape.
7. Instruction sheet, 20 copies of report forms.

1.2. Other materials

1. One mosquito cage	2. 10% sugar water solution
3. Live female mosquitoes (140 healthy specimens needed for testing)	4. Cotton wool, Rubber bands
5. Wet towel	6. Paper cups
7. Digital thermometer	8. Covering nets
9. Hygrometer	

1.3. Test procedures

1. Roll seven sheets of clean white papers (12 x 15 cm) separately to make them cylinder shape and insert each into seven holding tubes (one per tube) and secure them in position with a steel spring-wire clip. Then the tubes should be attached to slides.
2. Collect at least 140 female mosquitoes with the aspirator provided (Fig.1A). Damage resulting from careless handling of mosquitoes during collection may produce misleading high mortalities. Mosquitoes should be collected in groups of not more than 10 (Fig.1, B) and gently transferred to the holding tubes through the filling-hole in each side (Fig.1, C) to give 20 per tube. (Where possible collect this number from the same locality and monitored over time to examine the trends.)
3. Once the mosquitoes have been transferred, the slide unit has to be closed and the holding tubes set in an upright position for one hour. After one hour, replace any knocked-down, dead or damaged mosquitoes with healthy ones.
4. Seven additional exposure tubes have to be prepared in much the same way. Line each of the five (5) red-dotted exposure tubes with a sheet of insecticide-impregnated paper, while the 2 yellow dotted control exposure tubes should be lined with oil-impregnated papers; each has to be fastened into position with a copper spring-wire clip.
5. Attach the five exposure tubes to the vacant position on the slides, and with the slide unit open the mosquitoes need to be blown gently into the exposure tubes. (Fig. 1, D) Once all the mosquitoes are in the exposure tubes, the slide unit has to be closed and detach the exposure tubes and set them upright. Fill the two control tubes with mosquitoes in the same way.
6. Keep mosquitoes in the exposure and control tubes for one hour. Make sure that the tubes are

- set in an upright vertical position with the mesh-screen on top (Fig.1, E).
7. Transfer back the mosquitoes to the holding tubes at the end of the 1-hour exposure period, by reversing the procedure outlined in step 5. Set all the holding tubes upright, with the mesh-screen on top. Soak a pad of cotton-wool in 10% sugar solution and place on mesh-screen (Fig. 1, F).
 8. Maintain mosquitoes in the holding tubes for 24 hours (the recovery period). During this time, it is important to keep the holding tubes in a shady, sheltered place free from extremes of temperature (an insectary is ideal). If conditions are very hot and dry, a moist chamber may be prepared by suspending damp toweling in a container, temperature and humidity should be recorded during the recovery period. If necessary, the tubes should be protected from ants by placing them on a platform standing in a pan of water. Temperature and humidity should be recorded during the recovery period.
 9. At the end of recovery period (i.e. 24 hours post-exposure), count and record the number of dead mosquitoes as in table 1.

Table 1. Classification of adult mosquitoes in bioassays

Alive	Moribund*	Dead*
<ul style="list-style-type: none"> • Can stand on and fly in a coordinated manner 	<ul style="list-style-type: none"> • Cannot stand (e.g. has 1 or 2 legs) • Cannot fly in a coordinated manner • Lies on its back, moving legs and wings but unable to take off • Can stand and take off briefly, but falls down immediately 	<ul style="list-style-type: none"> • No sign of life; immobile; cannot stand

* Knocked down after 60 minutes or dead after 24 hours of exposure

10. If mosquito mortality in the control tubes exceeds 10%, correct the mortalities of all treated groups using Abbott's formula (below). Discard the test and repeat if the corrected mortality in the control tubes exceeds 10%.

$$\text{Corrected mortality (\%)} = \frac{(\% \text{ mortality with treated paper} - \% \text{ mortality with control})}{(100 - \% \text{ mortality with control})} \times 100$$

11. If supplementary tests (biochemical or molecular) are necessary after completing the susceptibility test, transfer each mosquito (dead or alive) to an individual, clearly labelled Eppendorf tube. Refrigerate and store the tubes until they can be processed for supplementary testing

