BIOECOLOGY AND CHEMICAL CONTROL OF AEDES MOSQUITOES IN DHAKA CITY

MD. GOLAM SHAROWER

DEPARTMENT OF ENTOMOLOGY
SHER-E-BANGLA AGRICULTURAL UNIVERSITY
SHER-E-BANGLA NAGAR, DHAKA-1207

JUNE, 2016

BIOECOLOGY AND CHEMICAL CONTROL OF AEDES MOSQUITOES IN DHAKA CITY

BY

MD. GOLAM SHAROWER

Registration No. 13-05793

A thesis
Submitted to the faculty of Agriculture,
Sher-e-Bangla Agricultural University, Dhaka,
In partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY
IN
ENTOMOLOGY
SEMESTER: JANUARY-JUNE, 2016

Approved by

_____

Prof. Dr. Md. Abdul Latif
Supervisor and Chairman
Advisory Committee

_____

Prof. Dr. Md. Razzab Ali
Member
Advisory Committee

_____

Prof. Dr. Md. Mizanur Rahman
Member
Advisory Committee

_____

Prof. Dr. M. Salahuddin M. Chowdhury
Member
Advisory Committee

Dr. Md. Abdul Latif
Professor
Department of Entomology
Sher-e-Bangla Agricultural University
Sher-e-Bangla Nagar, Dhaka-1207
Bangladesh
Mob: **+8801552-373406**
E-mail: mdalatif15@yahoo.com

CERTIFICATE

This is to certify that the thesis entitled ***"BIOECOLOGY AND CHEMICAL CONTROL OF AEDES MOSQUITOES IN DHAKA CITY"*** *submitted to the faculty of Agriculture, Sher-e-Bangla Agricultural University (SAU), Dhaka in partial fulfillment of the requirements for the degree of* ***DOCTOR OF PHILOSOPHY IN ENTOMOLOGY,*** *embodies the result of a piece of bona fide research work carried by Md. Golam Sharower, Registration No.* **13-05793** *under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.*

I further certify that such help or source of information availed of during the course of this investigation has dully been acknowledged.

Date: June, 2016
Place: Dhaka, Bangladesh

Professor Dr. Md. Abdul Latif
Supervisor and Chairman
Advisory Committee

ABBREVIATIONS USED IN THIS TEXT

Abbreviation	Full Word	Abbreviation	Full Word
AChE	Acetylcholinesterase	ICDDRB	International Centre for Diarrhoeal Disease Research, Bangladesh
ai	Active ingredient	IR	Infrared
BI	Breteau Index	km	Kilometer
cc	Cubic centimeter (liquid materials)	L	Liter
CI	Container Index	LC_{50}	Median Lethal Conc.
cm	Centimeter	LD_{50}	Median Lethal Dose
cm	Centimeter	LSD	Least significant difference
cm^2	Square centimeter	m	Meter
cm3	Cubic Centimeter (Solid materials)	m^2	Square meter
^0C	Degree Celsius (Centigrade)	mg	Milligram
DCC	Dhaka City Corporation	min	Minute
df	Degree of freedom	ml	Milliliter
DF	Dengue fever	pH	potential of Hydrogen
DHF	Dengue hemorrhagic fever	RCBD	Randomized Complete Block Design
diam	Diameter	RH	Relative humidity
DNCC	Dhaka North City Corporation	SAU	Sher-e-Bangla Agricultural University
DSCC	Dhaka South City Corporation	SD	Standard deviation
EC	Emulsifiable Concentrate	SE	Standard error
et al.	and others (at elli)	SUP	Shape Use Material
^0F	Degree Fahrenheit	µg	Microgram
ft	Foot, feet	ULV	Ultra Low Volume
g	Gram	µ	Micron
HI	House Index	viz	Videlicet
hr	Hour	WHO	World Health Organization

ACKNOWLEDGEMENTS

All the gratefulness is due to the almighty Allah, the cherisher and sustainer of the world. His blessings have enabled the author to complete his dissertation leading to Doctor of Philosophy degree.

The author expresses his heartiest gratitude sincere appreciation, indebtedness and deep sense of respect to his adorable teacher, venerable Supervisor and Chairman of the Advisory Committee Dr. Md. Abdul Latif, Professor, Department of Entomology, Sher-e-Bangla Agricultural University for his planning, painstaking and scholastic guidance, support, extraordinary kind concern, everlasting encouragement, inestimable cooperation and intellectual encircling the research work till final preparation of the thesis.

He expresses his profuse gratitude, cordial appreciation and gratefulness to his thoughtful, creative members of the advisory committee Professor Dr. Md. Razzab Ali and Professor Dr. Md. Mizanur Rahman in the Department of Entomology, Sher-e-bangla Agricultural University, for their valuable suggestions, guidance constant encouragement and inestimable during the entire period of study.

Cordial thank giving due to the scholar member of the advisory committee, Dr. M. Salahuddin M. Chowdhury, Professor, Department of Plant Pathology, Sher-e-bangla Agricultural University, for his valuable suggestions, comments and advice during the entire period of study.

The author expresses his heartiest gratitude sincere appreciation, indebtedness and deep sense of respect to the Prof. Dr. Md. Kamal Uddin Ahamed Honuorable Vice-Chancellor, Sher-e-Bangla Agricultural University, for his support, extraordinary kind concern, everlasting encouragement and inestimable cooperation during the entire period of study.

The author acknowledges his sincere gratitude to The Ministry of National Science and Technology (NST) authority for providing a fellowship for PhD study.

The author acknowledges his sincere gratitude to The Ministry of National Science and Technology (NST) authority for providing a fellowship for PhD study.

He also expresses his profuse gratitude to the authority of North South University to allow leave for the completion of his degree.

With due regards, he thanks the Chairman, Department of Entomology, Sher-e-bangla Agricultural University, for the facilities provided, in carrying out this work. He also

acknowledges with deep regards the help and cooperation received from his respected teachers and stuff of the Department of Entomology, Sher-e-bangla Agricultural University while carrying out this work.

He expresses his sincere and deepest sense of gratitude to his respected teacher Professor Dr. Md. Mohsin Ali Sardar, former professor in the Department of Entomology, Bangladesh Agricultural University, Mymensingh, for inestimable cooperation and intellectual encircling the research work till final preparation of the thesis.

He expresses his sincere and deepest sense of gratitude to his respected teacher Professor Dr. Md. Mohsin Ali Sardar, former professor in the Department of Entomology, Bangladesh Agricultural University, Mymensingh, for inestimable cooperation and intellectual encircling the research work till final preparation of the thesis.

He expresses his heartiest gratitude sincere appreciation, indebtedness and deep sense of respect to his father Md. Ashraf Ali Fakir and his mother Mrs. Asya Ashraf for their sincere and affectionate support and love, extraordinary kind concern, everlasting encouragement and inestimable cooperation during the entire period of study.

Last but not the least his dearest thanks goes to his beloved wife 'Tahsina Sharower Ratna' for her interest in his work, her encouragement and love, which immensely contributed to his success.

Finally the author is deeply indebted to his loving son 'Raain' who must have missed his warm company and total attention during the whole study period. Their big sacrifice, patience and cooperation made it possible to complete his degree successfully.

The Author

BIOECOLOGY AND CHEMICAL CONTROL OF AEDES MOSQUITOES IN DHAKA CITY

ABSTRACT

A comprehensive research was conducted during January, 2013 to December, 2015 to study the life history traits through the life cycle of the medically important mosquitoes *Aedes aegypti* (Linnaeus, 1762) and *Aedes albopictus* (Skuse,1894) and their breeding sites, seasonal distribution affecting occuarence of dengue disease in different areas of Dhaka city and chemical control approach. The period of development from the egg to adult stage for aedes mosquitoes was 8.37±0.18 days for male and 9.5± 0.24 days for female. Female aedes mosquitoes fed with chiken blood showed the highest mean longivity which was 26.23±2.17days while 10% sucrose fed male recorded 19.23±2.21days which was the shortest mean survival period. Depending on the gonotrophic cycle of aedes mosquitoes their number of eggs and longevity varied.This research investigated aedes mosquito container productivity of each type and identified breeding sites of aedes larvae. Of total 9,222 households inspected, 1,306 (14.2%) were positive for aedes larvae breeding. Out of total 38,777 wet containers examined in the houses, 2,272 (5.8%) were infested with aedes larvae. Water holding containers, such as tyres, tanks, earthen jars, flower pots and drums were found to be the most common containers for aedes mosquitoes breeding. Tyres in outdoor, tanks in indoor and flower pots in rooftop locations were also important containers for the highest larval breeding. Factors such as independent household, presence of a water storage system in the house, and fully/partly shaded outdoors were found significantly associated with household infestation of aedes larvae. All these containers exhibited risk of breeding aedes mosquitoes. To evaluate ecological variation of their population density in different parts of the Dhaka city among its eight major divisions average highest density of both these mosquitoes at all the life stages viz. eggs, larvae, pupae and adults was in Tejgaon division with 2960±9.82, 2329 ±4.36, 1786.33±35.92 and 1369.67±16.50 respectively and the lowest density of 1556±51.39, 1122.67±32.88, 764±34.39 and 570.67±7.02 in Lalbagh division. The seasonal distribution of the aedes mosquitoes showed that the highest density of eggs, larvae, pupae and adults was found in June respectively following May with their mean numbers of 556±103.94, 451.76±103.42, 356.72±102.06 and 291.44±91.85respectively. The abundance of these mosquitoes was related to prevailing rainfall and temperature in these months. The highest LD_{50} value of used petroleum oil as diesel was 12.57 after 6hr following kerosine and the lowest LD_{50} value of organophosphorous insecticide as temephos was 1.24 after 24 hr of 1^{st} instar larvae. The efficacy of the the tested insecticides decreased with developing larval instars and the LD_{50} values thus increased. The pyrethroid insecticides such as pralemethin, deltamethin and permethrin killed almost at similar level, but their effectiveness appeared to be next to organophosphorous insecticide temephos.

CONTENTS

LIST OF TABLES

LIST OF TABLES (Cont'd)

LIST OF FIGURES

LIST OF FIGURES (Cont'd)

LIST OF FIGURES (Cont'd)

LIST OF FIGURES (Cont'd)

LIST OF PLATES

LIST OF PLATES (Cont'd)

LIST OF APPENDICES

CHAPTER I

INTRODUCTION

The mosquito is a deadly vector of human diseases including malaria, chikungunya and dengue fever. There are currently more than 3,000 mosquito species in the world grouped in 39 genera and 135 sub-genera, of which about 100 are vectors of human diseases (Reinert 2001, Clements 1992). Dengue viruses are transmitted through biting of the *Aedes aegypti* and *Aedes albopictus* mosquitoes (Rigau-Perez *et al.* 1998) during feeding on blood from human body. In Bangladesh 113 mosquito species were recorded (Ahmed 2007). Most of them transmit different kinds of diseases namely dengue fever, chikungunya and yellow fever by *Aedes* sp., malaria by *Anopheles* sp., Japanese encephalitis by *Culex* sp. etc. Among these diseases, dengue fever and dengue hemorrhagic fever (DHF) are the most important viral diseases of public health significance in many tropical and subtropical regions including Bangladesh. Dhaka is located in the heart of Bangladesh at 23°42′0″N 90°22′30″E, on the Southern banks of the Buriganga River. The city lies on the lower reaches of the Ganges Delta and covers a total area of 360 square kilometres (140 sq mi).

Although serological studies and virus isolation conducted during the outbreak of “Dacca fever” in 1964 due to dengue viral infection, sporadic

cases and small outbreaks went unreported until it took heavy death toll in 2000 (5,555 cases and 93 deaths); 2001 (2,430 cases and 44 deaths) and 2002 (6,104 cases and 58 deaths) in Bangladesh (Anon. 2009, Aziz *et al.* 1967).

Subsequent entomological and serological studies have indicated the continued presence of the mosquito vector and dengue virus in Bangladesh (Khan and Ahmed 1986, Islam *et al*. 1982, Gaidamovich *et al*. 1980, Khan 1980).

The magnitude of DF was largely unknown until an epidemic of DF and DHF broke out in June, 2000. Since then, it has become endemic in Bangladesh (Anon. 2001). The cases are being encountered each year particularly in urban areas. In 2000, the overall Breteau Index (BI) was 22.6 (ranging from 0.0 to 94 in different wards of Dhaka city). Above 20 BI was observed in 46 wards out of 90 wards in Dhaka city of Bangladesh (Chowdhury *et al.* 2000). The high BI for Dhaka City was also reported by the Health Department of Bangladesh Government during 2000. The number of dengue cases increased in 2000 not only in Dhaka city but also in other cities, viz. Chittagong, Khulna, Barisal and Rajshahi in Bangladesh (Ahmed *et al.* 2001, Yunus *et al.* 2001).

The increased dengue cases in this part of the world is due to unplanned urbanization, concurrent population growth, increased travel by airplane, non effective mosquito control program (Kindhauser 2003, Singh 1996), inadequate water supply, solid wastase management (Anon. 1995), increasing resistance of vectors and pathogens, decreasing number of effective insecticides, global warming (Yap *et al*. 2003) etc. These have led to increase of new mosquito breeding sites especially for the *Aedes* mosquitoes.

The reproduction of *Ae. aegypti* and *A. albopictus* occurs all the year round in both tropical and subtropical zones and their abundance is associated with rainfall (Micieli and Campos 2003, Moore *et al.* 1978). It is known that insects are exceedingly sensitive to environmental factors viz. temperature, humidity, rainfall and the species of tropical and temperate regions show great variations in their abundance in different seasons (Samways 1995). Bangladesh as a subtropical country, where temperature varies in different seasons and rainfall occurs in a particular period. Thus, bioecologylogy and behavior of mosquitoes can be different from other countries. Not only that the incidence of their populations vary season to season in a particular year in Dhaka city of Bangladesh due to variations of different ecological factors viz. temperature, relative humidity, rainfall and intensity of sunlight. So far

it is known, no information was found about the relationship of biological behavior of these mosquito species with these ecological factors. Little works on mosquito biology in relation to environment were done in Bangladesh. Thus it is felt to undertake the research works on biological and ecological aspects as bioecology on *Aedes* mosquito to know their seasonal abundance and distribution to control them effectively.

The management of dengue fever, chikungunya and other mosquito-borne diseases primarily depends on the control of the vector mosquitoes by using insecticides. The efficacy of many of these compounds has not been reported against mosquitoes. Because the resistance of mosquitoes against organochlorine, organophosphate, carbamate and pyrethroid insecticides has been a serious problem in confronting of several important species of mosquitoes (Ponlawat *et al.* 2005, Braga *et al.* 2004, Rodriguez *et al.* 2001, Vaughan *et al.* 1998). On the other hand, the indiscriminate use of chemicals for the control of mosquitoes creates health hazards, mosquito resistance to insecticides and the destruction of different types of beneficial wildlife such as predators, parasitoids etc. In addition chronic inhalation of mosquito coil smoke in humans may also be harmful (Okine *et al.* 2004). A few insecticides remain available for vector control (Brogdon and McAllister

1998, Gratz and Jany 1994). Among them very few insecticides are being offered as replacements.

Therefore, investigation is an urgent need to identify the potentiality of these materials in mosquito control for effective control measures as new tool for reducing the incidence of dengue cases.

Objectives

With the above points in view, the study was made on the following objectives-

i) To study the biology of aedes mosquitoes in the laboratory.

ii) To explore the breeding sites of aedes mosquitoes in Dhaka city.

iii) To study the distribution and seasonal abundance of aedes mosquitoes in Dhaka city and its effects on dengue disease.

iv) To evaluate the effectiveness of some insecticides against larvae and pupae of aedes mosquitoes.

CHAPTER II

REVIEW OF LITERATURE

An update knowledge regarding the current status of aedes mosquitoes, their biology, breeding sites, seasonal distribution and chemical control practices against them has been attempted. Available and accessible sources of information have been thoroughly reviewed and summarized as properly as possible. It is noted that most of the available information originated from outside of Bangladesh because there have been done least research regarding aedes mosquitoes in Bangladesh. However, care was taken to consider information that has relevance to and can be applicable in the context of aedes mosquitoes in Bangladesh. The information is accumulated and summarized in the following subheading: i) species history ii) classification iii) biology iv) container effect v) seasonal distribution vi) abiotic factors and vii) chemical control of aedes mosquitoes.

Species history of aedes mosquitoes

While the official common name for this species is the “yellow fever mosquito”, today it is of most public health concern as the major vector of dengue and zika fever (Judd 2008). Due to an effective vaccine, yellow fever is of less concern worldwide, although cases still occur (Barret and Higgs

2007). Generally, *Ae. aegypti* is important in spreading viral diseases such as yellow fever, dengue fever and Chikungunya.

Kropelin (2006) reviewed many of the ideas about the history of *Ae. aegypti's* distribution throughout the world given the information at that time. It is almost certain that the ancestor of the domestic form of *Ae. aegypti* lived in sub-Saharan Africa. The larval habitat was likely tree holes and non-human animals provided blood meals. Today, this ancestral form still exists in forests and vegetated ecotones in sub-Saharan Africa (Nene *et al.* 2005) and is called by a subspecies name, *formosus.* In addition to laying eggs in tree holes and preferring non-human blood, morphologically this form is much darker than the form adapted to human habitats, although this morphology based on scaling patterns is quite variable (Pape 2004) and, as will be clear later, is decoupled from the behavioural traits associated with urban vs. sylvan breeding in different parts of the world.

Two scenarios have been put forward for the origin of the light-coloured domestic subspecies, *Ae. aegypti aegypti* (for ease of communication, from here on we refer to forest-breeding populations in sub-Saharan Africa as the classically defined *formosus* subspecies as Aaf and the light coloured populations outside of Africa as Aaa. However, as will become clear, this

simple dichotomy masks the true complexity of the species). Almost certainly *Ae. aegypti* came to the New World on ships where conditions were such as to select for a domestic type. The two scenarios differ in whether the species had already become domesticated prior to spread (i.e., pre-adapted to human transport) or became domesticated in response to transport. The species was likely once more widespread including in forested northern Africa before the formation of the Sahara Desert. As the north part of the continent dried over the last 4,000-6,000 years forming the Sahara (Kropelin 2006), populations along the northern coast and around the Mediterranean would have become isolated from the sylvan form south of the Sahara. As the drying continued, the only reliable water sources for northern populations were those found in human settlements. Interestingly, a third subspecies, *Aedes aegypti queenslandensis*, was described as a particularly light coloured form found in the Mediterranean Basin (Nene *et al*. 2005). As *Ae. aegypti* has been eradicated in the Mediterranean Basin, it is not clear if *queenslandensis* still exists although we do know it was certainly a domestic form.

Whether the domestication event preceded or occurred simultaneous with its introduction into the New World, *Ae. aegypti* arrived soon after Europeans first arrived. Yellow fever was known in sub-Saharan Africa much before

1400, but was not known in the New World prior to European arrival. The first confirmed outbreak of yellow fever in the New World occurred in the Yucatan, in 1648 (McNeill 1976), although yellow fever may have been in Haiti as early as 1495 (Abercrombie, 2003). The early trade between the Old and New Worlds has been described as "triangular" (Adames and Galindo 2003). Ships from Portugal and Spain sailed to West Africa to acquire slaves, brought them to the New World where they were exchanged for goods that were brought back to Portugal and Spain. Whether the ships acquired *Ae. aegypti* in West Africa or already had the domestic form when they originated in Europe is not clear (Abercrombie 2003).

Systemic position of aedes mosquitoes

Kingdom	Animalia
Subkingdom	Bilateria
Infrakingdom	Protostomia
Superphylum	Ecdysozoa
Phylum	Arthropoda
Subphylum	Hexapoda
Class	Insecta
Subclass	Pterygota
Infraclass	Neoptera
Superorder	Holometabola
Order	Diptera
Suborder	Nematocera
Infraorder	Culicomorpha
Family	Culicidae
Subfamily	Culicinae
Tribe	Culicini
Genus	Aedes
Species	Species 1. *Aedes aegypti* (Linnaeus, 1762)
	Species 2. *Aedes albopictus* (Skuse,1894)

Aedini is the largest tribe in family Culicidae with 1,240 currently recognized species. The traditional classification of Aedini is based on the concept of recognizing few genera and numerous subgenera (Harbach 2016). Mattingly (2015) viewed the tribe as a natural group but noted that some members showed affinities with all other higher-level taxa of subfamily Culicinae. Species of the tribe are extremely varied, and many are difficult to identify to genus because of overlapping suites of shared anatomical features. More recently, Faran (2015) investigated the relationships among 20 species of *Aedes* representing six traditionally recognized subgenera, including *Aedes* (1 species), *Aedimorphus* (1), *Diceromyia* (2), *Halaedes* (3), *Ochlerotatus* (10) and *Stegomyia* (3), based on sequence data for the cytochrome oxidase. Hence, combinations of characters are required to define the majority of the genera, subgenera and Species (Pratt and Moore 2014). General features of the tribe include the presence of toothed ungues (tarsal claws) and a pointed abdomen in most females. Although toothed ungues are not universally present, they are not found in any other tribe of Culicinae. Larvae have relatively short, stout siphons with a single pair of seta 1-S (except for species of Aedes mosquitoes and Ochlerotatus subgenus Rusticoidus) inserted well above the base, usually beyond the middle of the siphon. A comb is always present on segment VIII and the ventral brush is

usually represented by five or more pairs of setae. Based on the assumption that annectent forms may have been derived by hybrid origin, Belkin (Bram 2013*)* believed that Aedini differentiated in the Indomalayan area of the Old World where the majority of annectent forms presently exist. The traditional classification of Aedini before the end of the twentieth century included nine genera and 50 subgenera (Kausar *et al.* 2012). *Aedes* was by far the largest genuswith approximately 1,000 species divided between 41 subgenera (Shepard *et al*. 2012). Reclassification of genus *Aedes* began with the elevation of *Verrallina* and *Ayurakitia* to generic status (Cook *et al*. 2011)) and the subsequent separation of the remaining subgenera into genera *Aedes* and *Ochlerotatus* (Cook *et al*. 2011). Whereas the generic status of *Verrallina* and *Ayurakitia* was readily accepted, the elevation of *Ochlerotatus* to generic status was widely condemned by taxonomists and medical entomologists, particularly in North America. Critics suggested that this change should not have been made without strong corroborating evidence from other character systems, presumably principally molecular data. As the following discussion will show, analyses of molecular data are lending support to taxonomic changes based on morphology. Anderson *et al*. (2010) examined the phylogenetic relationships of six mosquito species belonging to genera *Aedes* (3 species), *Armigeres* (1 species) and *Culex* (2

species) by comparing chromosomal rearrangements based on shared restriction fragment length polymorphisms. Genus *Aedes* was paraphyletic, with two species of *Stegomyia Ae. aegypti* (Linnaeus) and *Ae. albopictus* (Skuse) forming one clade and *Ae.* (*Protomacleaya*) *triseriatus* (Say) clustering with the two *Culex* species *Cx. pipiens* Linnaeus and *Cx. tritaeniorhynchus* Giles. The analysis of *white* gene sequences by Besansky and Fahey (2007) also indicated that *Aedes* was paraphyletic, with *Ae. triseriatus* clustered with *Haemagogus equinus* Theobald. Rey *et al*. (2006) used a 763-bp segment of the mitochondrial COI gene to examine the phylogenetic relationships of 14 species traditionally placed in genus *Aedes* subgenera *Aedes* (1 species), *Aedimorphus* (1), *Ochlerotatus* (11) and *Stegomyia* (2) members of superfamily Culicoidea *Hereditas*, 113, 139–144.

Biology of aedes mosquitoes

Egg: Mosquitoes (Family: Culicidae, Order: Diptera) exhibit a complete metamorphosis with four life stages: egg, larval, pupa, and adult. Larvae have also been found in fresh fruit husks, decaying fruit husks, puddles, bamboo holes, discarded containers, tyres, rocks holes and storage containers (Tristophers 2016).

Scientists assumed that removing *Ae. albopictus* adult females and their eggs from the field doesn't significantly affect the mosquito population size and

temporal dynamics collections were carried out in typical hot-spots of high Ae. albopictus density (Gontenille and Rodhain 2015).) in heavily infested areas (Yalliard 2014)).

Egg collections were carried out by ovitraps filled with 300 ml water and internally lined with a germination paper on which mosquito females lay their eggs (Taey 2013). *Aedes aegypti* is a holometabolous insect, meaning that it goes through a complete metamorphosis with an egg, larva, pupa, and adult stage.

After taking a complete blood meal, females produce on an average 100 to 200 eggs per batch; however, the number of eggs produced is dependent on the size of the bloodmeal. Females can produce up to five batches of eggs during a lifetime. A smaller bloodmeal produces fewer eggs (Jien 2010). Eggs are laid on damp surfaces in areas likely to temporarily flood, such as tree holes and man-made containers, and are laid singly, rather than in a mass. Not all the eggs are laid at once, but can be spread out over hours or days, depending on the availability of suitable substrates (Nasci *et al.* 2009). Most often, eggs will be placed at varying distances above the water line, and a female will not lay the entyre clutch at a single site, but rather spread out the eggs over two or more sites (Grangstrum and Chan 2011).

Eggs of *Aedes aegypti* are long, smooth, ovoid shaped, and approximately one millimeter long. When first laid, eggs appear white but within minutes turn a shiny black. In warm climates, such as the tropics, eggs may develop in as little as two days, whereas in cooler temperate climates, development can take up to a week (Rawley 2008). *Aedes aegypti* eggs can survive desiccation for months and hatch once submerged in water, making the control of *Aedes aegypti* difficult (Toekiman 2008).

Female mosquitoes lay 50-500 eggs either in the water or on sites that are likely to flood, depending on the species (Clements 2000). Eggs of most mosquitoes hatch within two days to a week, although the eggs of some mosquitoes can survive for months before hatching during drought or cold seasons (Clements 2000).

Larva: The larvae of most species live in small or shallow bodies of still water including puddles, shallow pools, sheltered stream edges, water-filled tree-holes, or man-made containers (Clements 2000). Most mosquito larvae live on the particulate matter in the water pool such as bacteria, diatoms, algae, and particles of decayed plant material (Clements 2000). Larval maturation can take as little as four or five days in tropical mosquitoes, allowing them to exploit temporary bodies of water (Clements 2000). After adults emerge, it may take a day or two before they are ready to mate

(Clements 2000). Both males and females consume plant sugar as their main source of energy, but the females of the subfamilies Anophelinae (including the mosquitoes that transmit malaria) and Culicinae (including the genus *Aedes*) also require protein from blood meals to lay eggs (Clements 2000). In fact, multiple studies have found that *Aedes aegypti* females can live exclusively, or almost exclusively, on human blood making them particularly good vectors for disease (Soreta 2007). Most species have preferred times (e.g., morning or evening) for mating, feeding, and laying eggs (Clements 2000). Life spans of mosquitoes range from a few days to months (Clements 2000).

Pupa: As ecdysis approaches the final or pupal ecdysis, larva becomes plump and increasingly turgid (Tristophers 2016). The larva tends to cease feeding and to remain at rest at the surface. When first emerged, the pupa is white, but in a short time shows pigment changes (Gontenille and Rodhain 2015). They are comma shaped (Yalliard 2014) and also called “tumblers”. The pupal stage is quite short and usually last 1 to 2 days (Taey 2013). The mosquito pupa is active, unlike pupa of the most insects (Urancy *et al.* 2012).