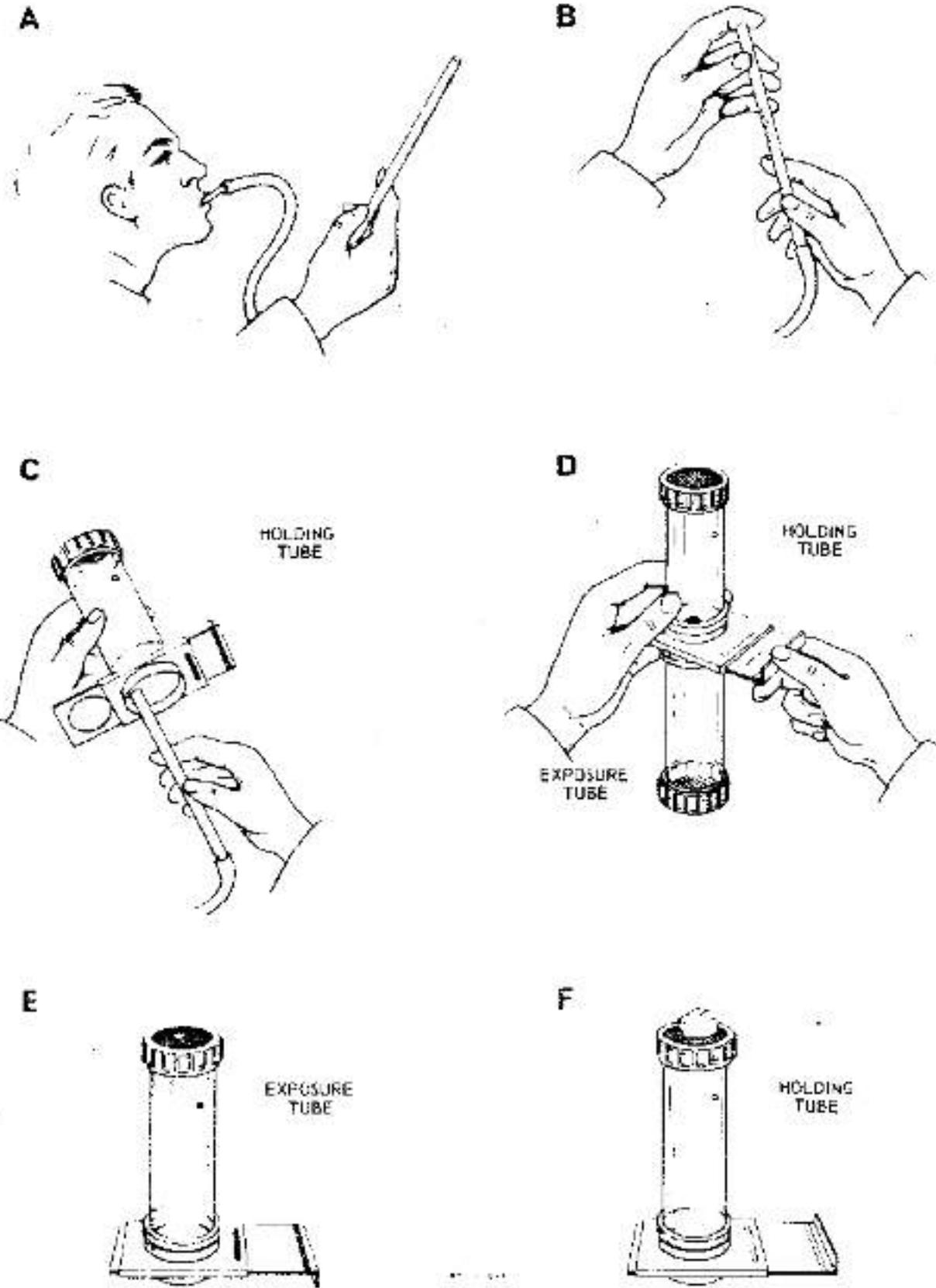


Fig.1 Method for determining the susceptibility or resistance of adult mosquitoes
 Source: WHO/VBC/81.806

When testing pyrethroids, timed observation of the rate of knock down (kd) should be made routinely after 10, 15, 20,30,40,50 and 60 minutes of exposure.

1.4. Calculation of mortality and knock-down rates and Interpretation of susceptibility test results

- Observed mortality = $\frac{\text{Total number of dead mosquitoes}}{\text{Total sample size}} \times 100$

If mosquito mortality in the control tubes exceeds 10%, correct the mortalities of all treated groups using Abbott's formula (below). Discard the test and repeat if the corrected mortality in the control tubes exceeds 10%.

$$\text{Corrected mortality (\%)} = \frac{(\% \text{ mortality with treated paper} - \% \text{ mortality with control})}{(100 - \% \text{ mortality with control})} \times 100$$

- Mortality between 98–100%: Susceptibility is indicated
- Mortality less than 98%: Resistance suggested. Further tests are needed to verify.
- Mortality between 90%–97% (corrected if necessary): Presence of resistant genes in the vector population must be confirmed. The confirmation of resistance may be obtained by performing additional bioassay tests with the same insecticide on the same population or on the progeny of any surviving mosquitoes (reared under insectary conditions) and/ or by conducting molecular assays for known resistance mechanisms. If at least two additional tests consistently show mortality below 98%, then resistance is confirmed.
- Mortality less than 90%: Confirmation of existence of resistant genes in the test population with additional bioassays may not be necessary, as long as a minimum of 100 mosquitoes were tested. However, further investigation of the mechanisms and distribution of resistance should be undertaken.

1.5. Insecticide impregnated papers and discriminating concentrations

Table 2 Discriminating concentrations and exposure time of insecticides used for *Aedes* mosquitoes

Insecticide class	Insecticide	Discriminating concentrations	Exposure period (hours)	Control paper
Pyrethroids	Cyfluthrin	0.15% ^b	1	Silicone oil
	Deltamethrin	0.03% ^a	1	Silicone oil
	Lambdacyhalothrin	0.03%	1	Silicone oil
	Permethrin	0.25%	1	Silicone oil
	Etofenprox	0.5% ^b	1	Silicone oil
	Alpha-cypermethrin	0.03% ^a	1	Silicone oil
Organophosphate	Fenitrothion	1%	1	olive oil
	Malathion	0.8%	1	olive oil
	Pirimiphos methyl	0.21% ^b	1	olive oil

^a Tentative ^b Determined for Anopheles mosquitoes, tentative for *Aedes*.

Source : (WHO/ZIKV/VC/16.1)

NOTE: Manufacture date and expiry date mention in the box; all papers have to be used before the expiry date.

1.6. Conditions and precautions in use.

◆ **Mosquito sampling and rearing**

Larval stages are easier to collect from the most productive breeding sites, and should be kept alive and taken to a local or centralized insectary facility for rearing. Usually the second or F2 generation is used, as enough number of larvae or adult mosquitoes are needed for the necessary tests. Only female mosquitoes should be used in the tests. It is recommended that susceptibility tests be performed on non-blood fed females of 3–5 days old.

◆ **Conditions**

The optimum conditions for phenotypic susceptibility tests are $27 \pm 2^\circ\text{C}$ temperature, $75 \pm 10\%$ relative humidity and low illumination that are usually maintained in an insectary. Where such infrastructure is not available, the tests should be done indoors in a building free from insecticidal contamination while maintaining optimum humidity and temperature using local procedures and avoiding extreme illumination and wind. Where possible, subsequent comparison test should be made under similar conditions of temperature and humidity

◆ **Multiple use of the impregnated papers**

The efficacy of impregnated papers declines with the number of uses and the number of mosquitoes tested. This is especially true of the pyrethroid-impregnated papers. The current recommendation is that no insecticide-impregnated paper should be used more than 6 times. Pyrethroid papers should not be used more than 5 times.

After the impregnated paper has been removed, the package should be resealed carefully with the plastic tape. The packages should be kept in a cool place.

2. Monitoring of susceptibility levels in larval mosquitoes to insecticides

The purpose of the susceptibility test is to detect the presence of resistant individuals in a mosquito larval population as soon as possible so that alternative control plans can be made in time to deal with the situation when the insecticides in question is no longer having the desired effect.