Adult: The adult life span can range from two weeks to a month depending on environmental conditions (Urancy *et al.* 2012). *Aedes aegypti* comes in

three polytypic forms: domestic, sylvan, and peridomestic. The domestic form breeds in urban habitat, often around or inside houses. The sylvan form is a more rural form, and breeds in tree holes, generally in forests, and the peridomestic form thrives in environmentally modified areas such as coconut groves and farms (Grangstrum and Chan 2011).

In searching for resting places adult mosquitoes frequent a wide range of places. Mosquitoes are generally found in areas where the air is relatively static and the humidity high (Anon. 2012). During day time most mosquito species prefer to rest in dark places and avoid light (Nasci *et al.* 2009). *Aedes aegypti* adults prefer to rest inside the house where it is dark (Rawley, 2008). Toekiman (2008) found most of them resting in temporary objects (on clothing and mosquito nets) while a few percentage was found resting on furniture and other semi-permanent articles In Malaysia, *Aedes albopictus* adults have been found resting outdoors in clearing and rubber plantations (Clements 2000).

The models estimated the presence of adult biting females also at zero mean number of eggs/day, as also observed during the experiment. This is counterintuitive, as each adult female released tens of eggs each gonothrophic cycle, and questions the widely accepted concept that ovitraps

are a very sensible tool to detect the presence of adult females (Rawley 2008).

In tropical regions, the life span of adult mosquitoes ranges from a few days to several weeks and it is frequently longer in temperate regions. The life span of female for species that overwinter as adults may approach one year (Clements 1992). The longevity of *Aedes albopictus* under natural environment is not fully known but it is expected to be shorter than under laboratory conditions (Grangstrum and Chan 2011). Laboratory studies showed that male and female *Aedes* mosquito survive an average of 20 to 30 days respectively (Anon. 2012).

Productive containers in different locations

Analyses revealed that water storage containers, such as tyres, earthen jars, tanks, flower pots and drums were consistently more likely to contain aedes larvae. Similar results were found in previous studies (Koenraadt *et al.* 2007 and Maciel-de-Freitas *et al.* 2007). Indoor tanks and drums were the most productive; while outdoor tyres and earthen jars were the most productive. Rooftop earthen jars, flower pots and drums were highly productive. Although present in abundance, buckets did not contribute much to larval production. Understanding the cultural traditions of owning and using containers is important to identify the essential containers in different

locations. Dhaka city has a scarcity of domestic water supply, and 87.7% of the municipal water supply is mainly derived from groundwater (Anon. 2011). Most of the city dwellers store supplied pipe water. They either send the pipe water directly to rooftop tanks or store it in underground reservoirs and pump it to the rooftop tanks. Underground reservoirs are categorized here as outdoor tanks. As the municipal water supply is not guaranteed all the time, people store water in drums, earthen jars, buckets, and indoor tanks for use in emergencies and tyres and flower pots are kept in outdoor and rooftop respectively where stagnant water reserved. Tanks in outdoor locations and rooftop are normally kept covered and closed; therefore, these reservoirs are protected from mosquitoes. Buckets are relatively smaller in size compared to other water storage containers and are frequently used for washing clothes, cleaning house, and transferring water from one place to another. These practices would reduce the chance of larvae breeding in buckets. Previous studies also reported that weekly cleaning of the water-holding containers was effective in the control of larval production (Arunachalam *et al.* 2010, Phuanukoonnon *et al.* 2005). However, apparently unattractive or frequently cleaned containers, if present in large numbers, may still serve as potential breeding sites for a large portion of the aedes population. On the other hand, drums, earthen jars, and indoor tanks

are bigger in size than buckets and contain a large volume of water. Water in these containers is never emptied and is replenished periodically. A study in Rio de Janeiro found that open-mouthed and large containers are the most suitable or larval production (Maciel-de-Freitas *et al.* 2007). Moreover, containers outdoors like tyres, earthen jars, cans and bottle and flower pots on rooftops are not always covered, sometimes unintentionally allowing them to collect rainwater and, therefore, making them perennial breeding sites for aedes mosquitoes (Strickman *et al.* 2003, Kittayapong *et al.* 1993). The most important breeding site in outdoor was tyres. Around 27% of tyres were found infested with Aedes larvae. In outdoor places 46.1% of the aedes larvae were found in tyres. Usually tyres are left abandoned. The collected rain water in tyres is an ideal source of *Aedes* larvae (Lloyd, *et al.* 1992). Some recent studies use different container parameters while evaluating container productivity. A study in Thailand developed a container-classification method that consists of the shape (S), use (U), and material (M) of the container (SUM-method). Size or volume of the container, exposure to sunlight, presence of abate, cover status, and filling methods of the containers were also considered to determine the container productivity for aedes larvae and pupae (Koenraadt 2007, Maciel-de-Freitas *et al.* 2007).

In this study there was no detailed information on these container parameters.

In this study, *Aedes aegypti* was found two times higher in number than *Ae. albopictus*. Moreover, *A. aegypti* was found the dominant indoor breeder, while *A. albopictus* showed higher affinity for outdoor containers. Previous studies on the habitation of aedes mosquitoes showed that *A. albopictus* usually seems to be restricted to wooded areas adjacent to human habitations. Conversely, *A. aegypti* can be found in a variety of urban habitats including the highly urbanized areas without wooded vegetation (O'Meara *et al.* 1995). Additionally, *A. aegypti* depends highly on human blood and tends to bite and rest indoors, whereas *Ae. albopictus* feeds on a variety of vertebrates outdoors (Scott *et al.* 1993) Therefore, *A. aegypti* predominates in highly urbanized areas, specially indoor containers.

Conversely, *Ae. albopictus* predominates outdoor containers. It seems that *A. aegypti* is better adapted than *A. albopictus* to the environment of crowded tropical cities like Dhaka. The present study found that indoor tanks were the highest productive containers for *A. aegypti*, while outdoor earthen jars were the highest productive containers (86%) for *A. albopictus*. Although a high percentage of tyres was found positive, they contained large numbers of

aedes larvae. One possible reason may be that they contained stagnant water with suitable temperature than other water storage containers.

Seasonal distribution

The geographic locations where *Aedes aegypti Aedes albopictus* live may depend much more on differing climatic requirements than on interactive competition. In a study on the island of Madagascar that compared temperature, number of dry months and millimeters of rainfall to abundance of *Ae. aegypti* and *Ae. albopictus,* **(**Lima *et al.* 2016**)** found that *Ae. albopictus* dominated places with more than 1,000mm of rainfall annually and no more than six dry months. *Ae. aegypti* was the predominate species in areas that received less than 2,000mm of annual rainfall and experienced up to nine dry months a year. Other studies have reported a correlation between rainfall and adult *Ae. albopictus* abundance **(**Hills *et al.* 2016**),** oviposition rates **(**Hahn *et al* 2015**)** and biting rates. At the end of the dry season in Chiang Mai, Thailand, only the eggs of *Ae. aegypti* were found in rural ovitraps, and the proportions of *Ae. albopictus* eggs increased during the rainy seasons **(**Capinha *et al.* 2014).

Similar results were found in urban ovitraps **(**Lucio *et al.* 2013**),** in cemetery vases and in tyres (Rey *et al*. 2012). In experiments involving mixed populations of *Ae. albopictus* and *Ae. aegypti*, Eisen and Moore (2013)

found *Ae. albopictus* was negatively impacted by interspecific competition under drying conditions and inversely impacted under fluctuations between wet and dry conditions. The reverse was true for *Ae. aegypti*. Khormi and Kumar (2014) attributed this effect which most greatly impacted the adult stage, on the effects of drying during the egg stage. In contrast, studies in Malaysia (Scott 2003) and in Japan (Lucio *et al.* 2013) showed no correlation between *Ae. Albopictus* population size and rainfall. In Houston, Texas, an explosion in the population of *Ae. aegypti* occurred after a severe flood in the summer of 2000 following a couple of years of record dry spells (personal observation and communication with Harris County Mosquito Control district personnel). The district reported very low to non-existent numbers of *Ae. aegypti* before the flood. The effects of temperature and humidity on the adults of these two species, as well as many others have been extensively studied by Moore *et al.* (2014) compared desiccation survival times of adult *Ae. albopictus* and *Ae. aegypti* under 90% and 70% relative humidity (RH) and 25°C. *Ae. aegypti* was found more resistant to desiccation than *Ae. albopictus.* Research by Eisen and Moore (2013) on the reactions of the mosquito, *Culex fatigans* to temperature and humidity indicates that adult females are very sensitive to changes in humidity and temperature. At 29°C *fatigans* could detect a difference of 1°C. All females

avoided very high and very low levels of temperature and humidity, with blood fed females showing the strongest reactions and hungry females showing the weakest.

Research has been conducted on temperature dependent development rates for *Ae. aegypti* by Brady *et al.* (2014) and for *Ae. albopictus* by Leisnham (2014). In each case the results varied with the origin of the mosquito. A study on physiological time by Brady *et al.* (2014) that pooled development rates for 54 species of insects and seasonal trends, suggests that very similar species with differing temperature optimums might experience species replacement seasonally in areas that have high summer temperatures. There has been minimal research on the effects of temperature and humidity on the eggs of *Ae. albopictus* and *Ae. aegypti*. In Japan, Lucio *et al.* (2013) measured survival times of eggs from several *Aedes* species including *Ae. aegypti* and *Ae. albopictus* under three different humidity conditions (42%, 68% and 88% RH) at 25°C. *Ae. aegypti* survived longer then *Ae. albopictus* at all humidity conditions. Lucio *et al.* (2013) attributed this to egg volume, with *Ae. aegypti* having the greatest egg volume and thus the greatest ability to resist desiccation. In Florida, Reiskind and Lounibos (2013) compared egg mortality rates of *Ae. aegypti* and *Ae. albopictus* under different temperature (22°, 24° and 26°C) and humidity (25%, 55%, 75%, and 95%

RH) combinations. Reiskind and Lounibos (2013) found the effects of temperature and humidity on egg mortality significantly different between the two species, with *Ae. Albopictus* experiencing much higher mortality at all combinations except at the highest humidity. Over a three-month period, they did not find a significant interaction between temperature and/or humidity and egg mortality of *Ae. aegypti* until the third month (90 days). This indicates the effects of temperature and humidity increase with time. *Ae. aegypti* eggs had a lower rate of mortality with a higher level of humidity. Hotez (2011) compared the resistance of *Ae. albopictus* and *Ae. aegypti* eggs to low humidity (60-70% RH) at 25°C over a four-month period. At all time intervals *Ae. albopictus* was found more resistant to desiccation, resulting in a higher percentage of hatched larvae. In addition, the percentage of hatching larvae increased to the first month for *Ae. aegypti* and to the second month for *Ae. albopictus* and then gradually dropped.

All of this research has broadened our knowledge, but the climate combinations evaluated span a very narrow range and cannot be reliably applied to Texas strains. Research by Chesson and Hahn *et al.* (2015)suggest fluctuating harsh or stressful conditions in addition to temporal niche opportunities may play a role in species replacement.

Thus, the purpose of the research herein was to assess how immature embryos (eggs) of *Ae. albopictus* and *Ae. aegypti* from Texas will be affected by humidity and temperature over a broad range of combinations over time. It is hypothesized that the eggs of *Ae*. *aegypti* and *Ae. albopictus* will have different percentages of eggs hatch at different levels of relative humidity and temperature, and that these differences will not be the same between the two species.

Effects of abiotic factors on aedes mosquitoes

A study from Indonesia showed a positive correlation between eggs in ovitrap and number of host-seeking *Aedes aegypti* females in BG sentinel traps (Lindsay and Birley 2016)). A negative association between the number of *Aedes* mosquitoes and rainfall was observed in Pulau Ketam. Wickerson *et al.* (2015) also reported a negative association between ovitrap index and high rainfall on the campus of Universiti Putra Malaysia (UPM), Selangor. The Breteau index (number of positive containers per 100 houses) reached its lowest value at the peak of the rainy season in a study from Jinjang, Kuala Lumpur (Vezzani *et al.* 2014). A study of *Ae. aegypti* egg numbers in Salta, Argentina found they remained low during the dry season, increased at the beginning of the rainy season and decreased at the end of the rainy season (Vecieli and Gampos 2013). The larvae were most abundant

during the wet season, with the largest number of positive containers, the highest larval index and largest number of high density sites (Saruah and Dutta 2012). Heavy rain and strong winds may disturb the flight activity of *Aedes* resulting in difficulties in finding hosts and suitable breeding sites (Anon. 2012). Another reason for the negative impact of heavy rain on the *Aedes* ovitrap index is the larvae were flushed out of the ovitrap and other potential containers during heavy downpours (Yang *et al.* 2011). Thus, the negative association between outdoor ovitrap index and rainfall was more pronounced than indoors since the rainfall exerted a greater influence on outdoor *Aedes* larvae than indoor *Aedes* larvae. A study from Kolej Mohamed Rasid, Malaysia also showed similar results (Moore *et al.* 2011).

A higher ovitrap index was found during the dry season in Chiang Mai, Thailand (Yang *et al.* 2009a), similar to this observations. The higher index may be due to attraction to the ovitrap caused by the the scarcity of other suitable breeding sites (Yang *et al.* 2009b). A study in Tubiacanga, Rio de Janeiro, Brazil also found the container index, Breteau index, pupae per hectare and pupae per person were higher during the dry season (Koenraadt and Harrington 2008).

In this study, a large amount of rainfall was followed 25-27 days later by a peak in the ovitrap index. A one month lag time between rainfall and peak in

the container index (number of positive containers per house) was also seen in Singapore (Regis *et al.* 2008). An 18 day lag time was seen under laboratory conditions, which may be explained by the time period between hatching of eggs and first oviposition (Regis *et al.* 2008). A development time of 26 days was found among aedes mosquitoes in the field in our study (Harizo *et al.* 2007). A study conducted in Taman Permai Indah, Penang, Malaysia also showed a significant positive association between ovitrap index for *Ae. albopictus* and a lag time after rainfall of two months; while the mean number of eggs was also significantly associated with a one month lag time after rainfall (Yuore *et al.* 2006). The findings show there is a correlation between rainfall and *Aedes* population numbers after a lag time.

Harizo *et al* (2007) found an *Ae. aegypti* population increased during the rainy season; however, *Ae. albopictus* population initially increased at the beginning of the rainy season, but then declined. *Ae. albopictus* needs a dry season to mature: eggs laid during the rainy season become mature during the next dry season until new rainfall (Harizo *et al.* 2007). In Manaus City, Brazil, *Ae. aegypti* reproduction was greater during the rainy season due to less use of water storage vessels, allowing for *Aedes* development (Esteva and Vargas 2003).

The second peak in the *Aedes* population in our study occurred 37 to 41 days after the rains. This can be explained by a second gonotrophic cycle, since the interval between the first oviposition and second oviposition in *Ae. albopictus* is approximately 17 days in the lab (Esteva and Vargas 2003).

Information regarding climate variations is useful for dengue outbreak prediction and disease prevention. Rainfall as an early indicator of vector reproduction has obvious advantages over late indicators, such as ovitrap indices, larval density, *Aedes* house indices and Breteau indices (Esteva and Vargas 2003).

Chemical control

Pyrethrum is a botanical insecticide, which is extracted from the plant of the Chrysanthemum genus (Shan and Zairi 2016). Basically, pyrethroid can be divided into four generations. The first generation was in the market in 1949 when allethrin was synthesized based on 22 chemical reactions.

The second generation that was introduced in 1965 is tetramethrin (Chandre *et al*. 2015) and fourth generation is 10 times more effective compared to the third generation pyrethroid. The fourth generation insecticides are cypermethrin, bifenthrin, deltamethrin and esfenvalerate (Thamise *et al*. 2014).

Pyrethroids can be further divided into two groups which are type I and type II. The effects of type I pyrethroid (permethrin) are hyperactivity and trembling (seizures) for type II pyrethroid (deltamethrin) the effect is paralysis of the insects. Pyrethroid's overall characteristics as an insecticide are very good and could be the most ideal insecticide, if resistance does not occur (Chandre *et al*. 2015).

Pyrethroids are neurotoxic to insects. Insects that have been exposed to pyrethroid will be agitated, hyperactive, uncoordinated and paralysed. For the flying insects, the knockdown effect is very fast. The symptoms will vary depending on the type and dose of pyrethroid used (Becker 2010).

Pyrethroids act on the nerve membrane and affect the sodium channels by nerve excitation that occurs as a result of changes in the nerve membrane permeabilities to sodium and potassium ions. Neuro-physiological changes that occur as a result of this action are repetitive firing and the prevention of neuro-muscular transmission (Stenersen 2004).

For the control of dengue vector, pyrethroids are normally used in thermal fogging or ultra-low volume sprays. However, pyrethroids are not recommended for the use for larviciding since there are concerns of development of vector resistance towards synthetic pyrethroids and are very toxic to aquatic animals (Chang *et al*. 2009).

Organophosphates

Organophosphate insecticides kill insects by binding to and inhibiting acetylcholinesterase (AChE) at synaptic junction of the insect nervous system. This overstimulation of the insects' central nervous system results in death (Chang *et al*. 2009). An example of members from the organophosphate group that is used in public health is malathion and fenitrothion for IRs or ULV while temephos is used as a larvicide (Chen *et al*. 2008a).

Temephos larval bioassays were conducted according to WHO guidelines (Chen *et al*. 2008b). Temephos was chosen for this experiment because it is used to control mosquito larvae in the Ministry of Health control programs (Methen *et al*. 2013).

Preliminary tests on lab strains were done to determine the range of temephos concentration to be used for the bioassay. For each concentration of temephos, 4 replicates of 25 larvae were used. From the preliminary test, 8 concentrations 0.20%, 0. 40%, 0.6%, 0.80%, 1.0%, 1.20%, 1.40% and 1.60% were used for both *Ae. Aegypti* and *Ae. albopictus*. Controls were done using water.

Diesel and Kerosene

The use of petroleum oils as mosquito larvicides in the United States dates to 1793 in Philadelphia (Devine *et al*. 2009). Early petroleum-based larvicides such as diesel oil, kerosene, or tar oils were odor-iferous and often discolored the water's surface for several days (Cratz 2014). Current products, although derivatives of petroleum distillates, are nearly odorless and clear (Dharshini *et al*. 2011). Pupicidal effects of larvicide oils had been documented by Devine *et al*. (2009).

EXPERIMENT 3.1

STUDY OF THE BIOLOGY OF AEDES MOSQUITOES

STUDY OF THE BIOLOGY OF AEDES MOSQUITOES

ABSTRACT

The biology of aedes mosquitoes was studied in the central laboratory of Sher-e-bangla Agricultural University, Sher-e-Bangla Nagar, Dhaka from April 2015 to October 2015. Eggs were collected from Sher-e-Bangla Agricultural University campus emerged as aedes mosquitoes both *Aedes aegypti* and *Aedes albopictus.* The gravid female laid eggs in cluster. Each cluster having 105-129 eggs with an average of 117.71 ± 9.12. Each female laid 3-4 clusters. Initially the colour of the egg was white and gradually turned into black. The incubation period of eggs ranged 48-72 h with mean of 60 ± 0.53. The development period from the first instar larva to adult stage for aedes mosquitoes was 8.37 ± 0.18 days for male and 9.5 ± 0.24 days for female. Female aedes mosquitoes fed with blood showed the highest mean longivity which was 26.23 ± 2.17days while male aedes mosquitoes fed with 10% sucrose recorded 19.23 ± 2.21days which was the shortest mean survival period. Depending on the

gonotrophic cycle for aedes mosquitoes their number of eggs and longevity varied.

3.1.1 INTRODUCTION

The primary vector of dengue is *Aedes aegypti,* which is also called the 'yellow fever mosquito', first described by Linnaeus (1762). It is a member of the genus *Aedes* (Chistophers 1960). *A. aegypti* originated from Africa but is now found in tropical and subtropical regions thoughout the world. The secondary vector of dengue is the 'Asian tiger mosquito', *Aedes albopictus.* It was first described by Skuse (1894) in Bombay, India as *Culex albopictus.* It is an invasive species originally from tropical and subtropical areas of South East Asia that has invaded many countries around the world (Watson, 1967). Apart from being responsible as dengue vectors, both *A. aegypti* and *A. albopictus* have also become efficient vectors of other human diseases including Chikungunya and yellow fever (Phillips 2008 and Hochedez *et al.* 2006) as well as some encephalitis viruses (Anon. 2012) and filariasis parasites (Cancrini *et al.* 2003). Adult *A. aegypti* is a small to medium-sized mosquito, approximately 3 to 6 millimetres in length, with two white stripes and a single curved line at each side forming a lyre shape on the dorsal

thorax. The abdomen is generally dark brown to black, and is covered with white scales in the form of stripes and spots which create the unique distinguishing pattern. Each tarsal segment of the hind legs also possesses white stripes (Lee *et al.* 2003). On the other hand *Aedes albopictus* is characterized by its black-and-white-striped legs, and small black-and-white-striped body (Chistophers 1960). The gravid female laid eggs in clusters under favourable climatic and environmental conditions, the life cycle of this species can occur in less than 10 days. The lifespan for adult mosquitoes typically ranges from 2 weeks to a month. (Maricopa 2006, Lee *et al.* 2003b). *A. aegypti* is a day-biting mosquito that prefers to feed on humans even if other hosts are available (Harrington *et al.* 2001 and Scott *et al.* 2000). This species breeds and rests close to human habitation. (Huber *et al.* 2008). From larva to emergence of adult aedes mosquitoes spent in aquatic environment (Anon. 2012).

Generally breeding sites of aedes mosquitoes are likely to be in close proximity to their blood-feeding habitat (Anon. 2012, Valerio 2010, Chaves *et al.* 2010, Ponlawat and Harrington 2005). It is well recognised that both *Ae. aegypti* and *Ae. albopictus* can feed multiple times; therefore this behaviour may increase opportunities for the transfer of arboviruses to other

vertebrate hosts (Delatte *et al.* 2010 and Harrington *et al.* 2001). Detailed study of the biology of this mosquito has been undertaken in this study.

Objectives

1. To study the reproductive potential and behaviour of aedes mosquitoes.
2. To investigate the duration of different life stages of aedes mosquitoes in the laboratory.

3.1.2 MATERIALS AND METHODS

The biology of aedes mosquitoes was studied in the Central Laboratory, Department of Entomology, Sher-e-Bangla Agricultural University (SAU), Dhaka during April to October 2015. An elaborate methodology for studying biology is presented herein.

3.1.2.1. Collection of aedes larvae

Larvae of aedes mosquitoes were collected from 10 locations (Figure 3.1.1) at Sher-e-Bangla Agricultural University such as in front of Agricultural Chemistry Department, behind the Horticulture Department, behind the Seraj-ud-doula Hall, behind the Sher-e-bangla Hall, behind the Kobi Kazi Nazrul Islam Hall, infront of the Kobi Kazi Nazrul Islam Hall, behind the Vice Chancellor Bhaban and Northern part of central mosque, in front of Krishok Ratno Sheikh Hasina Hall and in front of Soil Science Department in order to study the biology. One ovitrap was set in each location to attract the gravid female for egg laying (Plate 3.1.1). For preparation of ovitrap an earthen pot (20 cm diameter in middle and 12 cm in mouth) was placed by making a hole in soil. Then five liter water was kept in each earthen pot. Fifty gram (50.0 g) Yeast powder and 20.0 g sugar were added with water and stirred with stick for mixing properly. Earthen pot with yeast solution was considered as ovitrap and sugar was added for larval food. A

germination paper was placed in each ovitrap to support gravid female for egg laying. Ovitrap was monitored regularly to observe egg cluster and larvae of aedes mosquitoes. Egg cluster of aedes mosquito was observed just after 10 days of placement of ovitrap. Larvae of aedes mosquitoes with water were collected from these ovitraps. Larvae were also collected from natural breeding sites such as old tyres on the yard of central laboratory, tree holes in front of soil science department (Plate 3.1.2), flower pots and containers those held water. Larvae were picked up with water by using pipettes and droppers. All the samples were brought to the laboratory for the purpose of mass rearing.

Figure 3.1.1 Different locations of placing earthen containers in the Sher-e-Bangla Agricultural University.

Plate 3.1.1 Earthen pot placed in the campus of Sher-e-Bangla Agricultural University to collect aedes larvae.

Plate 3.1.2 Tree hole in front of soil science department, SAU.

3.1.2.2 Rearing of aedes mosquitoes

Larvae of aedes mosquito collected from 10 ovitraps (Plate 3.1.1) and natural sources (Plate 3.1.2) were brought to the Entomology Laboratory of Sher-e-bangla Agricultural University, Sher-e-bangla Nagar, Dhaka. Water containing larvae from plastic bottles were poured into enamel trays and fed with larval food containing grounded biscuit, beef liver, powdered milk and yeast at a ratio of 2:1:1:1 (Toma *et al*., 2003) until they pupate. Then pupae were collected by pipettes and kept in separate plastic pots filled with water and placed within a rearing cage (Plate 3.1.3).

After emergence, adults were picked up one by one using a manually operated aspirator. Adult *Aedes aegyptii* were identified and separated by observing their morphological characteristics mainly the pattern of scutum with lyre shaped white marking and long median longitudinal white stripe on the thorax (Plate 3.1.10). Then ten adult mosquitoes (5 males and 5 females) were kept together in each rearing cage. A chicken was kept at the bottom of the rearing case (Plate 3.1.4) for feeding blood by adult female mosquitoes. Legs and wings of the chicken were tied with rope for restriction its movement during feeding by female mosquitoes. Moreover, 10% sucrose solution soaked cotton mass was placed at the bottom of each cage for feeding of adult male. They were allowed to mate freely inside the rearing

cages at room condition having 27°C temperature and 75% relative humidity (RH). Mating frequency was recorded by counting how many times they mated. A wet filter paper folded into a shape of a cone in a Petri dish was placed into the cage for egg lying by female mosquitoes after two days of blood feeding. The filter paper was taken out of the cage after six hours and the cone was inverted making sure that the eggs were on the inner surface of the filter paper. It was dried at room temperature to ensure that the embryos developed well in the eggs and uniform hatching. Then 20 eggs were placed in each enamel tray with fresh water and monitored regularly to observe the incubation period properly. After hatching, water was changed and 10% sucrose solution was added for nourishing 1st instar larvae. This process was repeated upto the pupation. In the pupal period fresh water was added again to find clear emerging of adult. Just after the emergence adult took rest for 20-25 minutes. The adults were fed on chickens as their blood-meal source for female and cotton soaked with 10% sucrose solution for male (Clements, 2000).

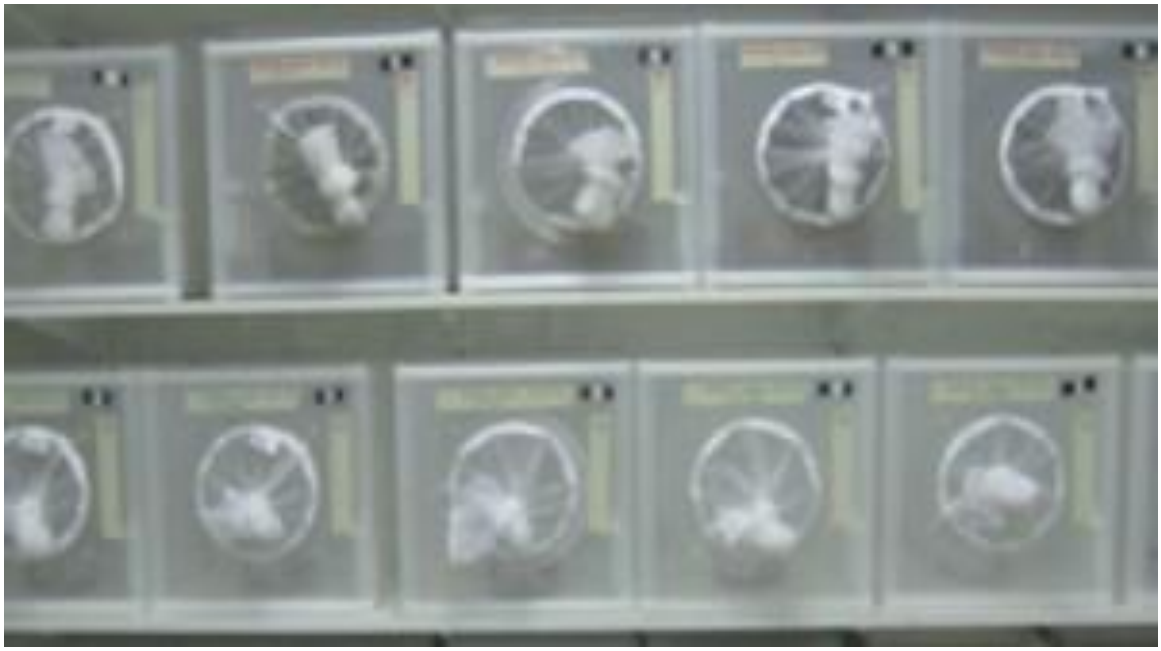


Plate 3.1.3 Aedes larvae rearing trays and adult cages.

Plate 3.1.4 Adults mosquitoes feed chicken blood in the cages.

3.1.3 RESULTS AND DISCUSSION

The results on the biology of Aedes mosquitoes in laboratory condition have been discussed herein under different sub-headings.

3.1.3.1 Mating of aedes mosquitoes

Mating frequency was recorded by counting how many times they mated. Mating of aedes mosquitoes occured 3-4 times for their total fecundity period with mean value 3.43 ± 0.53 (Table 1.3.1). Black *et al*. (1989) concluded that *Ae. aegypti* males are more sexually aggressive and frequent than *Ae. albopictus* males, but when harassed by *Ae. aegypti* males, there was no effect on oviposition rates of *Ae. albopictus* females. In contrast, laboratory studies with Louisiana strains of each species found male *Ae. albopictus* more sexually aggressive in attempting to mate with female *Ae. aegypti* as compared to *Ae. albopictus.*

3.1.3.2 Pre-oviposition and oviposition period and fecundity of aedes mosquitoes

Adult females took 6-8 days for pre-oviposition with a mean pre-oviposition period of 7.93±0.19 days (Table 3.1.1). The duration of oviposition was as short as 6 days to a maximum of 7 days with a mean 6.84±0.35 days (Table 3.1.1). Females laid eggs in batch and produced cluster of eggs. Each female produced 3-4 clusters (Plate 3.1.5) with an average of 3.43±0.53 clusters. Every cluster contained 105-129 eggs with mean of 117.71±9.12 per cluster.

A female laid a total of 327-516 eggs with a mean of 386.57 and standard deviation 64.63 (Table 3.1.1). Egg viability rate of aedes mosquitoes was 78-94% with a mean of 87.57 ± 5.19. Chistophers (2010) observed and reported that the egg viability ranged 55-98% depending on the food sources and environmental conditions (Plate 3.1.6)

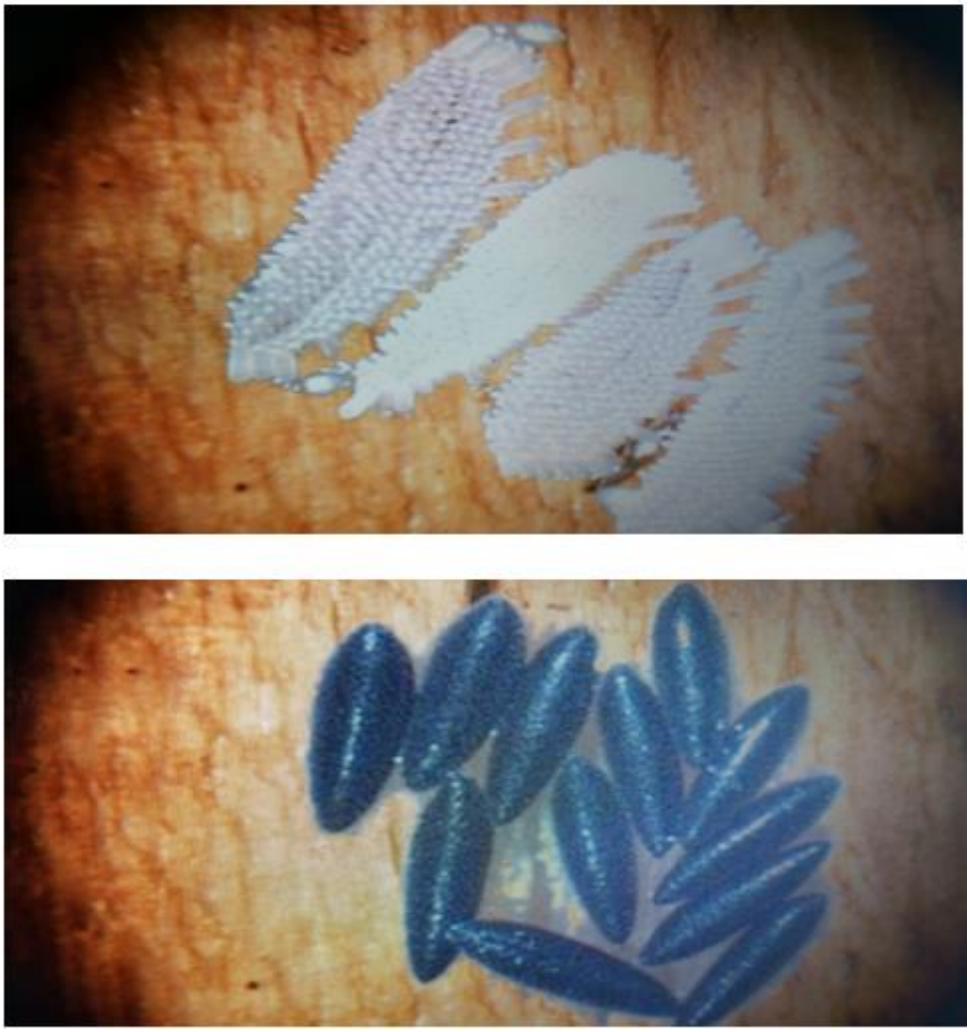


Plate 3.1.5 Eggs of *Aedes aegypti*, egg cluster above and individual eggs enlarged.

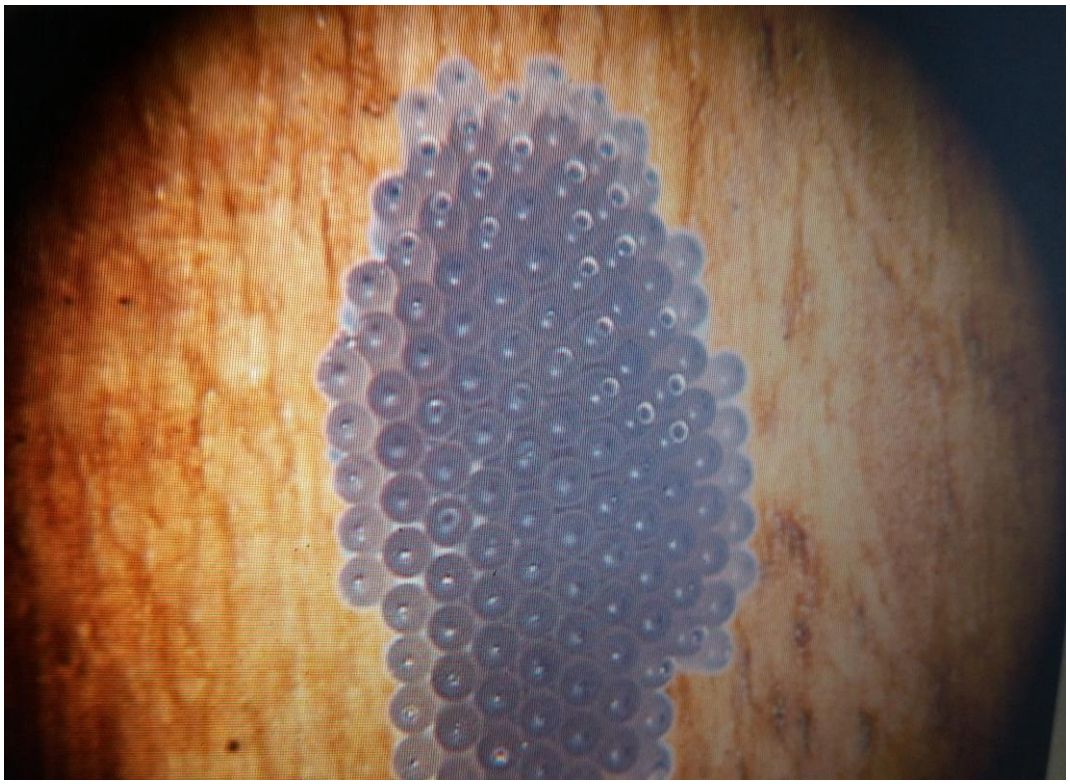


Plate 3.1.6 Eggs of *Aedes aegypti* showing black points of larval heads at hatching.

In this study a female aedes mosquitoes took 6-13 days to lay its full batches of eggs which was similar to that found by Toma *et al.* (2013) and Dieng *et al*. (2013). They found that the a female of aedes mosquito took 6-14 days to lay its full quota of eggs. Almost similar finding was reported by other authors. Slight variable observation was recorded by Chaves (2011) and Nguyen (2012), the mean of their observation of pre-oviposition period was 39.32±5.36 days. Difference could be due to environmental factors and impact of some chemicals in the oviposition sites. The incubation period ranged 48-72 hr with mean of 60±0.53 hr. The time required for both species to take a blood meal passed incubation period was observed to be the same by Soekiman *et al*. (1984), but Hein (1976) reported that *Ae. albopictus* takes longer to feed and having longer incubation period than *Ae. aegypti*.

Farajollahi and Nelder (2009) reported that a single female laid up to 300-600 eggs. Depending on field condition an aedes female mosquito was found to lay 354-597 eggs as reported by Vitek and Livdahl (2006). Variation could be due to weather condition. Takeda *et al* (2003) found that variation of number of eggs depended on available food contents of living habitat and species.

Studies comparing fecundity of the two species have produced conflicting results. Hein (1976), Sucharit and Tumrasvin (1981) and Black *et al*. (1989)

compared total life time fecundity of the two species, and they reported *Ae. aegypti* to be more fecund. Galliard (1962) found *Ae. albopictus* to be more prolific. Soekiman *et al*. (1984) observed that *Ae. aegypti* laid more eggs per batch than *Ae. albopictus* with a Java strain of both. In Hein's (1976) experiments, *Ae. albopictus* laid more eggs per milligram of blood ingested than did *Ae. aegypti*. Sames (1999) found egg production of *Ae. albopictus* dropped significantly at high temperatures, whereas *Ae. aegypti* had only a slight decrease.

Table 3.1.1 Reproduction periods, fecundity and incubation period of *Aedes aegypti* in the laboratory

Character	Unit	No. of observation	Range	Mean ±Sd
Mating frequency	No.	7	3-4	3.43 ± 0.53
Preoviposition period	Day	7	6-8	7.93 ± 0.19
Oviposition period	Day	7	6-7	6.84 ± 0.35
Postoviposition period	Hour	7	24-42	36.00 ± 6.00
Incubation period	Hour	7	48-72	60 ± 0.53
No. of cluster per individual	No.	7	3-4	3.43 ± 0.53
Number of eggs laid per cluster	No.	7	105-129	117.71 ± 9.12
No. of eggs laid per female	No.	7	327-516	386.57 ±64.63
Egg Viability	%	7	78 - 94	87.57 ± 5.19

3.1.3.3. Larval biology

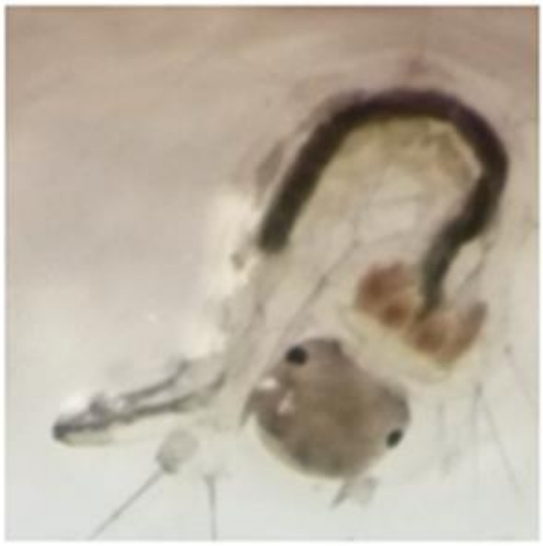
The first instar larva moulted to 2nd instar larvae (Plate 3.1.7A and B). The 2nd instar larvae mouted to 3rd instar (Plate 3.1.7C). The 3rd instar larvae moulted to 4th instar larvae (Plale 3.1.7D). The fourth instar larvae moulted to pupae (Plate 3.1.8) and adult emerged from pupae (Table 3.1.8). The duration of four larval instars was 6-7.5 days. Almost similar observation was found by Hawley (1988). He reported that mosquito larvae undergo four larval stages that required 5 to 10 days for completion. The variation of duration depends on temperature (Anon. 1972) or larval diets (Hawley 1988).

Larvae were emerged and grown in transparent water with some debris having bacteria. Similar studies were found on other research. Aedes mosquitoes larvae were never found in turbulent waters because the larvae were unable to withstand wave action (Bates 1970). The larvae commonly were found in water containing microflora and fauna and debris of plant and animal origin (Clements 1963).

Mosquito larvae moved mainly in two ways, by jerks of the body and by propulsion with the mouth brushes (Anon. 1972). Mosquito larvae normally dived to the bottom when the water surface was suddenly disturbed or if a shadow passes over them (Goma 1966 and Anon. 1972). According to

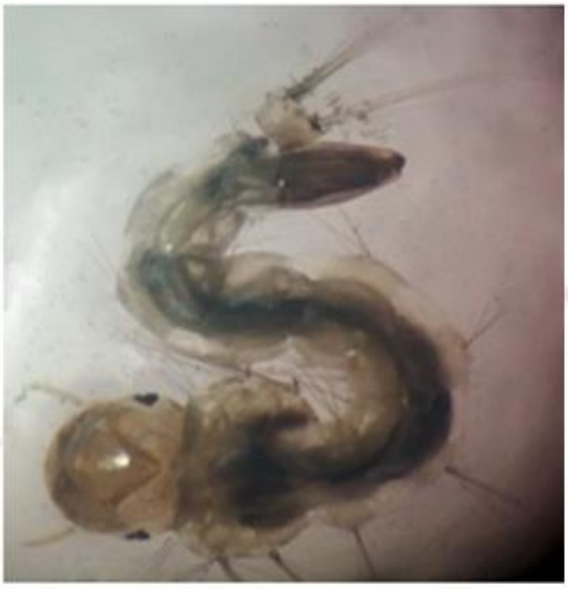
Chistopher (1960), in *Aedes aegypti* and *Aedes albopictus* and other mosquito species, there were four larval instars, each instar terminating with a moult or ecdysis. One of the first sign that ecdysis was about to take place in the appearance of dark bands across the thorax due to the circularly wrapped lateral hairs of the next instar shining though the cuticle.

A. First instar larva



B. Second instar larva

C. Third instar larva



D. Fourth instar larva

Plate 3.1.7 Different instars of *Aedes aegypti* larvae.

3.1.3.4 Pupal biology

After the fourth instar, *Aedes mosquitoes* entered into the pupal stage. Mosquito pupae were different from many other holometabolous insects in that the pupae were mobile and respond to stimuli. Pupae, also called "tumblers," did not feed and take approximately two days to develop (Plate 3.1.8). The pupal duration was ranging from 42 to 48 h with mean 45.43±2.27 h. Adults emerged by ingesting air to expand the abdomen thus splitting open the pupal case and emerging head first.

Similar result was found Dom *et al.* (2013) and Tilak *et al.* (2005). They found that the duration of aedes mosquito pupae was 2 days and pupae were moving but did not take food.

Plate 3.1.8 Pupa of *Aedes aegypti*, red arrow indicates respiratory trumpet of aedes pupa.

3.1.3.5 Adult biology

Just after emerging from pupa adult aedes mosquitoes took rest (Plate 3.1.9) for 20-30 minutes. Female mosquitoes lived longer than male mosquitoes. Longer developmental duration of female compared to male was due to higher longevity of 3rd instar larvae of female as compared to male. The longevity of adult female was 14-19 days with mean of 16.43 days with standard deviation ± 2.07. The life span of female ranged 22-29 days with a mean of 26.23 days having ± 2.17 standard deviation (Table 3.1.2). The mode of reproduction is sexual (Table 3.1.2).

The longevity of adult male was 8-14 days with a mean of 11 days. The life span of male ranged 16 to 23 days with a mean of 19.23 days having ± 2.21 standard deviation (Table 3.1.3). The mode of reproduction is sexual. Almost similar resuilt was revealed by conducting the research of Chen *et al* (2006). According to their observation the longevity of male and female of aedes mosquitoes depending on the species and the conditions, a female mosquito's average lifespan was anywhere from two to four weeks, while a male mosquito lived for one to two weeks.

Adult *Ae. aegypti* is a small to medium-sized mosquito, approximately 3 to 6 millimetres in length, with two white stripes and a single curved line at each side forming a lyre shape on the dorsal thorax (Plate 3.1.11). The abdomen

is generally dark brown to black, and is covered with white scales in the form of stripes and spots which create the unique distinguishing pattern. Each tarsal segment of the hind legs also possesses white stripes. On the other hand *Aedes albopictus* is characterized by its black-and-white-striped legs, and small black-and-white-striped body (Plate 3.1.11).

In terms of physical appearance, the mosquito's proboscis, a long, needle-like antenna that extends from the area of its mouth, is the best indication of the mosquitoes gender (Plate 3.1.12 and 3.1.13). Male aedes mosquitoes have a feather-like proboscis, while the proboscis of the female aedes mosquito was relatively smooth, not bushy. The hair on the antenna is called antennal flagellum. It is a sensory organ. Male aedes mosquitoes use their flagellum to help locate female aedes mosquitos by the (very) quiet buzzing sounds they emit.

Plate 3.1.9 A newly emerged adult of *Aedes aegypti*.

Table 3.1.2 Duration of different life stages of female *Aedes aegypti* in laboratory condition

Length of different stages	Unit	No. of observation	Range	Mean ±Sd
1st instar	Hour	7	36 - 48	44.57 ± 4.72
2nd instar	Hour	7	30 - 36	34.29 ± 2.93
3rd instar	Hour	7	48 - 54	47.28 ± 2.93
4th instar	Hour	7	36 - 54	46.28 ± 4.72
Pupa	Hour	7	42 - 48	45.43 ± 2.27
Adult female	Day	7	14 - 19	16.43 ± 2.07
Life span	Day	7	22-29	26.23 ± 2.17

Table 3.1.3 Duration of life stages of male *Aedes aegypti* in laboratory condition

Length of different stages	Unit	No. of observation	Range	Mean ± Sd
1st instar	Hour	7	36 - 42	44.57 ± 3.72
2nd instar	Hour	7	30 - 36	34.29 ± 2.93
3rd instar	Hour	7	42 - 48	46.28 ± 3.93
4th instar	Hour	7	36 - 42	46.28 ± 3.72
Pupa	Hour	7	42 - 48	45.43 ± 2.27
Adult male	Day	7	8 - 14	11.00 ± 1.91
Life span	Day	7	16-23	19.23 ± 2.21

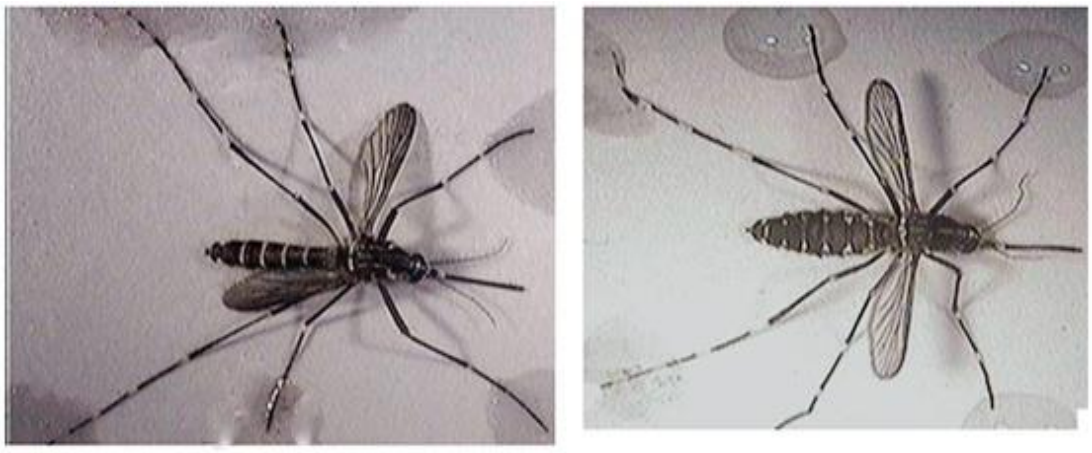


Plate 3.1.10 Adult *Aedes aegypti* (left) and *Aedes albopictus* (right).

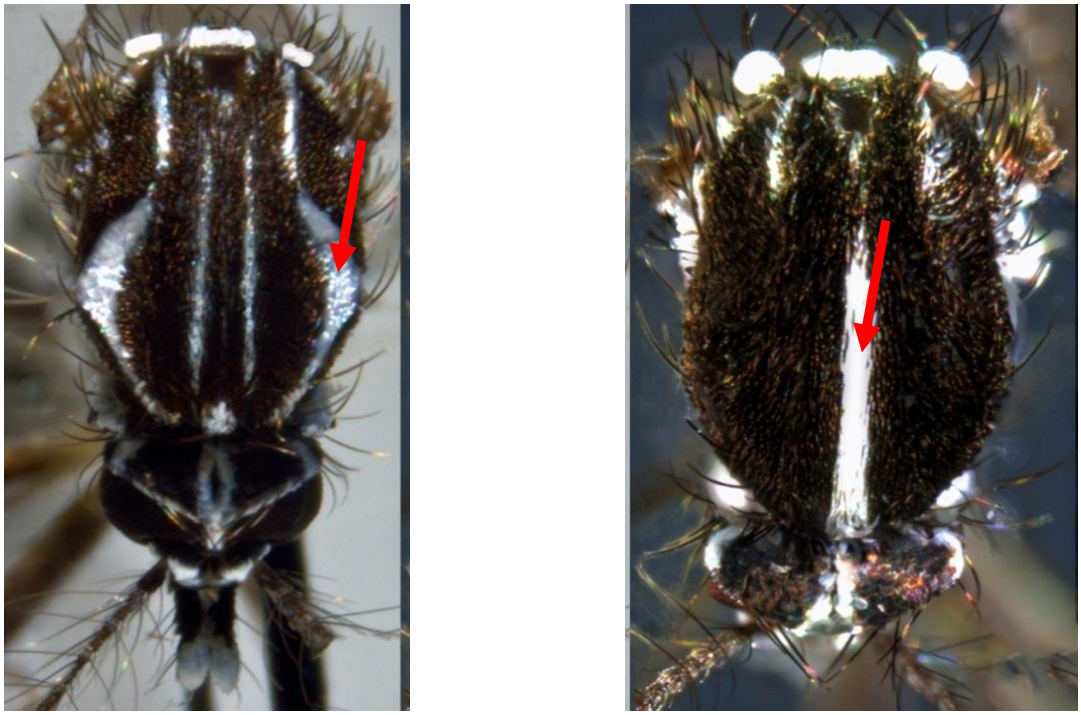


Plate 3.1.11 Thoraxes of *Aedes aegypti*, red mark indicates lyre shape on the dorsal thorax (left) and *Aedes albopictus* only a white stripe instead of lyre (right).

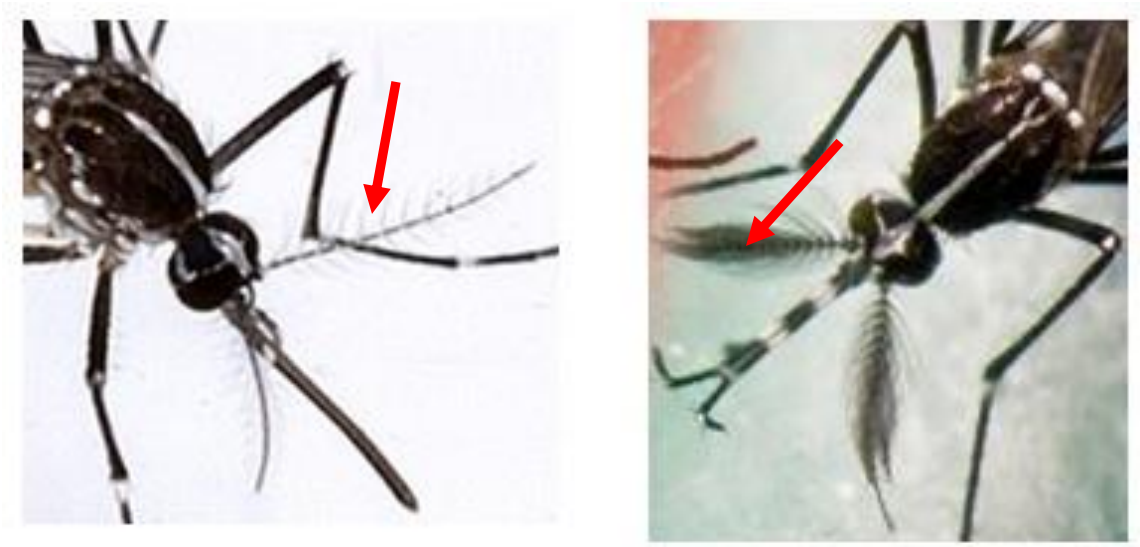


Plate 3.1.12 Antenae of female (left) and male (right) of *Aedes albopictus.*

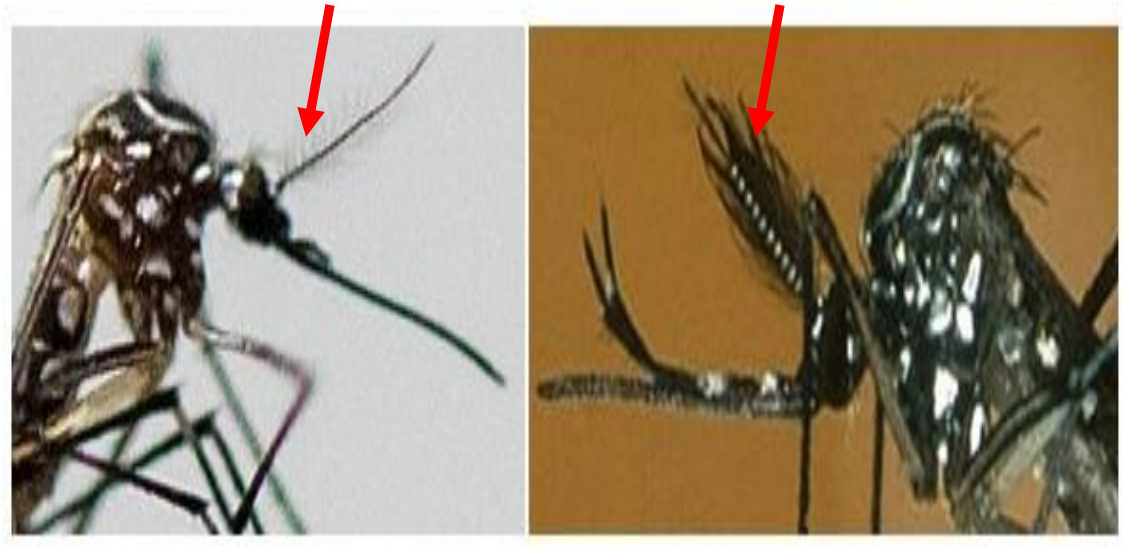


Plate 3.1.13 Antenae of *Aedes aegypti* male (right) and *female* (left) mosquitoes. (Red marker indicates less bushy antenna of female and bushy of male).