WHO standard larval susceptibility test kits use

2.1 Equipments:

- i. four 1-ml pipettes (red dot, yellow dot, black dot, white dot) for insecticides and 1 for ethanol
- ii. 5 rubber suction bulbs for above
- iii. 3 droppers with rubber suction bulbs (eye drops)
- iv. The following materials for use in making a strainer:
 - a. 2 wire loops
 - b. 1 piece of nylon netting (30 cm²)
 - c. 1 tube of cement (UHU)
- v. 1 polyethylene bottle, 50 ml

- vi. 1 instruction sheet
- vii. 20 report forms
- viii. 1 label

2.2 Other requirements

- 250 ml Beakers or disposable plastic cups
- Larval trays
- Measuring cylinder
- Thermometer

2.3 Insecticide solutions (in 50 ml bottle)

- i. Temephos 156.25 mg/l, 31.25 mg/l, 6.25 mg/l, 1.25 mg/l

2.4 Test procedures

Instructions to make a strainer.

Two pieces of netting be cut and cemented to opposite side of the large end of the wire loop. More cement should then be applied around the outside of the loops to join the 2 pieces of netting. When dry, the netting may be trimmed with scissors. The kit contains sufficient netting for replacement purposes.

- a) For a complete test with one insecticide, sufficient larvae should be collected from the field. 300 individuals from the same species should be selected; they should be in their 3rd or early 4th instars and should be retained in the water they were collected until selection for testing.
- b) Batches of 20 larvae should be transferred by means of a strainer to beakers each containing 200 ml of water. The average temperature of the water should be 25°C; it must not be below 20°C or above 30°C.
- c) Prepare the test concentration by pipetting 1 ml of the standard insecticide solution under the surface of the beakers and stirring vigorously for 30 sec. with the pipette, in preparing a series of concentrations, the most dilute should be prepared first. Four or more replicates are set up for each concentration and equal numbers of controls are set up simultaneously with water in which 1ml ethanol is added.
- d) Within 15-30 minutes of the preparation of the test concentration, larvae should be added.
- e) After 24 hr. exposure, larval mortality should be recorded. For slow acting insecticides, 48 hr regarding may be required. Moribund larvae are counted and added to dead larvae for calculating percentage mortality. Dead larvae are those that cannot be induced to move when they are probed with a needle in the siphon or the cervical region. Moribund larvae are those incapable of rising to the surface or not showing the characteristic of diving reaction when the water is disturbed.

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- f) Discard the larvae that have pupated during the test. If more than 10% of the control larvae pupated in the course of the experiment, the test should be discarded and repeated.
- g) If the control mortality is between 5%-20% the average observed mortality should be corrected by Abbott's formula

$$\text{Corrected mortality (\%)} = \frac{\% \text{ mortality in test} - \% \text{ mortality in control}}{100 - \% \text{ mortality in control}} \times 100$$

Table3. Tentative diagnostic dosage for larval mosquito

Insecticide	Diagnostic dosage (mg/litre)	Mosquito species
Temephos	0.012	<i>Aedes aegypti</i>

Source:WHO_TRS_818.pdf

2.5. General remarks

- a) The accuracy of the concentrations provided will be affected if the alcohol is allowed to evaporate from the standard solutions. The bottles should therefore be tightly stoppered after use. The contents should no longer be used when they have decreased below 5ml.
- b) The beakers should be carefully cleaned after use to remove traces of insecticides. They should be thoroughly rinsed, scrubbed with detergent and water. Pipettes should be thoroughly cleaned with acetone or alcohol.
- c) Distilled water, rain water, tap water, well or stream water can be used but it should be as free as possible from chlorine or organic contaminants.

3. Frequency and reporting system

3.1 Frequency of adult and larval susceptibility test

Insecticide resistance monitoring could be conducted across a network of sentinel site, with these sites selected so as to represent the range of ecological zones and dengue transmission intensities that occur in the country.

Testing could be repeated at least once in 3 months. (If observed any indication of resistance, frequency should be increased).

3.2 Reporting results of susceptibility testing.

The results of susceptibility testing should be submitted to the National Dengue Control Unit once in 6 months by Regional Malarial Officers/Entomologists to update the national level data base.

4. Cage Bioassays

4.1. Objective:

Caged bioassays are used to evaluate the efficacy of space spraying (fogging) operations.

- Space spray is transient and only mosquitoes flying at the time of the application are affected.
- The efficacy of space spray is greatly influenced by environmental and operational factors.

4.2. Equipments

1. Bio assay cages
2. Mosquito cages
3. Aspirator tubes
4. Paper cups

4.3. Test Procedure

- Mosquitoes for bioassay should be obtained from eggs collected in the field or field collected larvae. Larvae hatched from these eggs should be reared without crowding and with an adequate food supply to ensure uniformity of size.
- Shortly before the treatment, 20-25 females, 24-36 hours old and fed on a 10% sugar solution, should be transferred to each bioassay cage.
- It is recommended 100 mosquitoes to be tested, with 4-5 replicates. Two to three days old female mosquitoes should be used for bioassays.
- The cages should be transported to and from the field in mosquito cage protected from extreme heat.
- At least five houses in the treatment area should be used.
- As a minimum for evaluation of space sprays applied outdoors, cages should be located at each house at the following sites:
For indoors at an exposed site and in a sheltered site
For outdoor in front and at the rear of the houses
(Within 10m -50m different distances)
- The same number of cages should be exposed at similar sites in the untreated area.
- Thirty minutes after exposure, the cages should be removed and returned to the laboratory in their transport cages.

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- Then the mosquitoes are transferred to clearly marked clean holding cages, and are provided with sugar solution and maintained at ambient temperature.
- Mortality in all cages should be determined 24 hours after the spray application.
- Temperature and relative humidity should be recorded during the test (both the exposure and the holding periods). Ideal temperature for testing is $25 \pm 2^{\circ}\text{C}$, and should not higher than 30°C . Relative humidity should be 70-80%.
- Chambers should be hung vertically.
- Cages should be washed thoroughly before use in another experiment.

Interpretation of susceptibility test results

- Observed mortality = $\frac{\text{Number of dead mosquitoes}}{\text{Number of test mosquitoes}} \times 100$
- If control mortality is between 5-20%, the average observed mortality should be corrected by Abbott's formula:

$$\text{Corrected mortality (\%)} = \frac{\% \text{ mortality in test} - \% \text{ mortality in control}}{100 - \% \text{ mortality in control}} \times 100$$

4.4. Frequency of cage bioassay test

Test could be repeated at least once in 6 months (or accordingly MOH request, for clarification of usage of space spraying)

4.5. Reporting results of cage bioassays test.

The results of cage bio assay test should be submitted to the National Dengue Control Unit once in 6 months by Regional Malarial Officers/Entomologists update the national level data base.

QUARTELY STOCK RETURN OF INSECTICIDE

RMO/RFU/ENTOMOLOGIST:-

R.D.H.S :-

Month (Duration) :-

Year :-

Insecticide	Larvicides				Adulticides			
	Abate 1% SG (kg)	Abate 50 EC(L)	liquid Bti(L)	Technical Malathion (L)	Pesguard(L)	Delatacide(L)(L)
stocks on hand at the beginning of the month								
Stocks received during the Month								
Amount Stocks distributed during the month								
Balance at the end of month								
Three month average Distribution (current and past 2 months)								
Amount Required for the next 3 months								
Amount requested from NDCU								

.....
Signature of the RMO/MOIC-AFU/Entomologist

Date :

.....
Signature of the RDHS

Date :

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Quarterly Stock Return of Insecticide