EXPERIMENT 3.2

IDENTIFICATION OF VARIOUS BREEDING SITES OF AEDES MOSQUITOES IN DHAKA CITY

IDENTIFICATION OF VARIOUS BREEDING SITES OF AEDES MOSQUITOES IN DHAKA CITY

ABSTRACT

Dengue fever (DF), one of the most important emerging arboviral diseases, is transmitted through the bite of container breeding mosquitoes *Aedes aegypti* and *Aedes albopictus*. A household entomological survey was conducted in Dhaka from July, 2014 to June, 2015 to inspect water-holding containers in indoor, outdoor, and rooftop locations for aedes mosquitoes larvae for determining mosquito productivity of each container type and identifying some risk factors like stagnant water, position of containers etc. of households infested with aedes larvae. Of 9,222 households inspected, 1,306 (14.2%) were positive for aedes larvae. Of 38,777 wet containers examined, 2,272 (5.8%) had aedes larvae. Containers used to hold water, such as earthen jars, tanks, and drums were the most common containers for larval breeding. Tyres in outdoor and rooftop locations of the households were also important for larvae for living. Although present in abundance, buckets were of less importance. Factors such as independent household, presence of a water storage system in the house, and fully/partly shaded outdoors were found significantly associated with household infestation of aedes larvae.

3.2.1 INTRODUCTION

Dengue fever (DF) is one of the most important emerging diseases and serious public health concerns. It is found in tropical and sub-tropical regions around the world, predominantly in urban and semi-urban areas. The disease is now endemic in more than 100 countries in Africa, the Americas, the Eastern Mediterranean, Southeast Asia and the Western Pacific. The container breeding mosquito, *Aedes aegypti*, is the major global vector of dengue viruses, causing around 50 million infections annually (Kay and Nam 2005). Recently the number of reported cases has continued to increase as the disease spreads to new areas (Anon. 2014). In 2014, Japan experienced the first outbreak of dengue fever in almost 70 years. In Bangladesh, the first documented outbreak of dengue occurred in 1964. However, DF has become a serious public health threat in Bangladesh after the first large-scale outbreak in 2000 with 5,551 cases, among which 1,186 (37.6%) cases were dengue hemorrhagic fever. Since 2000, DF cases have been reported every year in all major cities of Bangladesh (Mahmood and Mahmood 2011). Dengue virus is transmitted to humans through the bite of infective female mosquitoes *Aedes aegypti* and *Aedes albopictus*. They breed mostly in artificial water holding containers, but have been reported in natural containers as well (Anon. 2012). In most areas there are a relatively

small number of containers that consistently serve as the primary shelter of aedes larvae, with other containers playing minor roles in mosquito production.

“Key containers” are the primary source of adult aedes mosquitoes (Lloyd 2003). The epidemiological importance of a container class depends on the productivity and the abundance of that specific class of container in the environment. Productivity of a container type depends on a variety of factors, such as size and shape, purpose of use, location (indoors, outdoors, under vegetation, etc.), method of filling (passively/actively rain-filled, manually filled, roof runoff, etc.), lid status (covered/uncovered), material with which the container is made (plastic, metal, cement/clay, etc.), temperature, availability of food, and competition among co-species (Barbosa *et al.* 1972, Moore 1972). Moreover, each ecological setting has its own unique set of key containers (Tun-Lin *et al.*1995).

The container breeding mosquitoes deposited their eggs on damp surfaces within artificial containers such as, cans, jars, earthen pots or rain-water containers. Old automobile tyres provide an excellent larval habitat and an adult resting site. In tropical climates, larvae are also found in natural water retaining in tree holes. The eggs of *Ae. aegypti* can resist desiccation up to 1 year. Eggs hatch when flooded by water that is deoxygenated (Womack

1993). Larval habitats are often shared with other container-breeding mosquitoes in the South-East Asia, such as, *Aedes albopictus,* (Womack 1993). Container breeding mosquito has been favored by poor urban drainage system (like Tejgaon of Dhaka city) such as, an unreliable or absent water supply, which forces residents to store water, or no refuse collection, which results in accumulations of water bearing discards suitable as larval habitats (Gubler 2002). Information on container breeding mosquitoes in Dhaka city is scanty. Survey report by Christophers, 1933 and Barraud 1934 from British India and later by Nasiruddin, 1957 revealed that the mosquitoes breed exclusively in tree-holes and bamboo stumps in Dhaka city. Khan, 1980 found only two mosquito species, *Aedes aegypti* (Linn.) and *Ae*. *albopictus* (Skuse) breeding in artificial containers. Rosenberg and Maheswary (1982) collected a few container habitat mosquitoes from Moulvibazar district, Bangladesh. In the recent years, the city dwellers are attracted more by canned foods and bottled drinks, which they randomly throw away after use, which in the rainy season become a good breeding ground for mosquito.

In Peru, for example, apparently “not useful” containers, located outdoors and passively filled with rainwater, represent the most important category for adult *Ae. aegypti* production (Morrison *et al.* 2004). In Mexico, tyres and

bottles were the most important class of container for the *Ae. aegypti* population (Lloyd *et al.* 1992), whereas in Vietnam water holding containers for household use, such as large concrete tanks and jars, were the main source of immature *Ae. aegypti* development (Kay *et al.* 2002). A "key container" survey for improved dengue vector surveillance and vector control was developed (1994–1997) and implemented on a regional basis in 1997 in Vietnam. This program was selected as one of the "best practices for environmental management of dengue (Lloyd 2003). By focusing on the containers that are consistent producers of larvae and houses that consistently accommodate aedes larvae, control measures can be tailored for the specific needs of the area and people. Once the most productive key containers are identified, targeted control of dengue vectors becomes more affordable and feasible. At the same time, targeted vector control can help minimize the use of chemicals that may be costly and have other long-term health and environment impacts. The aim of this study was to identify the containers which served as primary producers of aedes larvae during the dengue outbreak in the year 2000 in Dhaka. This study also aimed to identify some risk factors for households infested with aedes larvae.

Objectives

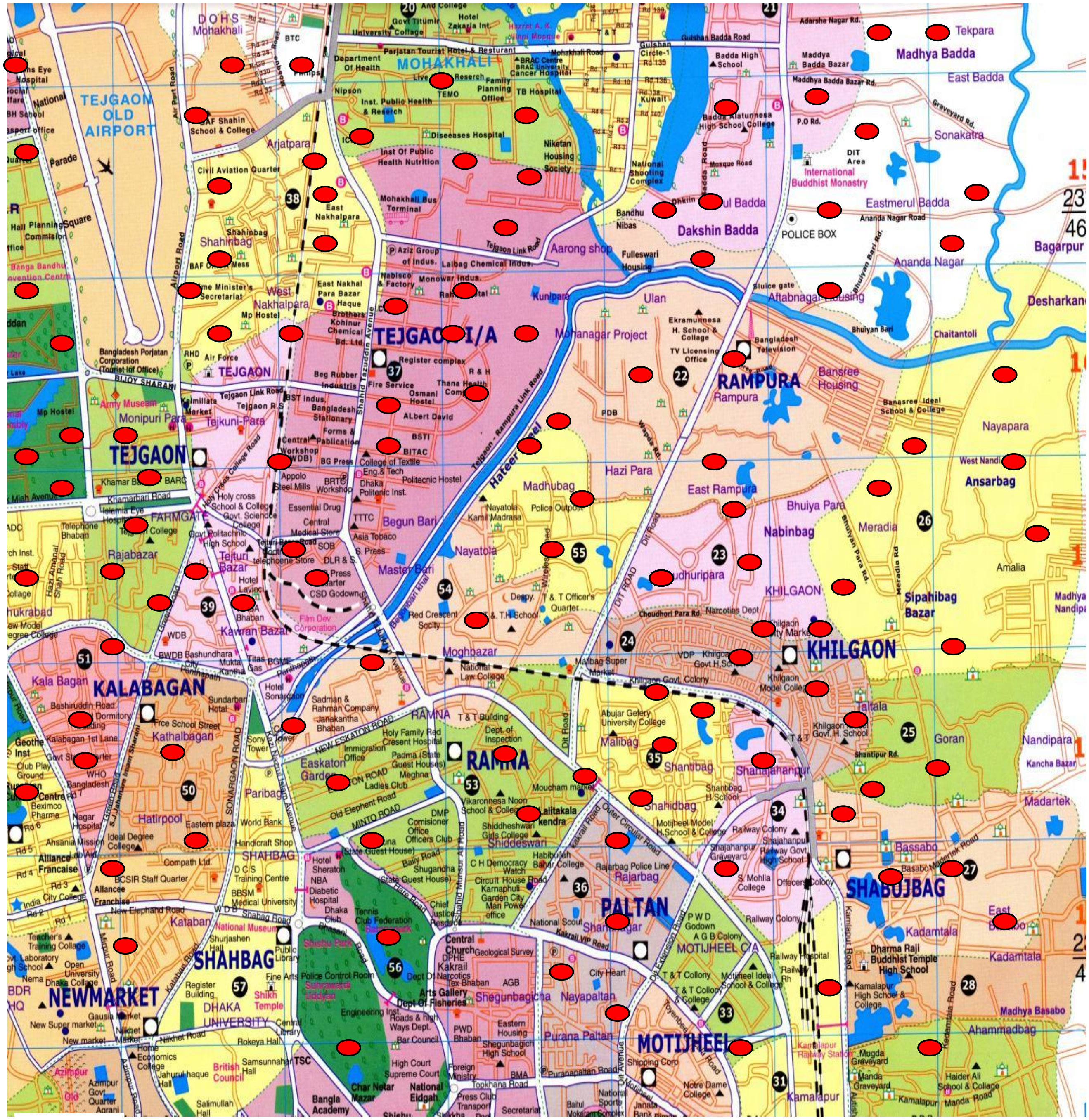
1. To study the breeding habitats in densely populated Dhaka city.
2. To identify the left over container productivity for the breeding of aedes mosquitoes.

3.2.2 MATERIALS AND METHODS

3.2.2.1 Selection of breerding sites

A household entomological survey was conducted in Dhaka city from July, 2014 to June, 2015 for the primary purpose to identify the habitats with high density of aedes mosquitoes in order to prevent the transmission of dengue (Ali *et al.* 2003). This study was conducted within the Dhaka City Corporation (DCC), now divided into Dhaka North City Corporation (DNCC) and Dhaka South City Corporation (DSCC) along with 92 small administrative units called wards. According to 2001 population census, DCC had 1,107,000 households. Dhaka meets all the criteria for rapid breeding of aedes mosquito as the temperature and high rainfall with rapid urbanization and dense population (Hossain *et al.* 2000). For the field survey, around100 households (range 100–119) were selected from each of the 92 wards on random sampling of households with available resources. In each ward, approximately 10 equally scattered points were marked in the map (Figure 3.2.1). All locations were identified by selecting a direction from the center of each selected point in the map by spinning a pen and to visit the required number of households in that direction. Information about distribution of housing types (i.e., independent house, multi-storied house, semi-permanent house, slum, and others) was collected from each of the

ward commissioner's office. A proportional number of households was then selected in each ward according to the distribution of housing types within that ward (e.g., if in a specific ward 10%, 20%, 30%, and 40% of the houses were of each respective housing type, then 10, 20, 30, and 40 households were selected, respectively, representing each type of house). In the study design method, a household was defined as one separate unit of accommodation (individual home or apartment), and the immediately surrounding premises, irrespective of the number of people residing within the unit. The houses were classified as per their construction.



Legend

Study area

Figure 3.2.1 Map of Dhaka City Corporation both North and South.

All three locations of each household, i.e., indoor, outdoor, and rooftop, were inspected during dawn and dusk for potential wet containers. All larvae that could not be identified in the households were collected in labeled specimen bottles and were reared up to the adult stage to identify species and recording data.

A total of 111 types of wet container were found in indoor, outdoor, and rooftop locations of the households. The containers were then categorized into 11 different groups: flower pots, buckets, water tanks, drums, tyres, discarded appliances, plastic bowls, earthen pots, coconut shells, cans and bottles, and others (Plate 3.2.1). All unusual and less abundant container types that eventually were found positive were classified as “others,” such as ant guard, air conditioner drip pan, refrigerator drip pan, polythene bag, bathtub, tree hole, bamboo stump, and leaf axil. Larvae were identified and counted by using magnifying glass. Aspirators and droppers were used to count the larvae. Although buckets, water tanks, drums, plastic bowls, and earthen jars mostly had a common purpose of use, i.e., water storage, It was opted to keep all the varieties instead of a common category in order to obtain a detailed profile of wet containers serving as potential breeding habitats of aedes larvae. Household infestation with aedes larvae was defined as a household having at least one container infested with atleast one aedes larvae.

Plate 3.2.1 Mosquito larvae breeding in various habitats: (a) Tyre (b) Discarded applinces, (c) plastic bowl, (d) tank, (e) earthen pot, (f) polythene sheet, (g) can and bottle, (h) flower pot, (i) drum, (j) bucket, (k) coconut shell.

3.2.2.2 Data Analysis

The larval survey data were calculated and analyzed in terms of different larval survey techniques like house index (HI), container index (CI), and breteau index (BI). The calculation of larval indices is based on the following mathematical formulae (Tun-Lin *et al.* 1995)

$$\text{House Index (HI)} = \frac{\text{Nnumber of houses infested}}{\text{Totol number of houses inspected}} \times 100$$

$$\text{Container Index (CI)} = \frac{\text{Number of positive containers infested}}{\text{Totol number of containers inspected}} \times 100$$

$$\text{Breteau Index (BI)} = \frac{\text{Number of positive containers}}{\text{Totol number of houses inspected}} \times 100$$

A descriptive analysis was done for the distribution of wet containers and aedes larvae in three locations. Firstly, the number of different wet containers in the three locations was listed to identify the most abundant container categories in different locations. Secondly, the percentage of each container category was calculated to determine their larval productivity. Thirdly, the contribution of each container category to total positive containers was calculated.

Finally, the percentage of aedes larvae in each container category was calculated. The relative frequency of each container category as an *Aedes*

larval breeding site in different locations was featured by two-dimensional presentation (Moore *et al.*, 1978). Slope = 1 was considered as the equality line. If the containers were equally utilized as breeding sites, all points fell on the equality line. If the percentage of positivity of any container category exceeded the percentage of contribution to total wet containers (slope > 1), the point for the container fell above the equality line. This container was then considered to be an essential container for *Aedes* larval breeding. Conversely, less importance was indicated for the container having a slope of < 1 (i.e., if the point falls below the equality line). Logarithm scale was used to show both larval incidences.

3.2.3 RESULTS AND DISCUSSION

3.2.3.1 Breeding sites of aedes mosquitoes

The results of the entomological survey in Dhaka city are outlined in Table 3.2.1. Of total 9,222 households inspected, 1,306 households (14.2%) were found positive for aedes larvae. Multi-storey houses were the highest in number with 39.6% following by semi-permanent houses of 30.4%, independent houses 20.5% and remaining slums and other houses. Household positivity rate was the highest in independent houses (18.6%) and the next slum houses (14.3%), semi-permanent houses (12.9%), and multi-storey houses (12.8%). Out of total 38,777 examined in all inspected households, aedes larvae were found in 2,272 wet containers (5.78%) (Table 3.2.1). Out of 38777 total wet containers the mean number of wet containers per household was 4.20 (SD = 4.99). The number of wet containers was abundant in outdoors (56.5%) following indoors (32.2%) and rooftop (11.3%) (Table 3.2.2). Among the outdoor containers, 7.8% containers were found positive as infested with aedes larvae. Among the indoor and rooftop containers, 3.1% and 3.9% of the containers were found positive, respectively. The overall House Index (HI) was 14.2. Breteau Index (BI) 24.6, and Container Index (CI) 5.9 (Table 3.2.1). All of the indices showed a

high level of risk for dengue transmission reported by Pan American Health Organization (1994).

Table 3.2.1 Types of household inspected and percentage of positive household infested by larvae of aedes mosquitoes in Dhaka city

House types	Total house inspected		Total positive house		Total container inspected (No.)	Total positive container (No.)	Risk indices		
	(No.)	(%)	(No.)	(%)			HI	CI	BI
Independent houses	1890	20.5	352	18.6	10742	669	18.62	6.23	35.40
Multi-storey houses	3651	39.6	466	12.8	11822	646	12.76	5.46	17.69
Semi-permanent houses	2801	30.4	364	12.9	7674	374	13.00	4.87	13.35
Slum houses	771	8.36	110	14.3	7764	463	14.27	5.96	60.05
Others	109	1.18	14	12.8	775	80	12.84	10.32	73.39
Total	**9222**		**1306**	**14.2**	**38777**	**2272**	**14.20**	**5.90**	**24.60**

HI= House Index, CI= Container Index, BI = Breteau Index

*Positive house means the house infested by aedes mosquitoes

Table 3.2.2 Percentage of the Number of different types of wet containers in different locations of households in determining aedes mosquitoes breeding habitat

Types of containers	Wet container inspected at different locations of households						Total container inspected	
	Indoor		Outdoor		Rooftop			
	No.	%	No.	%	No.	%	No.	%
Bucket	3753	30.03	2342	10.69	485	11.08	6580	51.8
Flower pot	2434	19.4	2546	11.62	1086	24.58	6066	55.6
Can and bottle	1565	12.52	3133	14.30	336	7.67	5034	34.49
Earthen jar	876	7.01	3641	16.62	501	11.44	5018	35.07
Drum	1532	12.26	1211	5.53	202	4.6	2945	22.39
Tank	654	5.23	1655	7.56	366	8.36	2675	21.15
Coconut shell	2	0.02	2623	11.98	135	3.0	2760	15
Plastic bowl	1305	10.44	1511	6.90	182	4.10	2998	21.44
Discarded appliances	127	1.02	757	3.46	270	6.10	1154	10.58
Tyre	159	1.27	851	3.89	277	6.30	1287	11.46
Others	592	4.74	1532	6.99	136	3.10	2260	14.83
Total	**12499**	**56.5**	**21902**	**32.2**	**4376**	**11.3**	**38777**	**100**

3.2.4.2 Outbreak of aedes mosquitoes in different houses

The houses that were infested by aedes mosquitoes consisted of independent house, multistoried house, semipermanant house, slum house and others. The investigations were carried out into three locations such as indoor, outdoor and rooftop of each of the houses. The number of houses visited contained different types of wet containers in which the larval development took place. These houses are regarded as positive houses.

3.2.3.1.1 Independent house: These were brick-built single family homes. These houses were either single floor or duplex surrounded by boundary

with plantation. The total number of containers inspected for aedes mosquitoes larvae in independent houses in indoor, outdoor and rooftop placement or location was 10742 and among them 669 were positive. The contribution of individual placement for aedes larvae as indoor, outdoor and rooftop of independent house was 5.42%, 9.8% and 4.32% respectively with total of 19.54% positive (Table 3.2.3).

3.2.3.1.2 Multistoried house: These were brick-built apartment houses having two or more floors. More than one family lives in these houses. The total number of containers inspected for aedes mosquitoes larvae in multistoried houses in indoor, outdoor and rooftop placement was 11822 among them 646 was positive. The contribution of individual placement for aedes larvae as indoor, outdoor and rooftop of independent house was 2.21%, 9.5% and 13.69% respectively with total of 25.4% positive (Table 3.2.3).

3.2.3.1.3 Semi-permanent house: The walls of this type of house are made of bricks, or cement concrete but the roofs are made of other materials, such as bamboo, tin, thatch, etc. The total number containers inspected for aedes mosquitoes larvae in semi-permanent houses in indoor, outdoor and rooftop placement for aedes larvae was 7674 and among them 374 was positive. The contribution of individual placement as indoor, outdoor and rooftop of

independent house was 3.03%, 5.84% and 4.76% respectively with total of 13.63% infested with aedes mosquitoes larvae (Table 3.2.3).

3.2.3.1.4 Slum house: Slum houses are poorly-built congested tenements, usually with inadequate infrastructure. Each slum area is designated by the local government. The total number containers inspected for aedes mosquitoes larvae in slum houses in indoor, outdoor and rooftop placement was 7764 among them 463 was positive. The contribution of individual placement as indoor, outdoor and rooftop of independent house was 3.13%, 8.53% and 3.14% respectively with total of 14.81% positive (Table 3.2.3).

3.2.3.1.5 Others: Others include schools, institutions, offices, factories, mosques, markets, etc. The total number of containers inspected for aedes mosquitoes larvae in independent houses in indoor, outdoor and rooftop placement was 775 and among them 80 was positive. The contribution of individual placement as indoor, outdoor and rooftop of independent house was 2.03%, 5.59% and 2.1% respectively with total of 9.72% infested with aedes mosquitoes larvae (Table 3.2.3).

Table 3.2.3 Types of wet containers inspected in indoor, outdoor and rooftop of various houses and positive containers infested by larvae of aedes mosquitoes in Dhaka city

House type	Household Location	Total container inspected (No.)	Positive container inspected (No.)	Positive container inspected (%)
Independent Houses	Indoor	2345	97	5.42
	Out door	6532	513	9.8
	Roof top	1865	59	4.32
	Sub total	**10742**	**669**	**19.54**
Multi-stored houses	Indoor	2812	60	2.21
	Out door	6269	553	9.5
	Roof top	2741	33	13.69
	Sub total	**11822**	**646**	**25.4**
Semi-permanent houses	Indoor	2543	77	3.03
	Out door	4900	326	5.84
	Roof top	231	11	4.76
	Sub total	**7674**	**374**	**13.63**
Slum houses	Indoor	2265	71	3.13
	Out door	4067	347	8.53
	Roof top	1432	45	3.14
	Sub total	**7764**	**463**	**14.81**
Others	Indoor	100	50	2.03
	Out door	545	143	5.59
	Roof top	130	12	2.1
	Sub total	**775**	**80**	**9.72**
	Total	**38777**	**2272**	**5.78**
	Mean	2600.53	151.47	
	SD	± 20.88	± 69.56	

3.2.3.3 Distribution of wet containers in different household locations

The number of each container category inspected in the three locations or placements of houses is described in details below. There were 11 types of wet containers for breeding aedes mosquitoes in the houses (Appendix II and III). The larval development was apparently visible in these containers and these were holding water called wet containers suitable for larva development. Among the wet containers, buckets were the most abundant (n = 6580) following flower pots (n = 6066), cans and bottles (n = 5034), and earthen jars (n = 5018). Other water-holding containers, as drums (n = 2945), and tanks (n = 2675) were also high in number (Figure 3.2.2). Among indoor wet containers Buckets (30.03%), flower pots (19.4%), and drums (12.26%) were common while earthen jars (16.62%), cans and bottles (14.3%), and other wet containers (7.39%) were common among outdoor wet containers. Among rooftop wet containers, flower pots (24.59%), and earthen jar (11.44%) (Figure 3.2.3) were the most abundant.

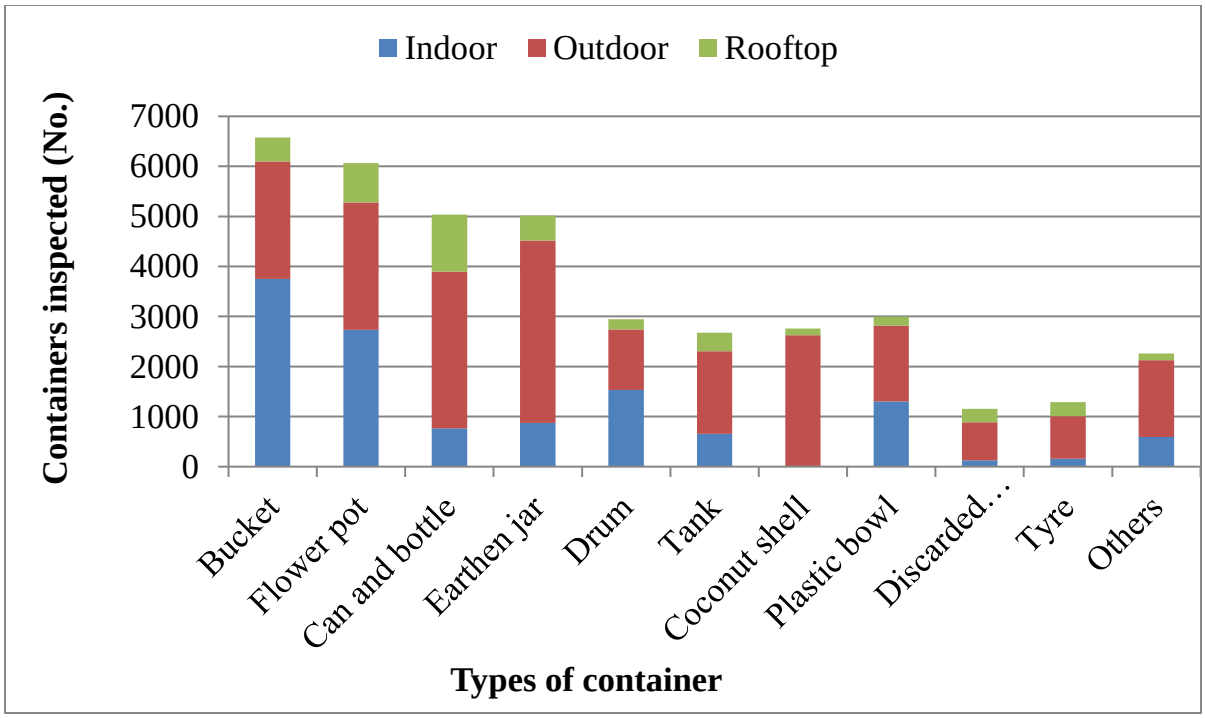


Figure 3.2.2 Total number of wet container inspected in three locations for aedes mosquitoes.

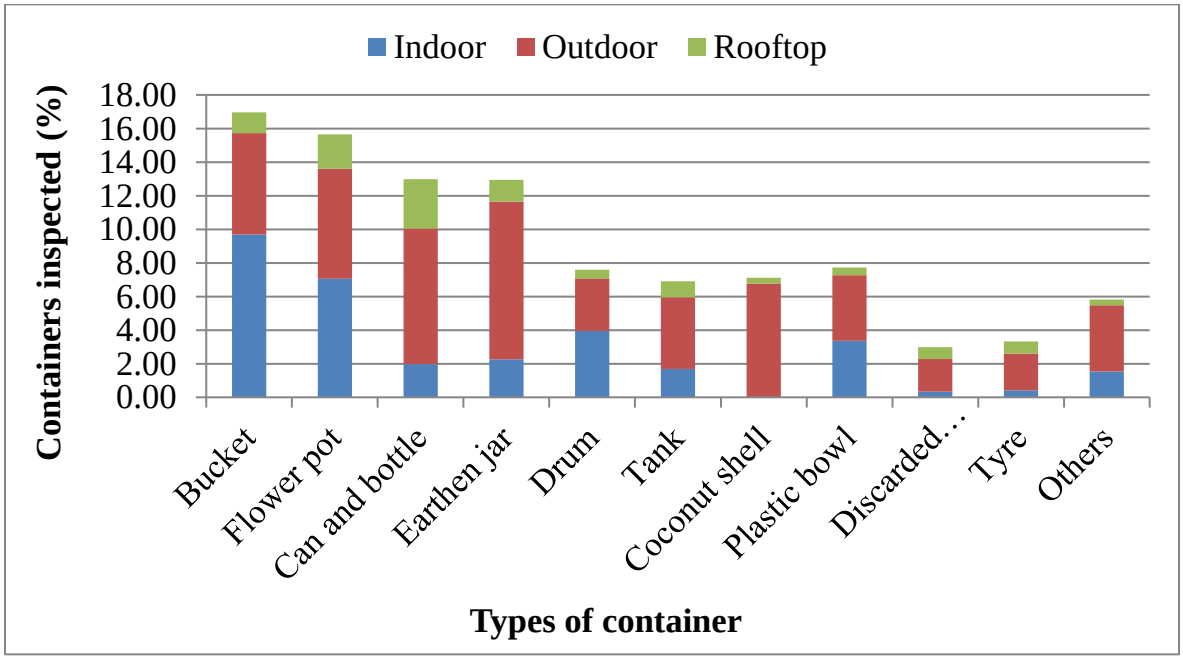


Figure 3.2.3 Percentage of wet containers inspected for larvae of aedes mosquitoes at three locations of household.

3.2.3.4 Productivity of different types of wet containers for aedes mosquitoes

The number of each container category inspected in the three locations is detailed in page 73 Among 2,272 positive containers in which larvae were observed and then recorded, tyres were the most abundant (n = 559) following earthen jars (n = 452), tanks (n = 282), can and bottle (n= 263), flower pot (n = 242), buckets (n = 123) and other water-holding containers together as rest of the numbers (n = 351) (Figure 3.2.4).

Figure 4.4 shows the percentage of each container category infested with aedes larvae. Among the positive containers inspected the highest percentage of positive containers was tyre (Figure 3.2.5) having 24.6%. Among the tyres inspected in three places, indoor, outdoor and rooftop, 23.28% was the highest in outdoor placement for aedes larvae. The next five highly positive containers were earthen jars (19.9%), tanks (12.4%), cans and bottles (11.58%), flower pot (10.65%) and drums (9.85%). Most of the positive tyres (23.28%), earthen jars (16.64%), cans and bottles (9.07%), flower pot (4.53%), tanks (2.68%) and drums (5.41%) were found outdoors (Figure 3.2.5).

In indoor placement tank was the highest positive container occurred 9.24%. Next to the tank three containers were drum (3.87%) and flower pot (3.26%). In roof top flower pot (2.86%) was the highest infested container.

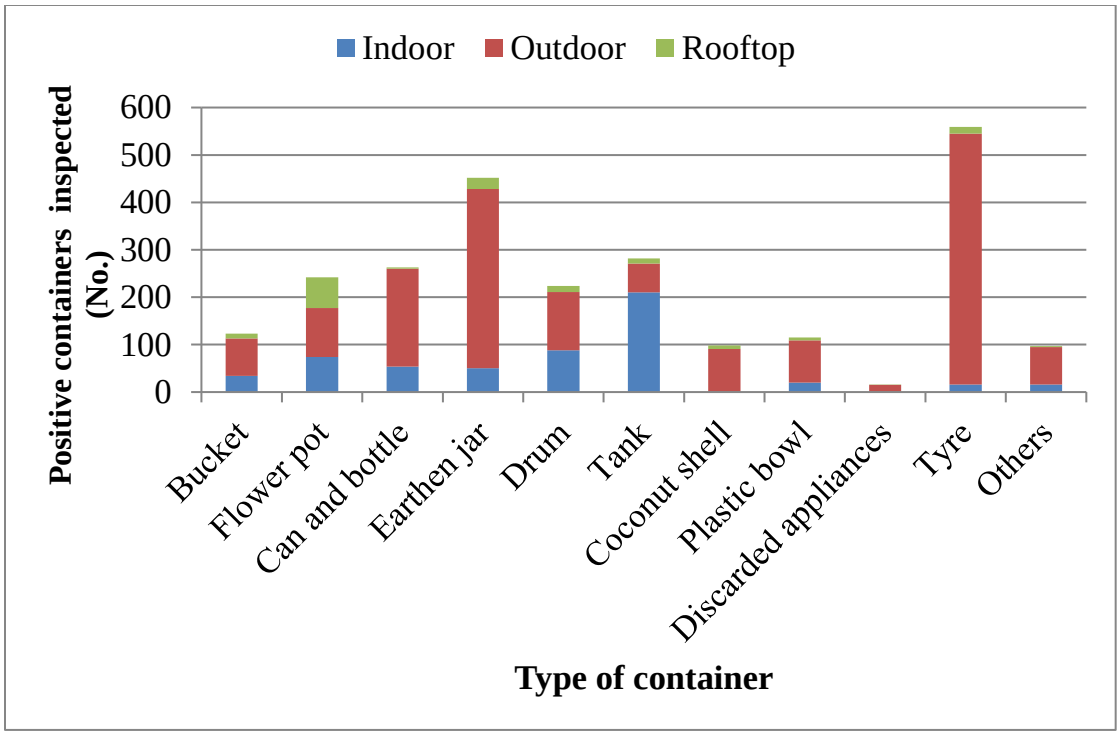


Figure 3.2.4 Number of positive container for aedes mosquitoes.

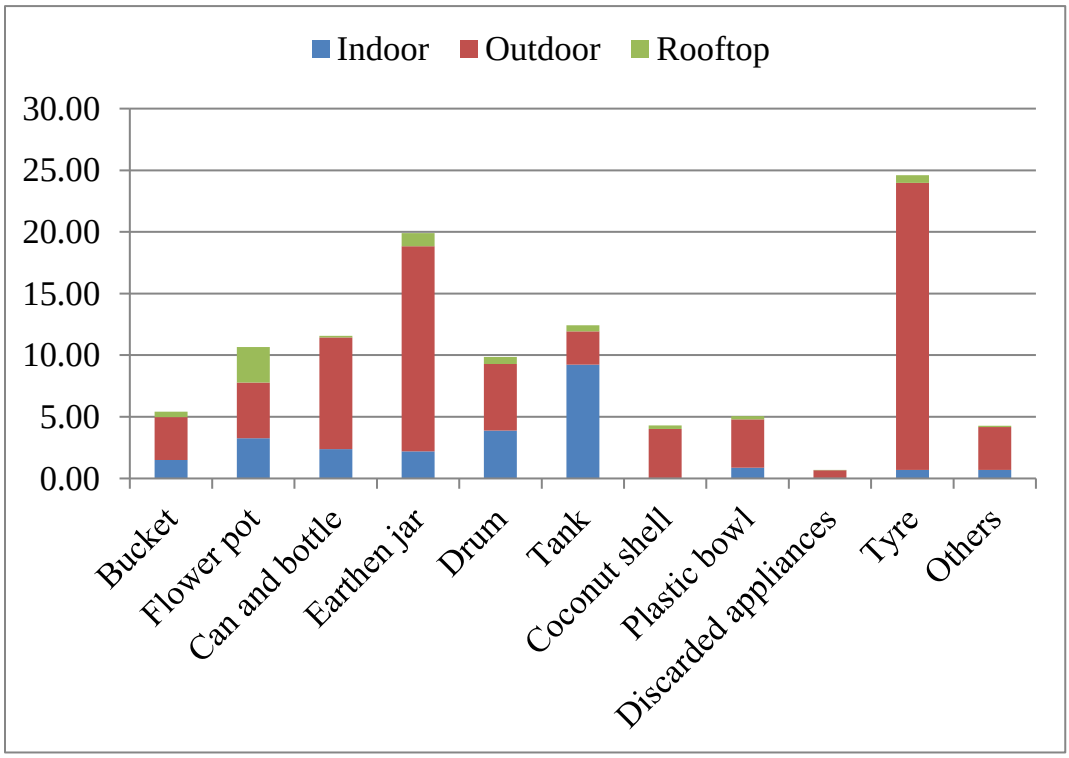


Figure 3.2.5 Percentage of positive containers infested with larvae of aedes mosquitoes at three locations of household.

3.2.3.5 Individual productivity of positive containers in respect to different placement

Among all the positive containers for aedes mosquitoes larvae surveyed in three distinct places as indoor, outdoor and rooftop, the highest breeding placement of aedes mosquitoes was in outdoor for most of the containers except tank. Figure 3.2.6 showed that tyres (94.63%) was the highest in outdoor and next to tyre coconut shell (91.84%), discarded appliance (87.5%), earthen jar (83.63%) and others (81.44%) among the productivity of individual containers and the lowest infested containers was tank (21.63%) in outdoor.

The second highest placement of positive containers for mosquitoes larvae was indoor occurred the highest value of tank (74.47%) and then drum (39.29%) and flower pot (30.58%).

Sequentially the lowest infested area was rooftop surveyed in three breeding sites. Results depicted in Figure 2.3.6 that flower pots (26.86%) were the highest productive containers in rooftop area and rest of the containers were negligible of aedes larval infestation in rooftop.

Individual container infestation of aedes mosquitoes in different location overall tyres, earthen jars, flower pots, tanks, and drums were found

essential containers for aedes larval breeding. Less importance was indicated for buckets, cans and bottles, and discarded appliances.

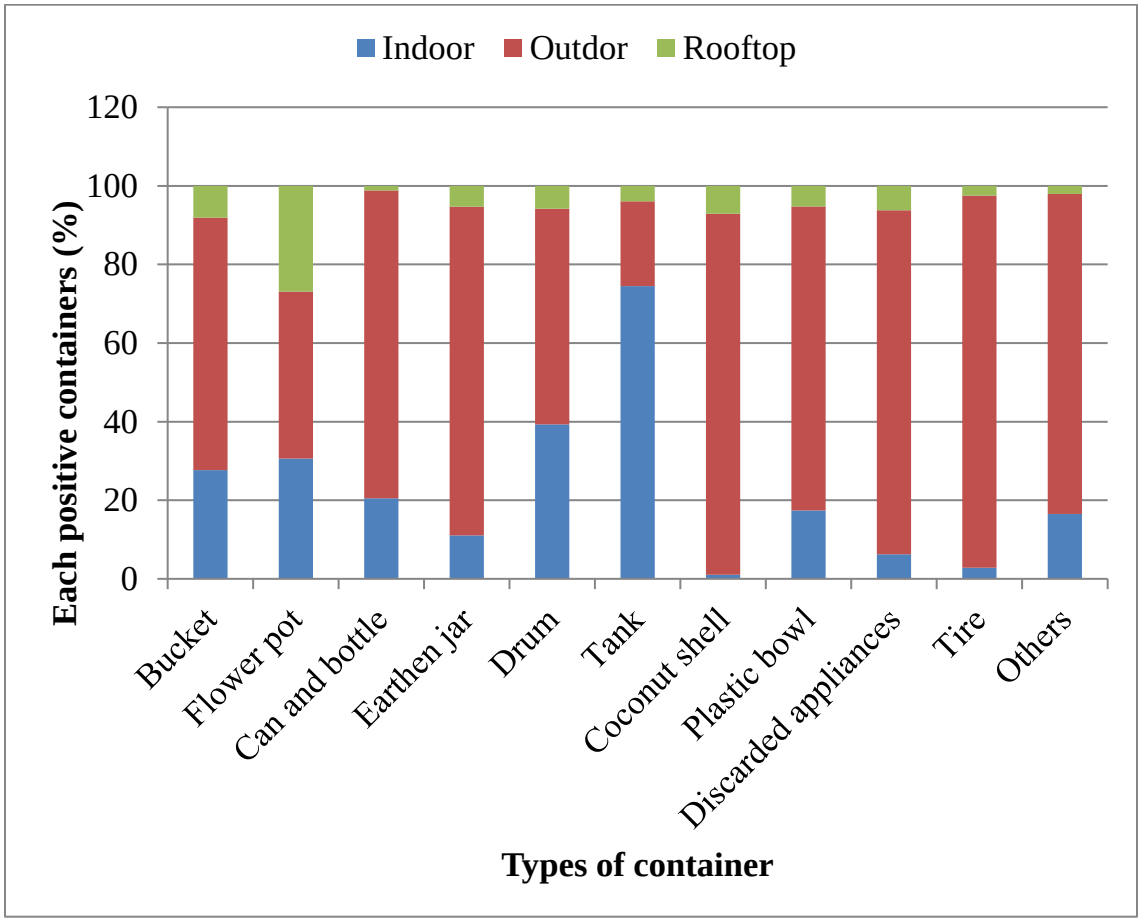


Figure 3.2.6 Percentage of each positive container for aedes mosquitoes at three locations of household.

3.2.3.6 Relative risk determination among breeding sites for aedes mosquitoes

Among indoor containers, tanks were found the most suitable container for aedes larval breeding. Tanks constituted only 5.23% of all wet containers in indoors but accounted for 9.24% of all positive containers in indoor placement. Similarly, drums and flower pots constituted 12.26% and 19.4% of all wet containers, respectively, but accounted for 3.87% and 3.26% of all positive indoor containers (Figure 3.2.7). Therefore, drums and flower pots might also be considered as essential containers indoors next to tank. On the other hand, buckets represented 30.03% of all indoor containers but accounted for only 1.50% of all indoor positive containers. Therefore, buckets fell below the equality line and considered as less importan. Tyres constituted only 3.89% of all outdoor containers but accounted for 23.28% of all outdoor positive containers. Earthen jars represented 7.01% and 16.64% of all outdoor containers and all outdoor positive containers respectively. Similarly, cans and bottles, flower pots and drums constituted 14.03%,11.62 and 5.53% of all wet containers, respectively, but accounted for 9.07% , 4.53% and 3.92 of all positive containers in outdoor(Figure 3.2.8). Therefore, tyres, cans and bottles, earthen jars, flower pots, and drums could be considered as essential containers in outdoors placement. Buckets outdoors, as indoors, were found less important for aedes larval

breeding. Buckets accounted for only 3.48% of all outdoor positive containers in spite of representing 10.69% of all outdoor containers. Among the outdoor containers, less importance was also indicated for tanks as well as discarded appliances. Tyres and drums were found the most important containers in the rooftop location (Figure 3.2.9). Flower pots constituted 24.58% and 2.86% of all rooftop containers and all positive containers, respectively. Earthen jars constituted 11.44% of all rooftop containers but accounted for 1.06% of all rooftop positive containers. Buckets represented 11.08% of all rooftop containers but accounted for 0.44% of all rooftop positive containers. Therefore, buckets in the rooftop location were found third essential containers. Similarly drum and tank were in same important area of aedes mosquitoes larvae breeding. Cans and bottles, tyres and coconut shells were in the border line and rest of the containers were less important.

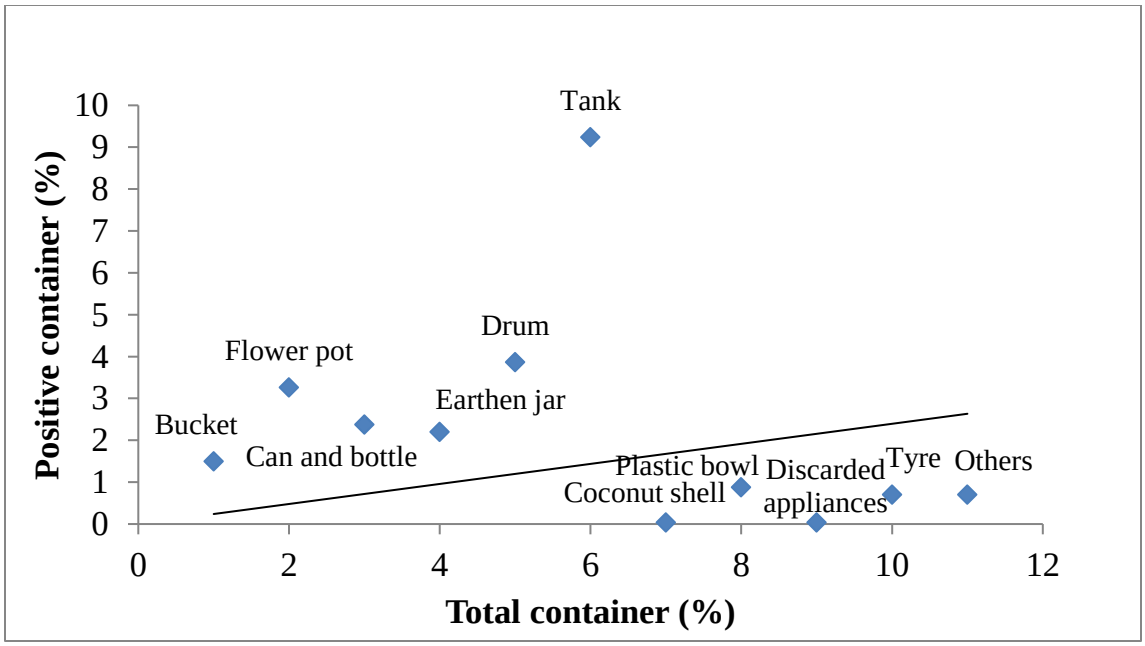


Figure 3.2.7 Two-dimensional presentation for relative risk of wet containers in indoors placement.

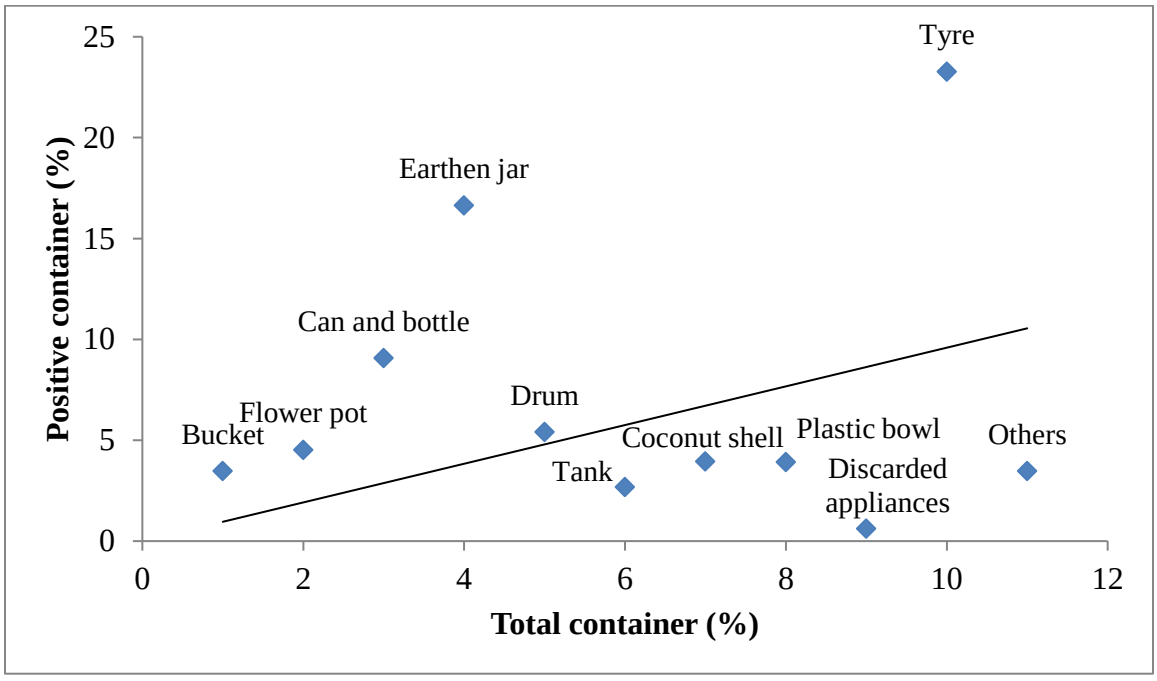


Figure 3.2.8 Two-dimensional presentation for relative risk of wet containers in outdoor placement.

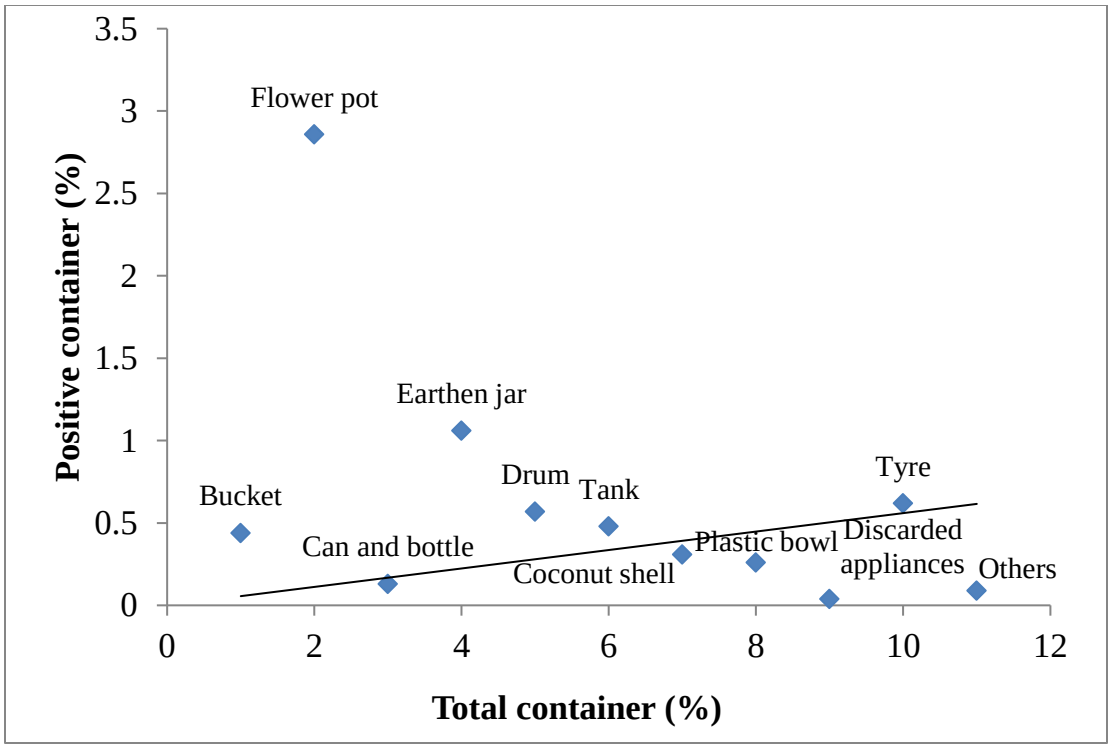


Figure 3.2.9 Two-dimensional presentation for relative risk of wet containers in roof top placement.

3.2.3.7 Larval population of two species of aedes mosquitoes in three locations of houses

Two species of aedes mosquito larvae were found to occur in different wet containers of three locations such as indoor, outdoor and rooftop of different houses of Dhaka city. These two species were *Aedes aegypti* and *Aedes albopictus.* Their total numbers are shown in logarithm scale in figure 3.2.10. A total of 3,027,867 aedes larvae encountered, among which 1,923,648 (63.5%) were *A. aegypti* and 110419 *A. albopictus.* The density of both types of larvae was higher in outdoors compared to the other two locations. The ratio of the total number of *Ae. Aegypti* larvae in the three locations was 8 : 39.6 : 1 (indoor : outdoor : rooftop) and *Ae. albopictus* was 0.9 : 276.7 : 1 (indoor : outdoor : rooftop). About 99% of *Ae. Albopictus* were found outdoors. The number of *Ae. aegypti* was higher than the number of *Ae. albopictus* in all three locations (92.7 : 1, 1.4 : 1, and 9.9 :1 indoors, outdoors, and rooftop, respectively).

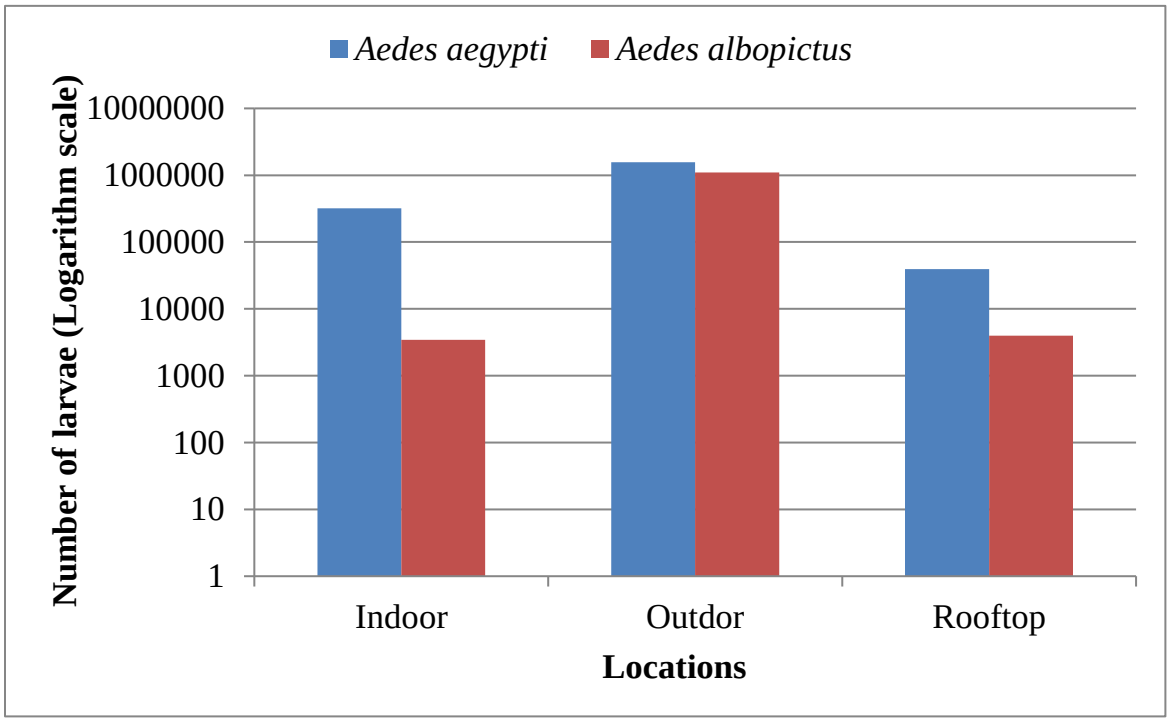


Figure 3.2.10 Incedence of two species of aedes larvae occurred in wet containers located at three locations in inspected households in Dhaka city.