BIBLIOGRAPHY

01. Anti Malaria Campaign,2009, Guidelines on the use of Pesguard FG 161 for thermal fogging/ULV application to control disease vector mosquitoes/flies, dated 22nd June 2009.
- 02 Anti Malaria Campaign, 2010, Guidelines for the application of Bactivec®(Cuban Bacillus thurengiensis) in dengue vector breeding sites.
03. Hapugoda Menaka D, De Silva N.R, Rajamanthri M.D.S, And Abeywickrema W. dengue vector surveillance in localities with reported dengue cases in Kurunegala and Gampaha district, Sri Lanka Association for the Advancement of science, Proceedings of the 57th Annual session of the 2001:5
04. Hapugoda M. D, Gunasekera, M.B., De Silva, N.R, Gunasena, S., Preethimala L.D., Dyanath M.Y.D and Abeywickrema,W., Detection of dengue virus in Aedes albopictus mosquitoes by reversed transcription polymerase chain reaction- lipid hybridization (RT-PCR-LH)base assay. The bulletin of the Sri Lanka college of microbiologists , 2003 , vol.1,issue 1:30-31
05. Louis VR ,Quiñonez C.A.M, Kusumawathie PHD,Janaki S, TozanY, Wijayamuni R, Smith AW, Tissera HA Characteristics of and factors associated with dengue vector breeding sites in the City of Colombo, Sri Lanka. Pathogens and Global Health, Volume 110, 2016.
06. Queensland Dengue Management Plan (DMP) 2010-2015, 2011.
07. Strategic Plan for Prevention and Control of Dengue Fever/Dengue Haemorrhagic Fever in Sri Lanka, 2011-2015.
08. World Health Organization (1957) Technical report series No.125, *insecticides seventh report of the expert committee.*, 7.http://apps.who.int/iris/bitstream/10665/40380/1/WHO_TRS_125.pdf
09. World Health Organization (1981) *Instructions for determining the susceptibility or resistance of adult mosquito to organochlorine, Organophosphate and carbamate insecticides establishment of base line* .WHO/VBC/81.805
10. World Health Organization (1986) , Arthropodborne and rodent borene viral diseases report of a WHO scientific group, Technical report series, 719, Geneevapg.32.
11. World Health Organization (1992) *Vector resistance to pesticides.* WHO/TRS/818. Fifteen Report of the WHO Expert committee on vector biology and control, [http:// whqlibdoc.who.int/trs/WHO_TRS_818.pdf](http://whqlibdoc.who.int/trs/WHO_TRS_818.pdf).

12. World Health Organization (1995) Guidelines for dengue surveillance and mosquito control. Western Pacific Education in action series no. 8, WHO regional office for the Western Pacific, Manila pg. 5-15
13. World Health Organization (2005) *Guidelines for laboratory and field testing of mosquito larvicides*, pp1-36. World Health Organization (Geneva).
14. World Health Organization (2009) *Dengue Guidelines for diagnosis treatment, prevention and control*. World Health Organization (WHO) and the special programme for Research and Training Diseases. Geneva, Switzerland (TDR). 1-160. WHO/HTM/NTD/DEN/2009.1
15. World Health Organization (2011) *Comprehensive guideline for prevention and control of Dengue and Dengue Haemorrhagic fever, revised and expanded edition*. p60-65, WHO (India). SEARO Technical publication series No.60.
16. World Health Organization (2012 a) fact sheet, WHO recommended insecticides for space spraying against mosquitoes, <http://www.who.int/whopes/recommendations/wgm/en/>
17. World Health Organization (2012 b) *global plan for insecticide resistance management in malaria vectors who global malaria programme*. p1-14, WHO (Geneva).
18. World Health Organization (2013a) fact sheet, WHOPES-recommended compounds and formulations for control of mosquito larvae, <http://www.who.int/whopes/recommendations/en/>
19. World Health Organization (2013 b). *Test procedures for insecticide resistance monitoring in malaria vector mosquitoes*. pp1-30, World Health Organization (Geneva). http://apps.who.int/iris/bitstream/10665/80139/1/9789241505154_eng.pdf.
20. World Health Organization (2016), Monitoring and managing insecticide resistance in *Aedes* mosquito populations interim guidance for entomologists, WHO/ZIKV/VC/16.1.
21. Yapa Bandara A.M.G.M and Abeykoon A.R, *Aedes aegypti* and *Aedes albopictus* breeding sites in the Matale municipal Council area, Sri Lanka Association for the Advancement of science, Proceedings of the 58th Annual session of the 2002: 20