3.2.3.7.1 Larval population of aedes mosquitoes at indoor

The larval productivity of *Aedes aegypti* and *Aedes albopictus* in wet containers at indoor locations is listed in Table 3.2.4. The container productivity as percentage of larvae recorded in the indoor, tank showed the highest productivity of 80.2% larvae for *A. aegypti* and drum 10.1%. In flower pot and can and bottle, 5.2% and 3.1% respectively were found. Bucket, earthen jar and tyre containers showed very few numbers of larvae while other containers did not have any larvae. The larval population of

Aedes albopictus was less than that of *Aedes aegypti* and the highest productivity recorded 35.6% in drum and 33.5% in tank (Table 3.2.4). Floer pot and can and bottle contained 4.8% larvae and the remaining seven containers a very few to no larvae were found.

3.2.4.7.2 Larval population of aedes mosquitoes at outdoor

Data **(**Table 3.2.5) express the productivity of wet containers in outdoor location for *Aedes aegypti* and *Aedes albopictus* by the presence of their larvae. The overall productivity of the containers in outdoor was five times higher than that of indoor location. The container productivity as percentage of larvae recorded in the outdoor, tyre showed the highest productivity of 46.1% larvae for *A. aegypti* and earthen jar 20.9%. In can and bottle and flower pot, 12.3% and 9.0% respectively were found. Tank and bucket with almost same number of larvae as 3.1% and 3.0% respectively.Rest of the five containers containers showed very fewnumber of larvae. The larval population of *Aedes albopictus* was less than that of *Aedes aegypti* and the highest productivity recorded 86.2% in earthen jar and 3.5% in flower pot (Table 3.2.5). Can and bottle contained 3.0% larvae, tank with 2.8% and the remaining six containers a very few to no larvae were found.

3.2.3.7.3 Larval population of aedes mosquitoes at rooftop

Two species of aedes mosquitoes *Aedes aegypti* and *Aedes albopictus* larval productivity in wet containers in rooftop locations is listed in Table 3.2.6. The overall productivity of the containers in rooftop was eight times less than that of indoor location of *Aedes aegypti* larvae and almost similar to *Aedes albopictus* larval population. In case of total larval population in rooftop comparing with that of outdoor location, the container productivity in rooftop was 40 times less than that of outdoor location of *Aedes aegypti* and 320 times less than that of *Aedes albopictus* larval population. The larval productivity as percentage of larvae recorded in the rooftop, flower pot showed the highest productivity of 71.1% larvae for *Ae. aegypti* and earthen jar 8.3%. In bucket, drum and tank, 7.6% and 6.4% and 2.1%respectively were found. Rest of the six containers containers showed very fewnumber of larvae. The larval population of *Aedes albopictus* was less than that of *Aedes aegypti* and the highest productivity recorded 30.14**%** in earthen jar and 27.22% in flower pot (Table 3.2.6). Drum contained 18.66% larvae, tank 5.51, bucket with 4.67%, tyre 4.41, can and bottle 4.02%, coconut shell 2.07 %, plastic bowl 1.56% and discarded appliances 1.1% respectively and in the remaining one container a very few larvae were found.

Table 3.2.4 Container productivity for two species of aedes larvae in indoor locations of household in Dhaka city

Type of containers	Population of *Ae. aegypti*		Population of *Ae. albopictus*	
	Total larvae		Total larvae	
	(No.)	(%)	(No.)	(%)
Tank	255161	80.2	1149	33.5
Drum	32159	10.1	1222	35.6
Flower pot	16659	5.2	126	4.80
Can and bottle	9712	3.1	126	4.80
Bucket	7852	0.38	17	0.54
Earthen jar	1134	0.01	21	0.67
Tyre	23	0.01	45	1.72
Polythene sheet	12	0.00	16	0.50
Plastic bowl	11	0.00	14	0.45
Discarded appliances	05	0.00	13	0.41
Coconut shell	00	0.00	00	0.00
Total	**318226**	**100.00**	**3434**	**100.00**

Table 3.2.5 Container productivity for two species of aedes larvae in outdoor locations of household in Dhaka city

Type of containers	Population of *Ae. aegypti*		Population of *Ae. albopictus*	
	Total larvae		Total larvae	
	(No.)	(%)		(No.)
Tyre	721182	71.12	4464	0.4
Earthen jar	328079	8.33	945044	86.2
Can and bottles	192673	7.58	33059	3.0
Flower pot	141372	6.38	38151	3.5
Tank	48211	2.13	30380	2.8
Bucket	47239	0.92	1452	0.1
Coconut shell	24013	0.89	1243	0.14
Plastic bowl	1987	0.77	1123	0.12
Discarded appliances	1543	0.69	543	0.11
Drum	1231	0.58	493	0.05
Polythene sheet	0	0.45	0	0
Total	**1565894**	**100**	**1096821**	**100.00**

Table 3.2.6 Container productivity for two species of aedes larvae in rooftop locations of household in Dhaka city

Type of containers	Population of *Ae. aegypti*		Population of *Ae. albopictus*	
	Total larvae		Total larvae	
	(No.)	(%)		(No.)
Flower pot	28113	71.1	420	27.22
Earthen jar	3294	8.3	465	30.14
Bucket	2996	7.6	172	4.67
Drum	2521	6.4	288	18.66
Tank	841	2.1	85	5.51
Tyre	245	0.64	68	4.41
Can and bottle	132	0.34	62	4.02
Coconut shell	95	0.25	32	2.07
Plastic bowl	87	0.23	24	1.56
Discarded appliances	79	0.21	17	1.10
Polythene sheet	45	0.12	10	0.65
Total	**39528**	**100.00**	**3964**	**100.00**

3.2.3.8 Productive containers in different locations

Our analyses revealed that water storage containers, such as tyres, earthen jars, tanks, flower pots and drums were consistently more likely to contain aedes larvae. Similar results were found in previous studies (Koenraadt *et al.*, 2007 and Maciel-de-Freitas *et al.*, 2007). Indoor tanks and drums were the most productive; while outdoor tyres and earthen jars were the most productive. Rooftop earthen jars, flower pots and drums were highly productive. Although present in abundance, buckets did not contribute much to larval production. Understanding the cultural traditions of owning and using containers is important to identify the essential containers in different locations. Dhaka city has a scarcity of domestic water supply, and 87.7% of the municipal water supply is mainly derived from groundwater (Anon. 2011). Most of the city dwellers store supplied pipe water. They either send the pipe water directly to rooftop tanks or store it in underground reservoirs and pump it to the rooftop tanks. Underground reservoirs are categorized here as outdoor tanks. As the municipal water supply is not guaranteed all the time, people store water in drums, earthen jars, buckets, and indoor tanks for use in emergencies and tyres and flower pots are kept in outdoor and rooftop respectively where stagnant water reserved. Tanks in outdoor locations and rooftop are

normally kept covered and closed; therefore, these reservoirs are protected from mosquitoes. Buckets are relatively smaller in size compared to other water storage containers and are frequently used for washing clothes, cleaning house, and transferring water from one place to another. These practices would reduce the chance of larvae breeding in buckets. Previous studies also reported that weekly cleaning of the water-holding containers was effective in the control of larval production (Arunachalam *et al* 2010 and Phuanukoonnon *et al.*, 2005). However, apparently unattractive or frequently cleaned containers, if present in large numbers, may still serve as potential breeding sites for a large portion of the aedes population. On the other hand, drums, earthen jars, and indoor tanks are bigger in size than buckets and contain a large volume of water. Water in these containers is never emptied and is replenished periodically. A study in Rio de Janeiro found that open-mouthed and large containers are the most suitable or larval production (Maciel-de-Freitas *et al.,* 2007). Moreover, containers outdoors like tyres, earthen jars, cans and bottle and flower pots on rooftops are not always covered, sometimes unintentionally allowing them to collect rainwater and, therefore, making them perennial breeding sites for aedes mosquitoes (Strickman *et al.,* 2003 and Kittayapong *et al.,* 1993). The most important breeding site in outdoor

was tyres. Around 27% of tyres were found infested with Aedes larvae. In outdoor places 46.1% of the aedes larvae were found in tyres. Usually tyres are left abandoned. The collected rain water in tyres is an ideal source of *Aedes* larvae (Lloyd, *et al.*, 1995). Some recent studies use different container parameters while evaluating container productivity. A study in Thailand developed a container-classification method that consists of the shape (S), use (U), and material (M) of the container (SUM-method). Size or volume of the container, exposure to sunlight, presence of abate, cover status, and filling methods of the containers were also considered to determine the container productivity for aedes larvae and pupae (Koenraadt, 2007 and Maciel-de-Freitas et al., 2007). In this study there was no detailed information on these container parameters.

3.2.3.9 Aedes larval population

In this study, *Aedes aegypti* was found two times higher in number than *Ae. albopictus*. Moreover, *A. aegypti* was found the dominant indoor breeder, while *A. albopictus* showed higher affinity for outdoor containers. Previous studies on the habitation of aedes mosquitoes showed that *A. albopictus* usually seems to be restricted to wooded areas adjacent to human habitations. Conversely, *A. aegypti* can be found in a variety of urban habitats including the highly urbanized areas without wooded

vegetation (O'Meara *et al.,* 1995). Additionally, *A. aegypti* depends highly on human blood and tends to bite and rest indoors, whereas *Ae. albopictus* feeds on a variety of vertebrates outdoors (Scott *et al.*,1993) Therefore, *A. aegypti* predominates in highly urbanized areas, specially indoor containers.

Conversely, *Ae. albopictus* predominates outdoor containers. It seems that *Ae. aegypti* is better adapted than *Ae. albopictus* to the environment of crowded tropical cities like Dhaka. The present study found that indoor tanks were the highest productive containers for *A. aegypti*, while outdoor earthen jars were the highest productive containers (86%) for *A. albopictus*. Although a high percentage of tyres was found positive, they contained large numbers of aedes larvae. One possible reason may be that they contained stagnant water with suitable temperature than other water storage containers.

EXPERIMENT 3.3

DISTRIBUTION AND SEASONAL ABUNDANCE OF AEDES MOSQUITOES IN DHAKA CITY

DISTRIBUTION AND SEASONAL ABUNDANCE OF AEDES MOSQUITOES IN DHAKA CITY

ABSTRACT

The seasonal prevalence of *Aedes aegypti* and *Aedes albopictus* was studied in Dhaka city divided into eight divisions with 25 thanas from July, 2014 to June, 2015. The abundance of different stages such as eggs, larvae, pupae and adult mosquitoes was observed throughout the year with variation in different seasons. The outdoor survey using containers for breeding purpose showed that the eggs were the most abundant and larval, pupal and adult population respectively were less than that of eggs. The peak of the population of all stages occurred in June when the rainfall and temperature were the highest. From September to April population level remained low due to low rainfall and temperature. The seasonal weather conditions such as rainfall, temperature and humidity influenced significantly on the population abundance resulting positive correlation particularly with rainfall.The dengue disease occurred predominantly in rainy season and almost nil at winter season. This disease was most severe in the month of July and also June and August. The severity of dengue patients was decreasing from September to November.

3.3.1 INTRODUCTION

Two species of aedes mosquitoes such as *Aedes aegypti* and *Aedes albopictus* are recorded in Bangladesh (Yunus *et al.* 2001). Aedes mosquitoes are recognized as vector of dengue fever and its severe form is dengue hemorrhagic fever (DHF). Now, it causes a serious threat to many countries of Asia including Bangladesh. The increase of dengue cases in this part of the world is due to such factors as rapid population growth, expanding unplanned urbanization, inadequate water supply and difficulties in refuse disposal. These have led to an abundance of new mosquito-breeding sites especially for the urban vector mosquito, *Ae. aegypti*. Dengue fever was unknown in Bangladesh until an outbreak occurred in 1964, known as "Dacca fever" (Aziz *et al.* 1967). Several entomological studies also showed the presence of aedes mosquitoes in Dhaka City, but its number was few (Ameen and Moizuddin 1973, Nasiruddin 1952).

The first report of the presence of dengue vector mosquitoes in good numbers was recorded in an ovitrap survey in early 80s (Khan and Ahmed 1986). They set ovitraps in the new and old part of the city where 22 positive areas were found out of 23 areas checked. The real alarming information came from the spot checked by the WHO consultant in the different areas of Dhaka and Chittagong City when Breteau Index (BI) in Dhaka City was

30.8, a figure well above the risk levels (Knudsen 1997). A thorough check during and just after the outbreak in 2000, revealed the vector position in Dhaka city. The overall density of eggs, larvae, pupae and adults aedes mosquitoes ranged from 23.28 ± 14.19 to 556 ± 103.94, 12.36 ± 10.33 to 451.76 ± 103.42, 5.92 ± 7.11 to 356.72 ± 102.06 and 3 ± 4.79 to 291.44 ± 91.85 respectively in different areas of Dhaka city. Out of 92 wards of Dhaka city 46 wards were above 20 BI (Chowdhury *et al.* 2000). The health department of Bangladesh Govt. also reported a high BI for Dhaka City (BI-50).

The increased number of vector mosquitoes resulted in increased number of cases and finally caused the outbreak in the year 2000. The number of dengue cases increased in the year 2000 not only in Dhaka city but also in other cities, e.g. Chittagong, Khulna, Barishal and Rajshahi (Yunus *et al.* 2001, Ahmed *et al.* 2001). It is known that insects are exceedingly sensitive to temperature and rainfall; tropical and temperate species frequently showed great variations in abundance in different seasons (Samways 1995). The reproduction of *Ae. aegypti* and *Ae. albopictus* from tropical to subtropical divisions occurs all the year round and their abundance is associated with rainfall (Micieli and Campos, 2003, Kalra *et al.* 1997, Chadee 1992, Moore *et al.*1978). Bangladesh is situated in the subtropical

region where temperature varies in different season and rainfall occurrs in a particular period. The present yearlong survey was conducted to find out the seasonal density of eggs, larval, pupal and adult population of dengue vector depending on temperature fluctuation and rainfall. The adult mosquito is directly affected by temperature, relative humidity and rainfall, but larval life is mainly affected by rainfall and water temperature (Micieli and Campos 2003). In this study temperature of all wet container's water could not be taken due to resource constraint. The seasonal density here was calculated based on the rainfall only. This study was a part of detailed entomological surveillance activities carried out in Dhaka City. The other objective of the study was to see whether aedes population has established here in great density and going to spread the dengue as a perennial problem as it happened in other cities of South East Asian region (Rudnick and Lim 1986).

The present study was conducted to know the following objectives

Objectives

1. To find out the distribution pattern of aedes mosquitoes in Dhaka city.
2. To study the seasonal abundance of aedes mosquitoes in Dhaka city and its effects on dengue disease.

3.3.2 MATERIALS AND METHODS

The study was conducted to find out the distribution and abundance of aedes mosquitoes in Dhaka city from July 2014 to June 2015.

3.3.2.1 Locations for data collection

The locations for the study of seasonal abundance of aedes mosquitoes were randomly selected 25 thanas (Table 3.3.1) out of 49 in Dhaka city. Total 250 plastic containers were placed in 25 thanas (Figure 3.3.1 to 3.3.7) considering 10 plastic containers for each thana to estimate aedes mosquito density.

Table 3.3.1 List of 25 thanas under eight divisions of Dhaka Metropolita city

Divisions	Thanas
Tejgaon	Tejgaon, Sher-e-bangla Nagar , Mohammadpur
Mirpur	Mirpur, Pallabi, Kafrul
Gulshan	Gulshan, Cantonment, Khilgaon, Badda
Uttara	Uttara, Turaag
Ramna	Ramna, Shahbagh, Hazaribagh, Dhanmondi , Kamrangirchar
Motijheel	Motijheel, Paltan
Lalbagh	Lalbagh, Kotwali , Sutrapur
Wari	Demra , Shyampur, Shabujbagh

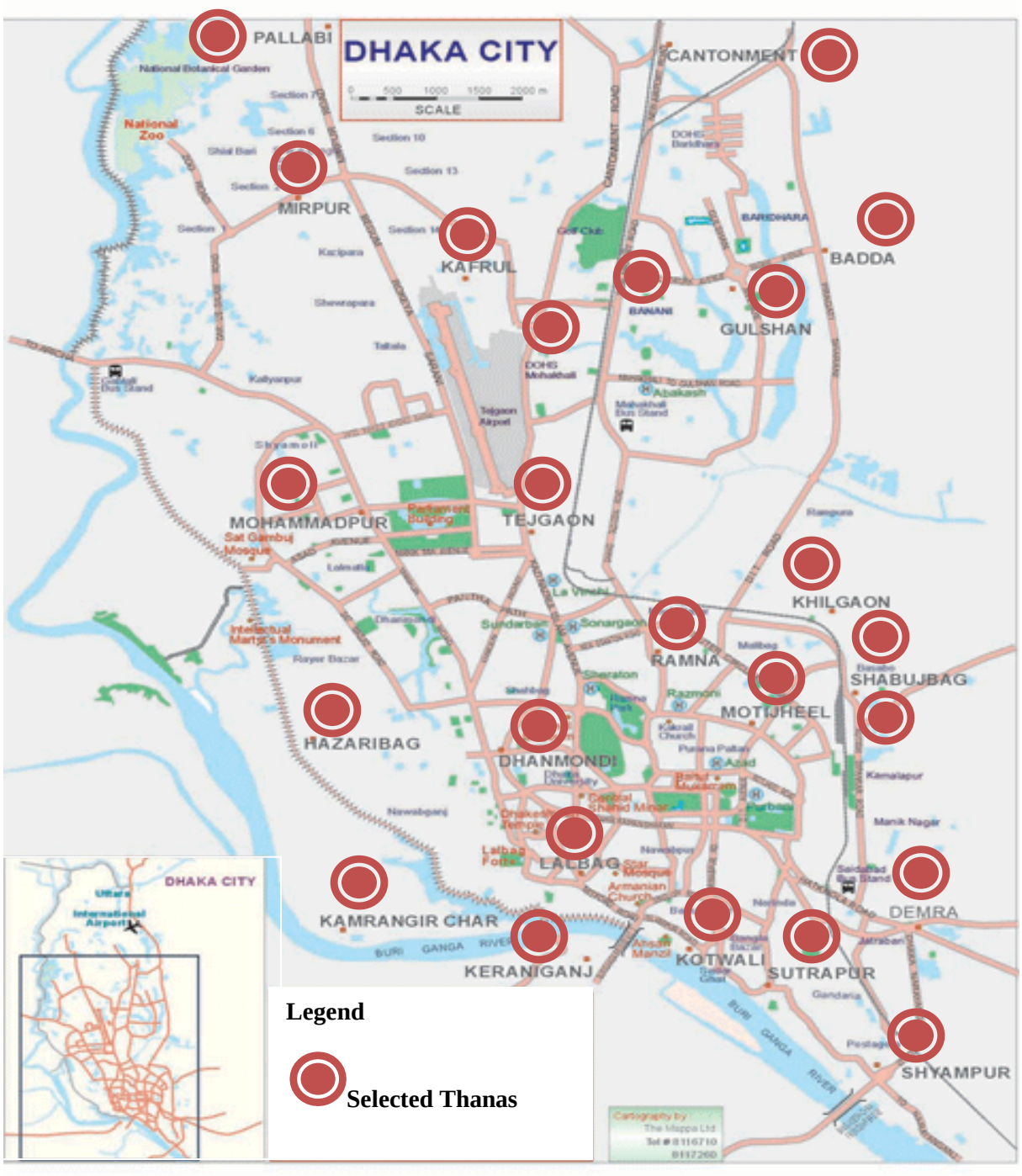


Figure 3.3.1 Dhaka Metropolitan City Map showing study locations.

Plate 3.3.1 Container used for collecting aedes mosquitoes.

Plate 3.3.2 Aedes mosquitoes ovitrap covered with net.

Plate 3.3.3 Containers placed in different places for oviposition of aedes mosquitoes.

Plate 3.3.4 Seperation of different stages (larvae and pupae) of aedes mosquitoes with aspirator.

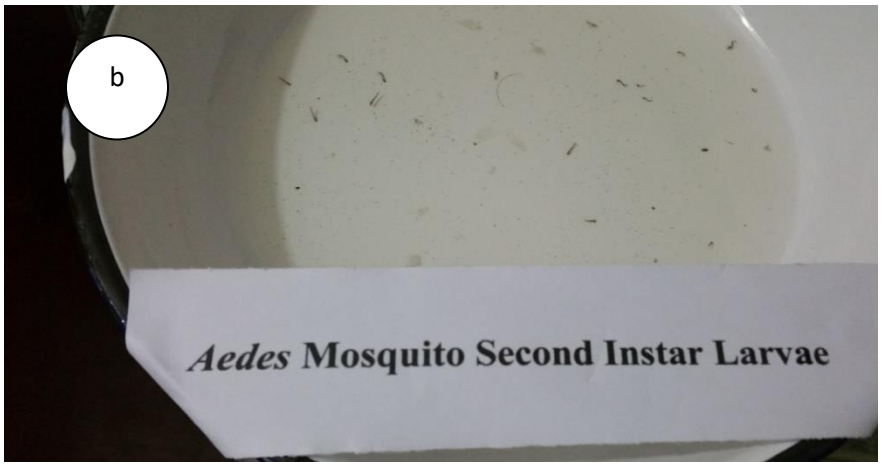


Plate 3.3.5 (a) 1st instar larvae and (b) 2nd instar larvae.

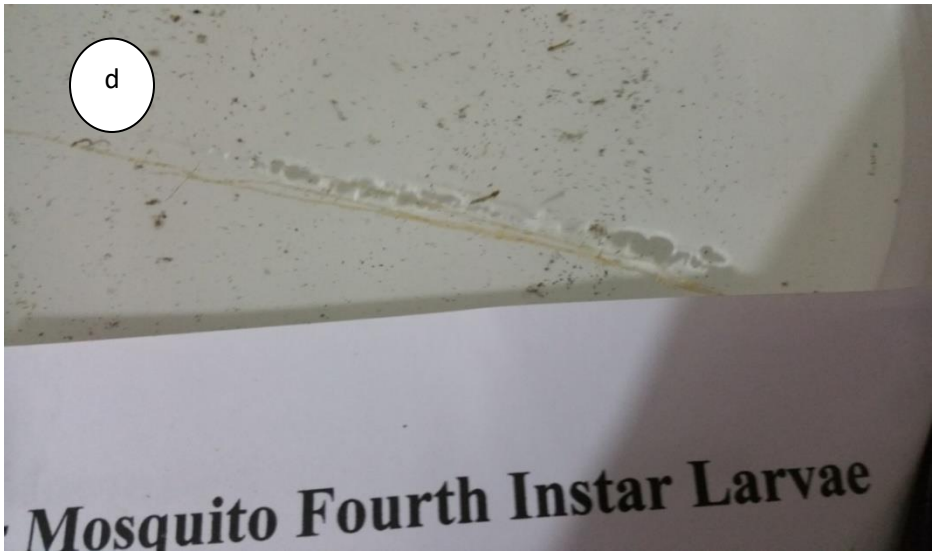


Plate 3.3.6 (c) 3rd instar larvae and (d) 4th instar larvae.

Plate 3.3.7 Accumulation of all stages of aedes mosquitoes larvae (left) and pupae (right).

3.3.2.2 Data collection

Ten plastic containers were placed beside each thana with water. The number of eggs, larvae, pupae and adults of aedes mosquitoes on respective locations were counted one by one using aspirator and droppers at 2 days interval. Temperature, humidity and rainfall of different months were recorded. The weather data on monthly average temperature, humidity and rainfall of different seasons were obtained from the weather office, Bangladesh Meteorological Department, Sher-e-Bangla Nagar, Dhaka. Relationship of aedes mosquitoe population with three weather factors was analyzed statistically. Their relationship was shown studying correlation coefficient and coefficient of determination.

The data were usually recorded at the time between 10 A.M and 12 noon.

3.3.2.3 Identification of aedes mosquitoes larvae

After hatching the first instar larvae from the eggs, they were developed into 2^{nd}, 3^{rd} and 4^{th} instars. The larvae of different instars were placed on slides with a few drops of water and were examined under a compound binocular microscope. The *Aedes sp*. were identified according to the method of Cheong (1986) and Anon. (1995). After examination the larvae of *Aedes* sp. were placed in a plastic container covered with mosquitoes net until adult

emergence. The numbers of adult male and female mosquitoes emerged were recorded.

3.3.2.4 Processing of the samples

After emerging adult mosquitoes the plastic containers were covered with a piece of fine mosquito net. The samples were observed every day for detecting the laying eggs. In the case of dry sample, water was added for the hatching of the eggs. After hatching, the larvae were counted for each collection container.

3.3.2.5 Survey of dengue patients in Dhaka city

For a comprehensive surveillance of dengue cases in Dhaka city 25 remarkable hospitals (Table 3.3.2) were surveyed. A pre-designed and structured questionnaire was used to collect data from the patients as well as from the personnels of respective hospital information center. Number of dengue patients in relation to the potential breeding sites of aedes mosquitoes in different seasons throughout the year was recorded from 25 hospitals of Dhaka City.

Table 3.3.2 List of hospitals surveyed for dengue patients in Dhaka city

SL NO.	Name of the hospital	Address
1	Bangabandhu Shiekh Mujib Medical University	Shahbagh, Dhaka.
2	BIRDEM	Shahbagh, Dhaka.
3	Shahid Suhrawardy Hospital	Sher-e-Bangla nagar, Dhaka.
4	ICDDRB	Mohakhali,
5	IBN SINA Hospital	Dhanmondi, Dhaka.
6	LABAID Hospital	Dhanmondi, Dhaka.
7	Gastroliver Hospital and Research Institute	Green Road Dhaka.
8	Sir Salimullah Medical College and Hospital	Mitford, Dhaka.
9	Squar Hospital	Panthopath, Dhaka.
10	United Hospital,	Gulshan, Dhaka.
11	Monoawara Hospital	Shidishwary,Dhaka.
12	Neurology Foundation and Hospital	Lake Circus, Kalabagan Dhaka.
13	Islami Bank Hospital 24/B,	Outer Cercular Road, Dhaka.
14	Green Land Hospital	Azampur,Uttara, Dhaka
15	Dhaka National Hospital Ltd	Dhanmondi R/A Dhaka.
16	Dhaka Medical College and Hospital	Polashi, Dhaka
17	Institute of Gerecitric Medicine	Agargaon, Dhaka.
18	Aysha Memorial Specialized Hospital	Mohakhali,Dhaka.
19	Ad-Din Hospital	Moghbazar, Dhaka.
20	Bangladesh Medical College and hospital	Dhanmondi, Dhaka.
21	Al- Helal Speacialist Hospital	Rokeya Sarani Senpara Mirpur, Dhaka,
22	Brighton Hospital	Sonargaon Road Hatirpool, Dhaka
23	Anowar Khan Modern Medical College and Hospital	Dhanmondi, Dhaka.
24	Popular Medical College and Hospital	Dhanmondi, Dhaka.
25	Apollo Hospital	Bashundhara, Dhaka.

3.3.3 RESULT AND DISCUSSION

3.3.3.1 Monthly population dynamics of aedes mosquitoes

A total of 250 wet (water filled) containers was placed in the surveyed area. The aedes mosquitoes appeared during all months of the year. The population was in January with mean of eggs, 23.28 ± 14.19 larvae, 12.36 ± 10.33 pupae, 5.92 ± 7.11 and 3 ± 4.79 adults (Figure 3.3.2). Their number gradually increased showing peak in June with 556 ± 103.94 ggs, 451.76 ± 103.42 larvae, 356.72 ± 102.06 pupae and and 291.44 ± 91.85 adults and decreased thereafter. The smallest population of aedes mosquitoes occurred in December although the number decreased in September to November almost similar to December. The winter months showed small population of aedes mosquitoes.

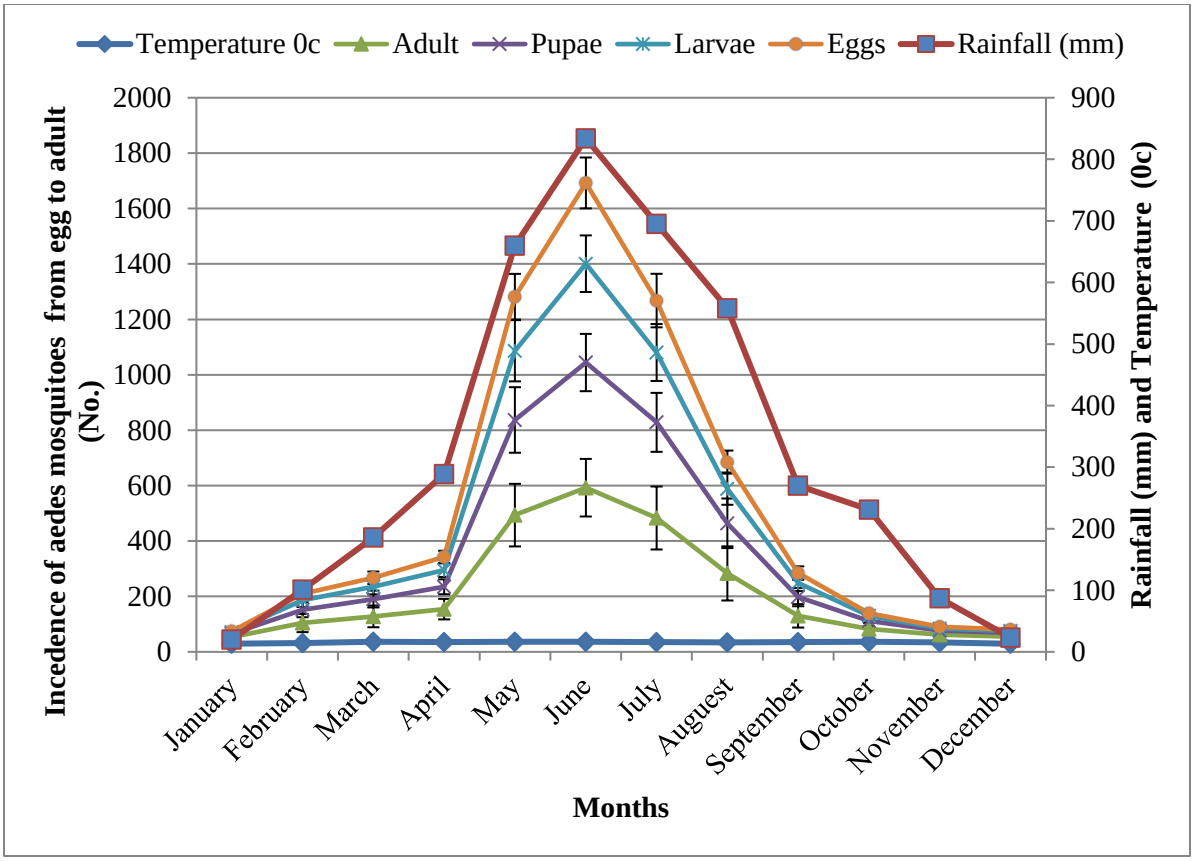


Figure 3.3.2 Mean number of aedes mosquitoes in relation to rainfall (mm) and temperature (°C) against the monthly sample collection.

The relation of increasing eggs density and frequencies of larvae, pupae and adults of aedes mosquitoes with meteorological condition especially rainfall and temperature are shown in Figure 3.3.3, where the seasonal pattern of *Ae. aegypti* and *Ae. albopictus* was fairly close to variations in rainfall. The heaviest rainfall occurring from May to August resulted in higher frequencies of eggs, larvae, pupae and adults of aedes mosquitoes in those month and highest in June. The highest monthly total rainfall (834mm) was recorded during June followed by May (660 mm) and August (558 mm).

There was little rain (in an average 149 mm) from January to April. In January and December rainfall was very low and the populations of aedes were also the lowest. The population peak (556±103.94) in June correspondent to the heaviest rainfall in June (834mm) and in August (558mm) and May (660 mm). The seasonal prevalence of dengue vector (*A. aegypti and A. albopictus*) was recorded in Dhaka city of Bangladesh. The mosquito population dynamics of this study showed similar seasonal pattern with other studies related with Aedes breeding in the world wide. More or less mosquito larvae were found in the whole study period because of rainfall. Vezzani *et al.* (2004) found the highest Ae. aegypti density with accumulated rainfalls above 650 mm. Micieli and Campos (2003) observed the close relation of the highest peak of *Ae. aegypti* population with high rainfall, and the population decreased for the months with less rainfall. In the present study the highest population density was observed at 834 mm rainfall and maximum larval population were from 450 to 834 mm rainfall. The main controlling factor identified here was rainfall. It was found that during the period of highest rainfall the larval production was also the highest. It may take some time to provide a suitable environment for the natural breeding of dengue vector mosquitoes.

Toma et al. (1982) found the greater larval abundance of *Ae. albopictus* in July and August in Japan and the USA. Akram and Lee (2004) recorded the peak of *Ae. albopictus* from May to July (34.0%, 35.1% and 30.9%) in South Korea when rainfall was higher than other seasons. They also observed that the population showed more variation in August as the month was marked with heavy rain. Alto and Juliano (2001) indicated high temperature combined with dry condition showed more variation in August as the month was marked with heavy rains significantly reduced adult production. In the present study seasonal prevalence of *Ae. aegypti* and *Ae. albopictus* in Dhaka City showed a peak in June. Some authors (Toma *et al.* 1982, Moore *et al.*1978) indicated that aedes abundance would be regulated mainly by temperature rather than precipitation. The successful hatching temperature for *Ae. aegypti* is above 17ºC (Micieli and Campos 2003, Campos and Macia 1996, Christophers 1960). But, in the present study period the temperature was always above this marginal level and this might cause increasing egg hatching at higher temperature in summer and thus higher population in this season followed by higher rainfall.

3.3.3.2 Monthly population distribution of aedes mosquitoes in eight divisions of Dhaka city during January to December

Population of aedes mosquitoes at eight divisions of Dhaka city throughout the year is described herein.

January 2015

The fluctuations of the population of aedes mosquitoe sthat occurred in the month of January, 2015 in eight divisions of Dhaka city viz. Gulshan, Uttara, Tejgaon, Mirpur, Ramna, Motijheel, Lalbagh and Wari. The highest density of eggs, larvae, pupae and adults of aedes mosquitoes were in Tejgaon division (46.33 ± 3.06, 26.33 ± 6.66, 13.67 ± 6.43, 5.67 ± 4.73 respectively). The lowest density of eggs, larvae, pupae and adults of aedes mosquitoes were in Lalbagh division (7.33 ± 1.53, 1.67 ± 0.58, 0.00 ± 0.00, 0.00 ± 0.00, respectively) (Figure 3.3.3).

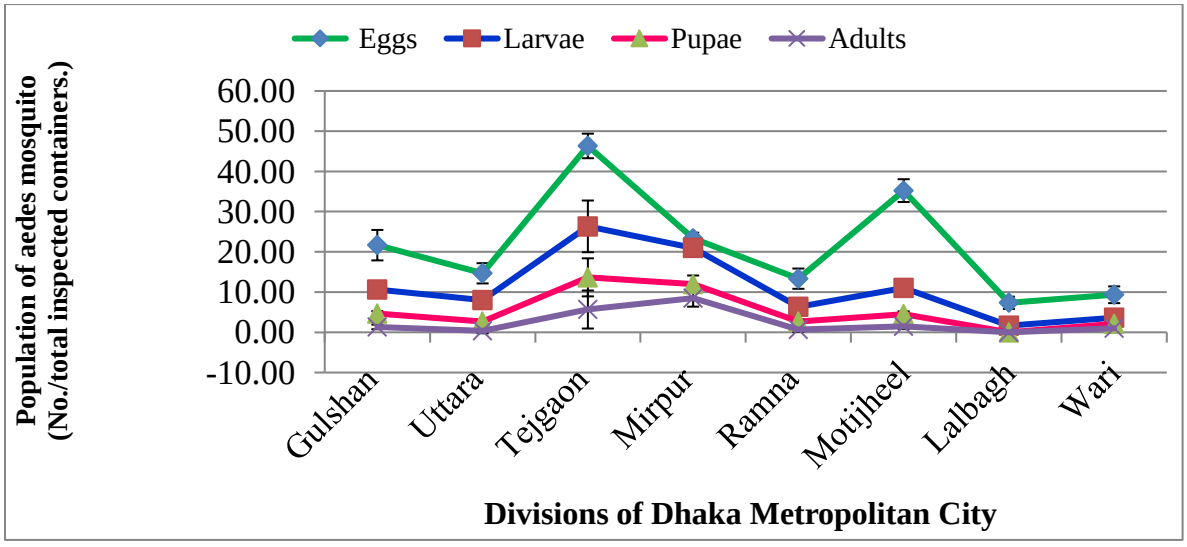


Figure 3.3.3 Distribution pattern of different life stages of aedes mosquitoes population in eight metropolitan divisions of Dhaka city in January, 2015.

February 2015

The fluctuations of the population of aedes mosquitoes that occurred in the month of February, 2015 in the different divisions of Dhaka city viz. Gulshan, Uttara, Tejgaon, Mirpur, Ramna, Motijheel, Lalbagh and Wari. The highest density of eggs, larvae, pupae and adults of aedes mosquitoes were in Tejgaon division (108.67 ± 15.31, 72.67 ± 21.50, 53.00 ± 25.87 and 38.67 ± 27.54 respectively). The lowest density of eggs, larvae, pupae and adults of aedes mosquitoes were in Uttara division (34.33 ± 3.06, 20.33 ± 1.15, 11.33 ± 0.58, 5.67 ± 0.58 respectively) (Figure 3.3.4).

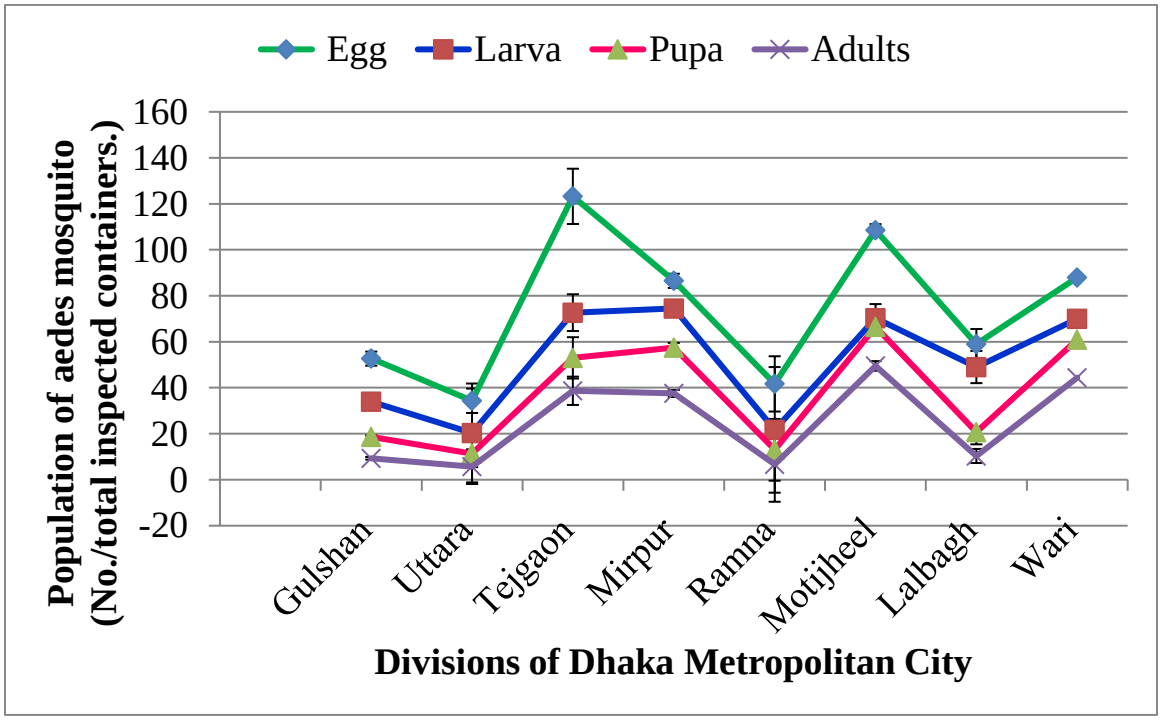


Figure 3.3.4 Distribution pattern of different life stages of aedes mosquitoes population in eight metropolitan divisions of Dhaka city in February, 2015.

March 2015

The fluctuations of the population of aedes mosquitoes that occurred in the month of March, 2015 in the different divisions of Dhaka city viz. Gulshan, Uttara, Tejgaon, Mirpur, Ramna, Motijheel, Lalbagh and Wari. The highest density of eggs, larvae, pupae and adults of aedes mosquitoes were in Tejgaon division (117.50±19.09, 77.00±14.1 4, 57.00±15.56 and 45.00±12.73 respectively). The lowest density of eggs, larvae, pupae and adults of aedes mosquitoes were in Uttara division (39.67 ± 6.81, 26.00 ± 7.00, 13.00 ± 6.93 and 9.33 ± 5.86 respectively) (Figure 3.3.5).

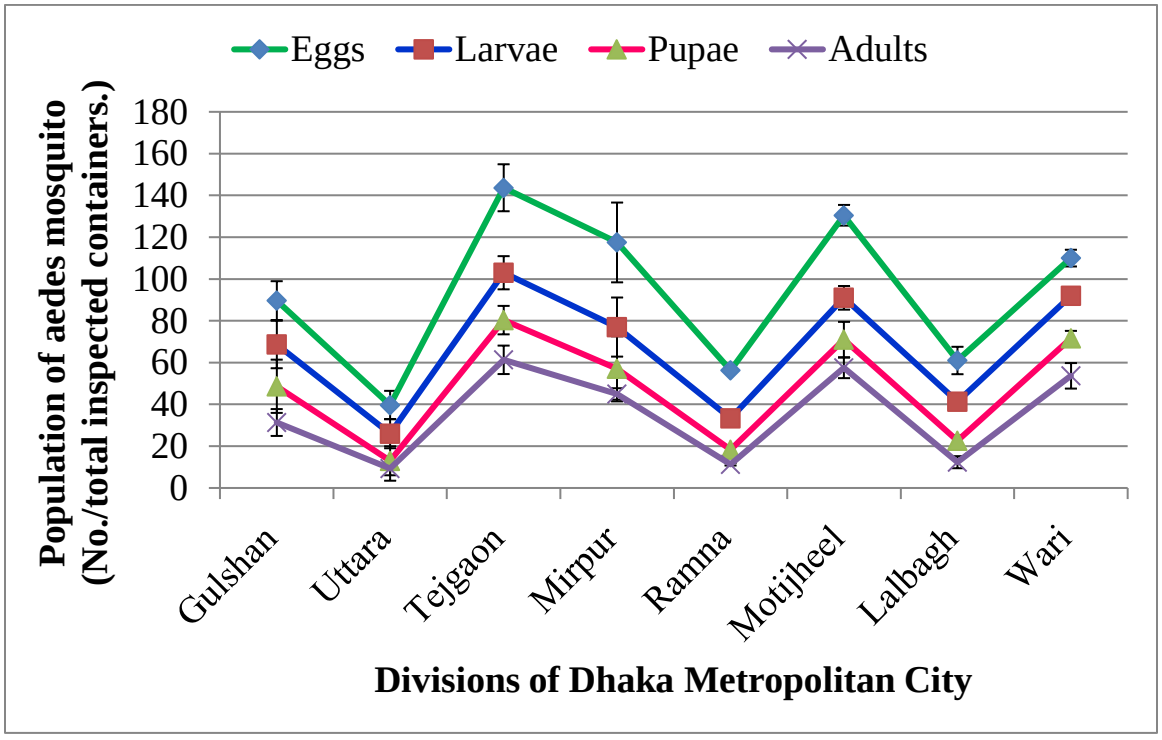


Figure 3.3.5 Distribution pattern of different life stages of aedes mosquitoes population in eight metropolitan divisions of Dhaka city in March, 2015.

April 2015

The fluctuations of the population of aedes mosquitoes that occurred in the month of April, 2015 in the different divisions of Dhaka city viz. Gulshan, Uttara, Tejgaon, Mirpur, Ramna, Motijheel, Lalbagh and Wari. The highest density of eggs, larvae, pupae and adults of aedes mosquitoes were in Tejgaon division (188.00 ± 29.46, 96.67 ± 23.25, 74.67 ±12.42 and 56.33±9.71 respectively). The lowest density of eggs, larvae, pupae and adults of aedes mosquitoes were in Lalbagh division (75.67±4.16, 55.00±2.65, 31.67±7.77 and 20.00±9.54 respectively) (Figure 3.3.6).

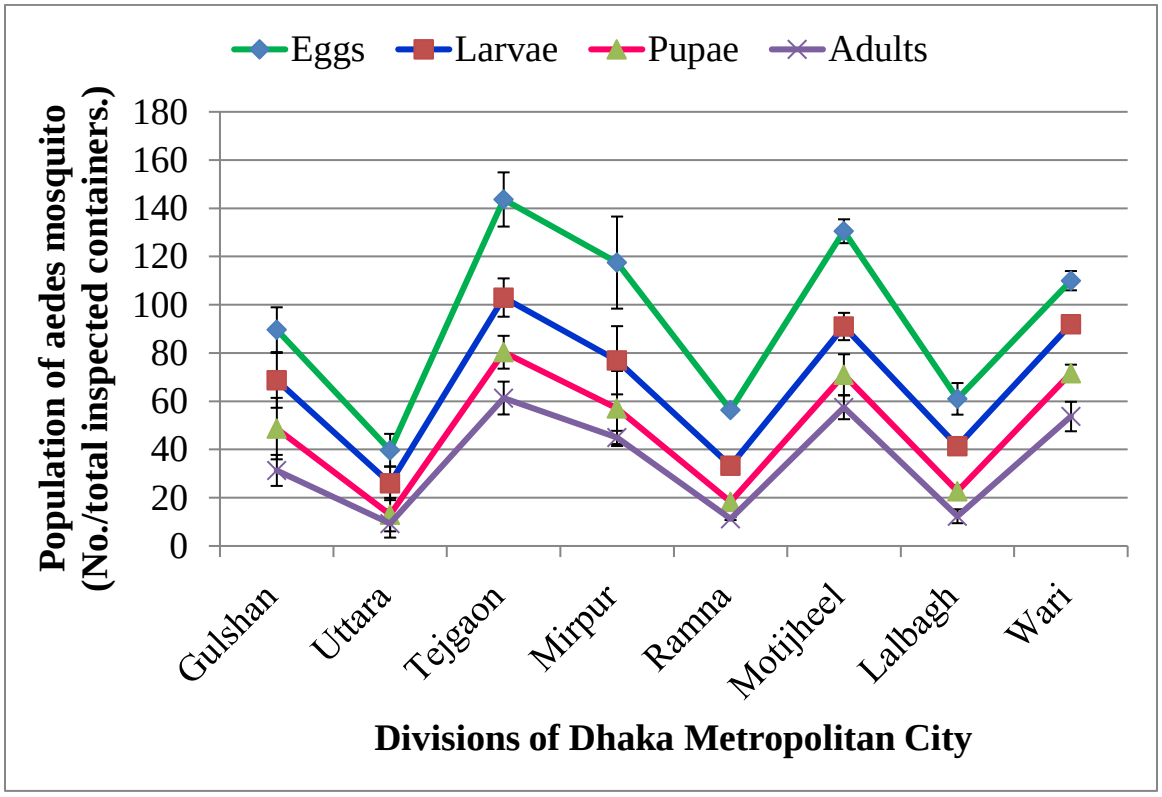


Figure 3.3.6 Distribution pattern of different life stages of aedes mosquitoes population in eight metropolitan divisions of Dhaka city in April, 2015.

May-2015

The fluctuations of the population of aedes mosquitoes that occurred in the month of May 2015 in the different divisions of Dhaka city viz. Gulshan, Uttara, Tejgaon, Mirpur, Ramna, Motijheel, Lalbagh and Wari. The highest density of eggs, larvae, pupae and adults of aedes mosquitoes were in Tejgaon division (562.67 ±21.08, 464.00 ± 13.86, 358.00 ± 6.08 and 108.67±5.86 respectively). The lowest density of eggs, larvae, pupae and adults of aedes mosquitoes were in Lalbagh division (325.33±16.65, 237.00±5.29, 141.33±5.69 and 98±3.21 respectively) (Figure 3.3.7).

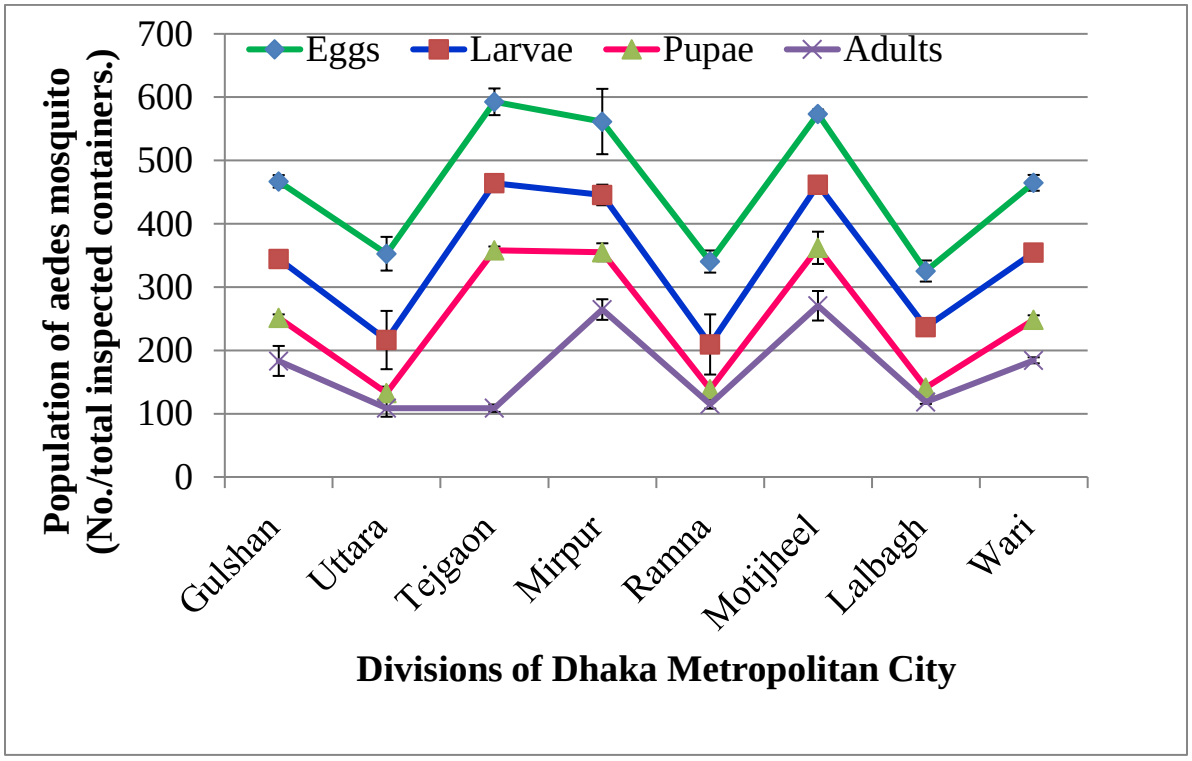


Figure 3.3.7 Distribution pattern of different life stages of aedes mosquitoes population in eight metropolitan divisions of Dhaka city in May, 2015.

June-2015

The fluctuations of the population of aedes mosquitoes that occurred in the month of June, 2015 in the different divisions of Dhaka city viz. Gulshan, Uttara, Tejgaon, Mirpur, Ramna, Motijheel, Lalbagh and Wari. The highest density of eggs, larvae, pupae and adults of aedes mosquitoes were in Tejgaon division (892.67±30.89, 644.11±31.47, 512.31±11.85, and 494.33± 5.51 respectively). The lowest density of eggs, larvae, pupae and adults of aedes mosquitoes were in Lalbagh division (7.33±1.53, 1.67±0.58, 0.00± 0.00, 0.00 ± 0.00 respectively) (Figure 3.3.8).

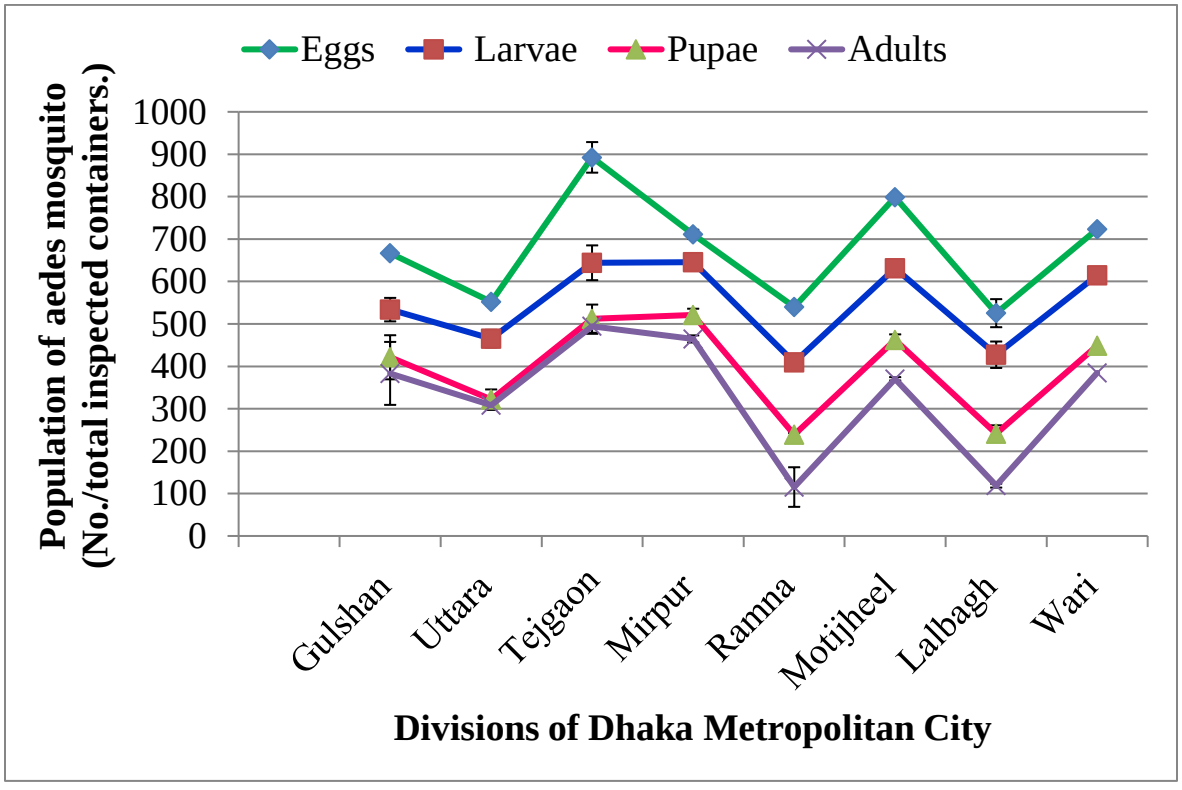


Figure 3.3.8 Distribution pattern of different life stages of aedes mosquitoes population in eight metropolitan divisions of Dhaka city in June, 2015.

July-2014

The fluctuations of the population of aedes mosquitoes that occurred in the month of July, 2014 in the different divisions of Dhaka city viz. Gulshan, Uttara, Tejgaon, Mirpur, Ramna, Motijheel, Lalbagh and Wari. The highest density of eggs, larvae, pupae and adults of aedes mosquitoes were in Tejgaon division (586.67±7.77, 470.00±20.66, 363.33±23.86 and 286.67±11.02 respectively). The lowest density of eggs, larvae, pupae and adults of aedes mosquitoes were in Lalbagh division (304.00 ±6.08, 218.67 ±13.65, 147.67±12.86 and 100.67±4.04 respectively). (Figure 3.3.9).

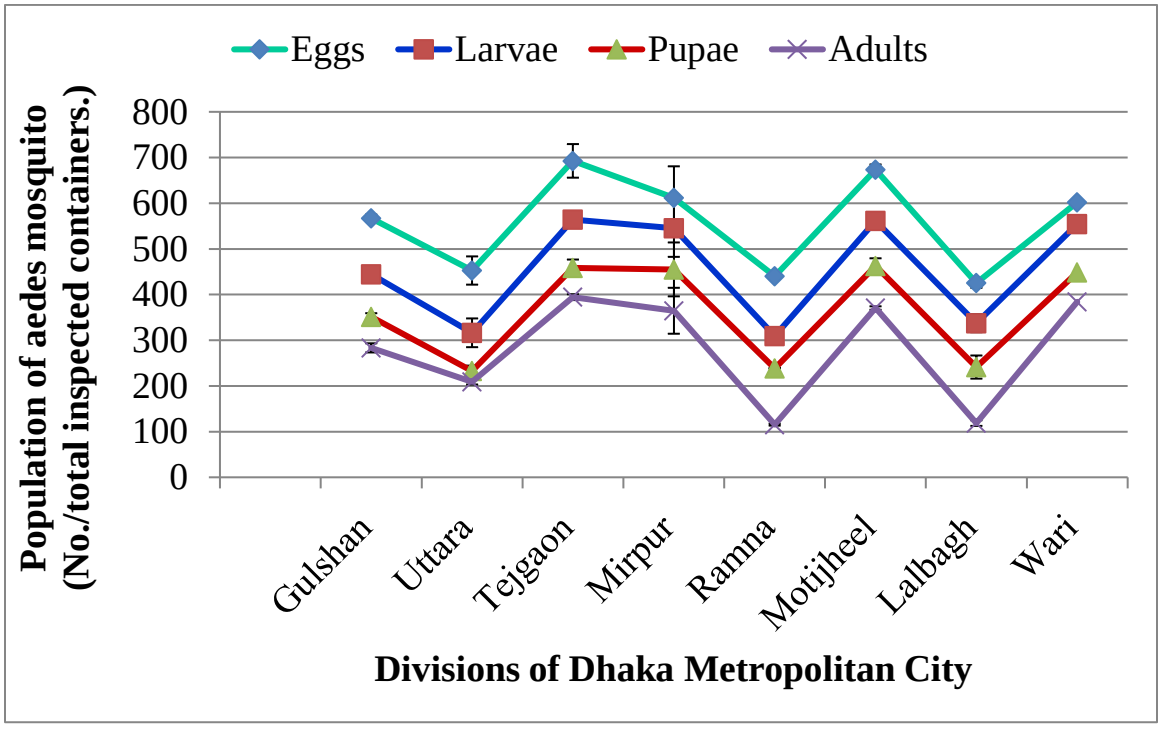


Figure 3.3.9 Distribution pattern of different life stages of aedes mosquitoes population in eight metropolitan divisions of Dhaka city in July, 2014.

August-2014

The fluctuations of the population of aedes mosquitoes that occurred in the month of Auguest, 2014 in the different divisions of Dhaka city viz. Gulshan, Uttara, Tejgaon, Mirpur, Ramna, Motijheel, Lalbagh and Wari. The highest density of eggs, larvae, pupae and adults of aedes mosquitoes were in Tejgaon division (381.33±5.51, 315.67±61.78, 219.33±57.74, 141.67±9.07 respectively). The lowest density of eggs, larvae, pupae and adults of aedes mosquitoes were in Lalbagh division (156.00±9.54, 107.67 ±1.73, 82.00 ±22.87, 61.33 ±14.22 respectively). (Figure 3.3.10).

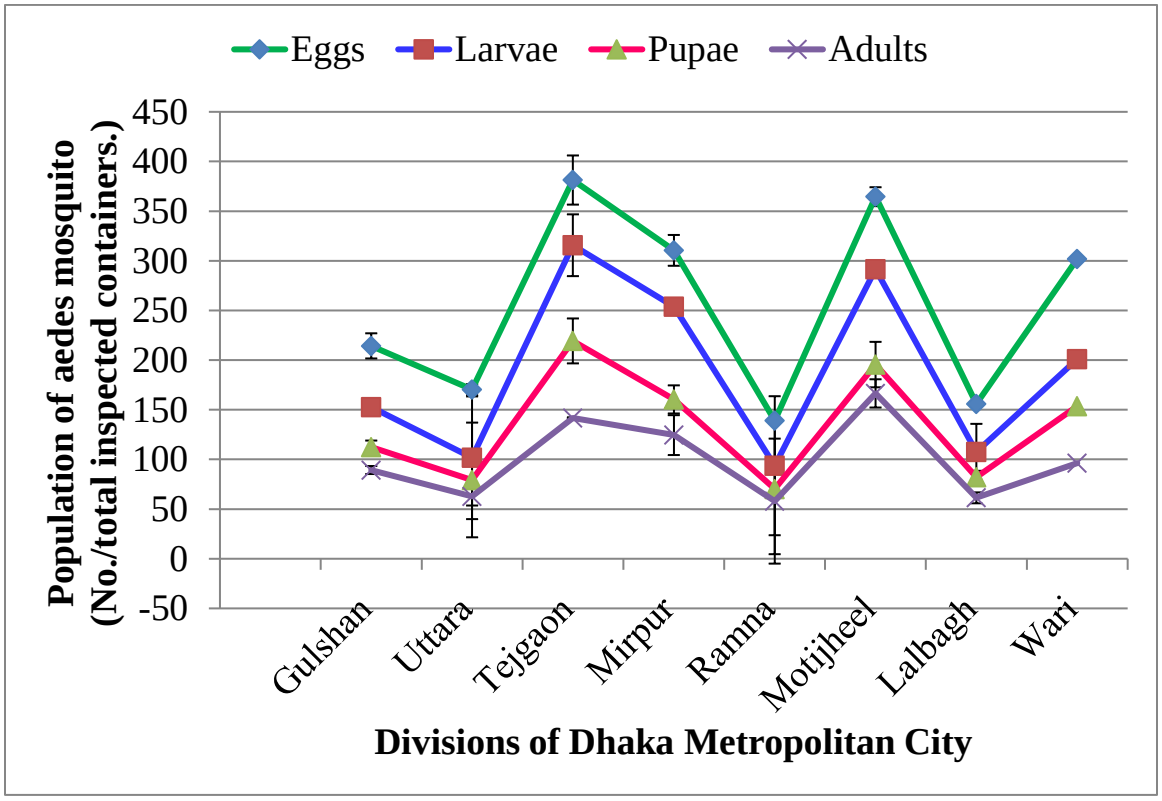


Figure 3.3.10 Distribution pattern of different life stages of aedes mosquitoes population in eight metropolitan divisions of Dhaka city in August, 2014.

September 2014

The fluctuations of the population of aedes mosquitoes that occurred in the month of September, 2014 in the different divisions of Dhaka city viz. Gulshan, Uttara, Tejgaon, Mirpur, Ramna, Motijheel, Lalbagh and Wari. The highest density of eggs, larvae, pupae and adults of aedes mosquitoes were in Tejgaon division (160.50±19.09, 117.00±8.49, 92.50±6.36 and 71.50 ± 6.36 respectively). The lowest density of eggs, larvae, pupae and adults of aedes mosquitoes were in Uttara division (33.67±2.08, 21.33±1.53, 9.67 ±1.53 and 4.3±1.53 respectively). (Figure 3.3.11).

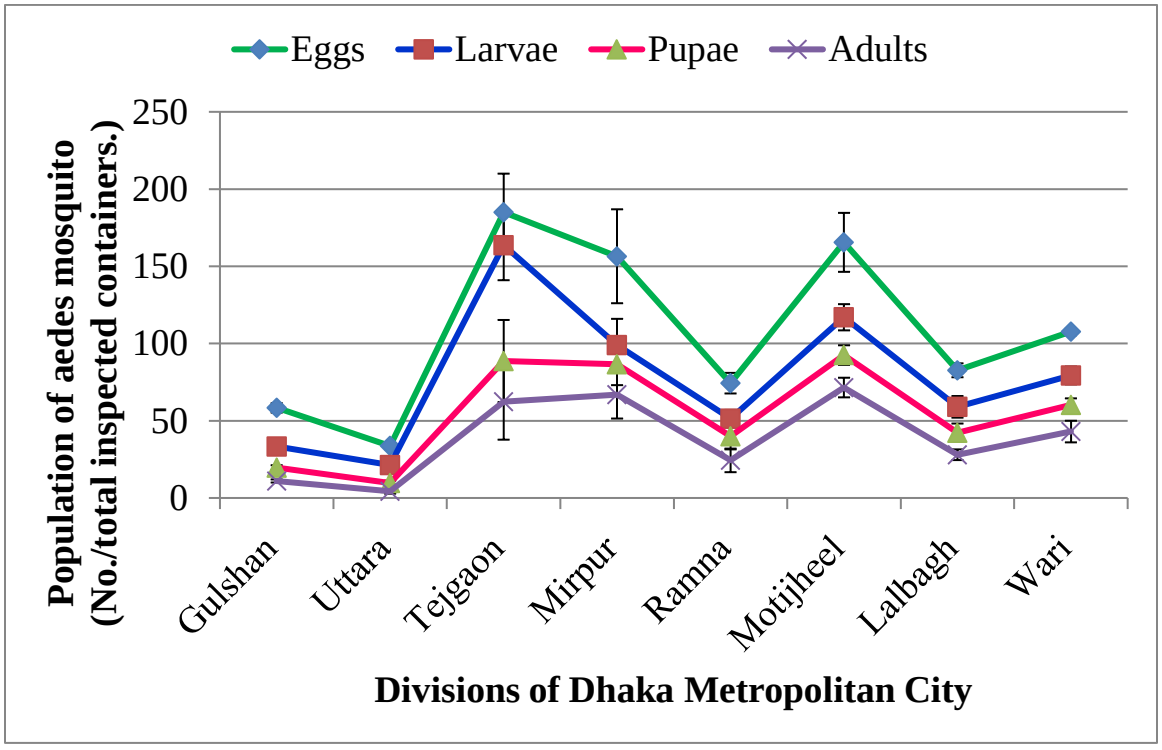


Figure 3.3.11 Distribution pattern of different life stages of aedes mosquitoes population in eight metropolitan divisions of Dhaka city in September, 2014.

October 2014

The fluctuations of the population of aedes mosquitoes that occurred in the month of October, 2014 in the different divisions of Dhaka city viz. Gulshan, Uttara, Tejgaon, Mirpur, Ramna, Motijheel, Lalbagh and Wari. The highest density of eggs, larvae, pupae and adults of aedes mosquitoes were in Tejgaon division (71.50±4.95, 46.00±14.14, 28.50 ±14.85 and 14.50 ±9.19 respectively). The lowest density of eggs, larvae, pupae and adults of aedes mosquitoes were in Uttara Division (16.00±3.00, 8.33±2.52, 3.33±2.08 and 1.33±1.15 respectively). (Figure 3.3.12).

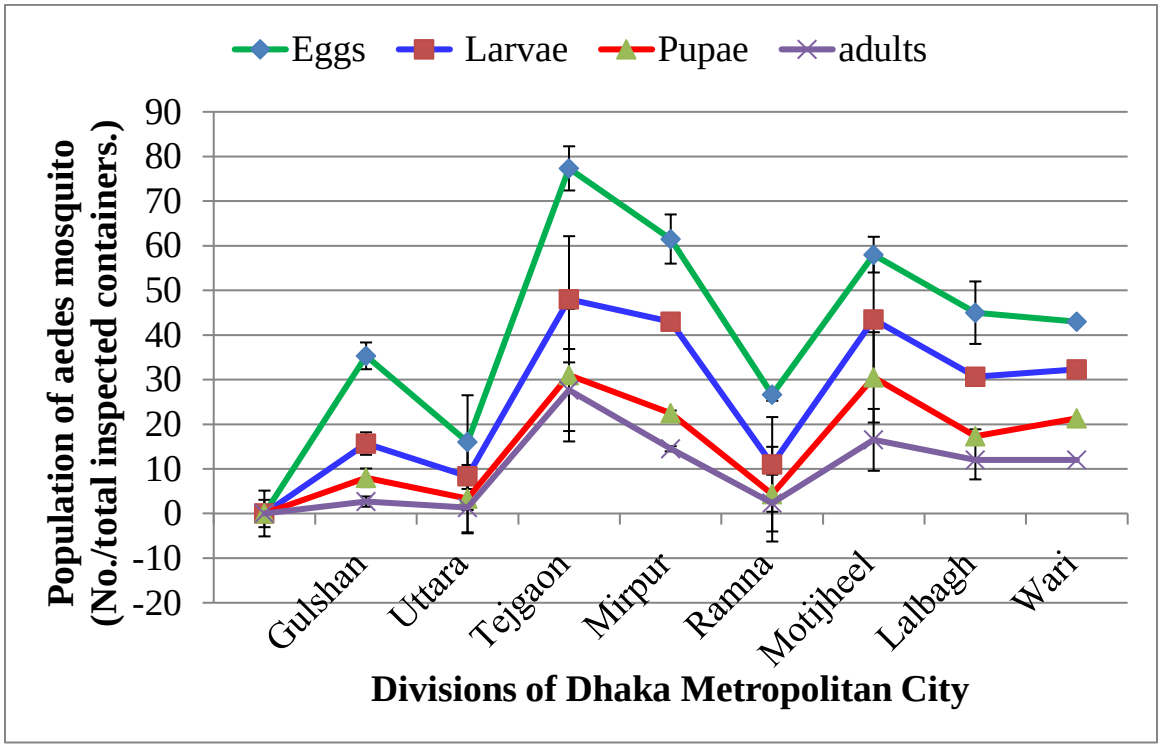


Figure 3.3.12 Distribution pattern of different life stages of aedes mosquitoes population in eight metropolitan divisions of Dhaka city in October, 2014.

November 2014

The fluctuations of the population of aedes mosquitoes that occurred in the month of November, 2014 in the different divisions of Dhaka city viz. Gulshan, Uttara, Tejgaon, Mirpur, Ramna, Motijheel, Lalbagh and Wari. The highest density of eggs, larvae, pupae and adults of aedes mosquitoes were in Tejgaon division (46.67±10.97, 27.33±7.57, 15.67±5.03 and 15.00±4.58 respectively). The lowest density of eggs, larvae, pupae and adults of aedes mosquitoes were in Lalbagh division (10.00 ± 1.00, 4.33 ± 1.53, 1.33 ± 0.58, 7.33 ± 8.74respectively). (Figure 3.3.13).

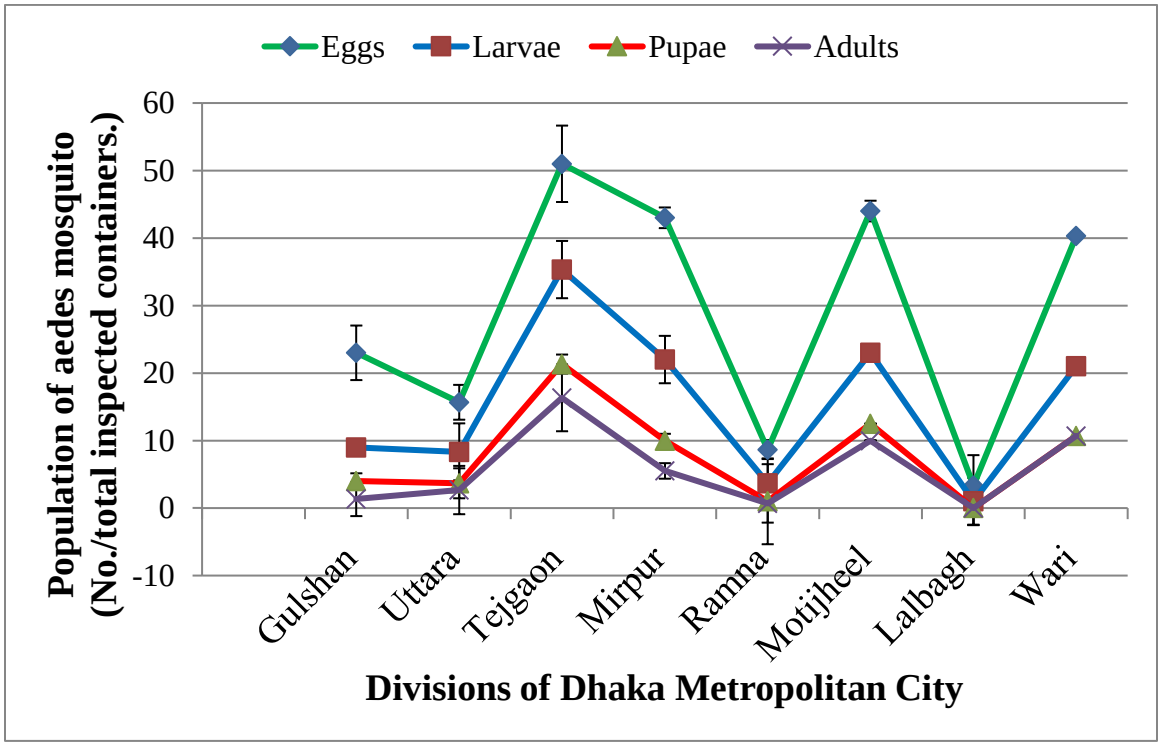


Figure 3.3.13 Distribution pattern of different life stages of aedes mosquitoes population in eight metropolitan divisions of Dhaka city in November, 2014.

December-2014

The fluctuations of the population of aedes mosquitoes that occurred in the month of December, 2014 in the different divisions of Dhaka city viz. Gulshan, Uttara, Tejgaon, Mirpur, Ramna, Motijheel, Lalbagh and Wari. The highest density of eggs, larvae, pupae and adults of aedes mosquitoes were in Tejgaon division (51.00 ± 24.58, 35.33 ± 21.22, 21.33 ± 12.22 and 16.33 ± 13.58 respectively). The lowest density of eggs, larvae, pupae and adults of aedes mosquitoes were in Lalbagh division (3.33 ± 1.53, 1.00 ± 1.00, 0.00 ± 0.00 and 0.00 ± 0.00 respectively) (Figure 3.3.14).

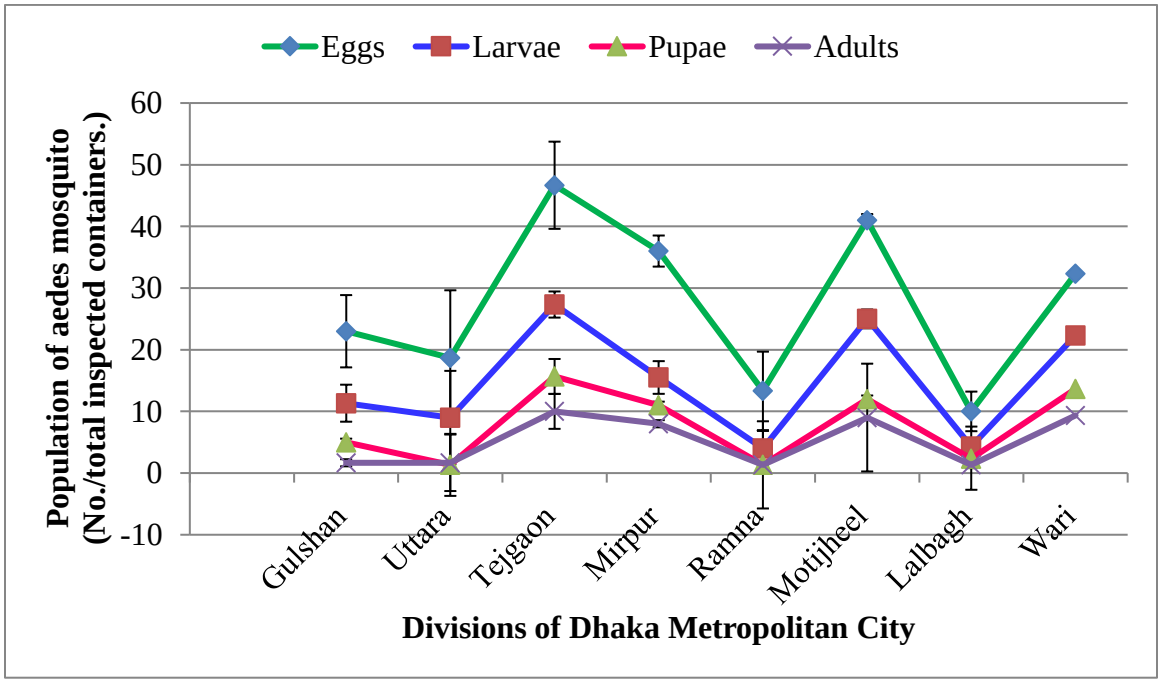


Figure 3.3.14 Distribution pattern of different life stages of aedes mosquitoes population in eight metropolitan divisions of Dhaka city in December, 2014.

Ahmed *et al.* (1990) found *Ae. Aegypti* in the month of January, February, March, April, May and October ; *Ae. albopictus* in February, March, April, May and October ; *Cx. quinquefasciatus* in January, February, March, October and November ; *Ae. subalbatus* in January, February, March, November and December. Ahmed *et al*. (1990) collected larvae from Saidabad, Mohakhali, Ramna Park and Mirpur Botanical Garden of Dhaka City. It was clearly shown during the present study that *Ae. albopictus* and *Ae. aegypti* were the dominant container breeder mosquito in Dhaka city. Khan (1980) found two mosquitoes *Ae. aegypti* and *Ae. albopictus* an Nasiruddin (1952) found only *Ae. aegypti* in Dhaka city. Rosenberg and Moheswary (1982) collected a few species mosquitoes from Bangladesh. Ahmed *et al.* (1990) found nine species of container habitat mosquitoes in Dhaka city; these were *Ae. aegypti*, and *Ae. Albopictus.*

3.3.3.3 Distribution of different stages of aedes mosquitoes in different divisions of Dhaka city

The present study revealed that the distributin of life parameters of aedes mosquitoes was apparently different, observed by counting the numbers occurring in containers in eight divisions of Dhaka city (Figure 3.3.16). The variation of number at different stages resulted the differences of mosquitoes population in different divisions. In all divisions the number of eggs were more abundant than other stages and the larvae were found less than that of eggs. These two stages showed variations in number in different divisions. The pupae occurred so abundant not as adults. The distribution pattern of the life parameters appeared to be eggs > larvae > pupae > adults.

Highest density of eggs, larvae, pupae and adults of aedes mosquitoes were found in Tejgaon Zone (2960.00 ± 29.82, 2329.00 ± 4.36, 1786.33 ± 35.92 and1369.67 ± 16.50 respectively) followed by Motijheel zone (2895.50 ± 33.23, 2281.50 ± 50.20, 1769.50 ±70.00 and 1315.00 ±120.21 respectively). In Mipur zone the mean values of eggs, larvae, pupae and adults of aedes mosquitoes were found 285.50 ± 109.60, 2208.00 ± 48.08, 1692.50 ± 36.06 and1342.060 ± 38.18 respectively, The lowest density of eggs, larvae, pupae and adults of aedes mosquitoes were found in Lalbagh Divisions (1556.00 ± 51.39, 1122.67 ± 32.88, 764.00 ± 34.39, 570.67 ± 7.02) followed by Ramna and Uttara zone (1610.33 ± 78.01, 1113.67 ± 33.50, 762.67 ±

73.38, 585.33 ± 47.50 and 1600.33 ± 28.54, 1105.67 ± 39.27, 712.00 ± 40.95, 497.67 ± 58.56 respectively).

Distribution pattern of mosquitoes could be explained by habitat preferences of the species. As the geographical distribution of a species in a given area without absolute barrier to dispersal, might be determined by environmental variations such as temperature and humidity (Micieli 2003, Samways 199). Ameen *et al* (1994) examined 1742 breeding sites of mosquitoes from Dhaka city and concluded that lowest density of mosquitoes occurred in lakes while highest in derelict ponds. *Ae. Aegypti* was previously found in Chittagong, Chandpur, Dhaka, Goalonda and Narayangonj (Barraud 1934). Later many workers collected this species only in Dhaka city (Ahmed *et al* 1990, Ameen and Hossain 1984, Khan 1980). In 1997, Knudsen first reported high density of this species in Dhaka city. Thus, the local distribution of the aedes mosquitoes was probably controlled by its reaction to environmental differences among the available range of habitats. As for example, Aedes species were found only in tyres and tree holes during the rainy season when the reservoir were filled with rainy water for a short period of time but Cx. quinquefasciatus was found in all kinds of habitats and abundantly in stagnant drains suitable for its regeneration (Ali *et al.* 1999). However, breeding habitats such as drains and coconut barks were the richest habitats

for the mosquitoes in the study areas while lowest mosquito density was recorded from Tree holes.

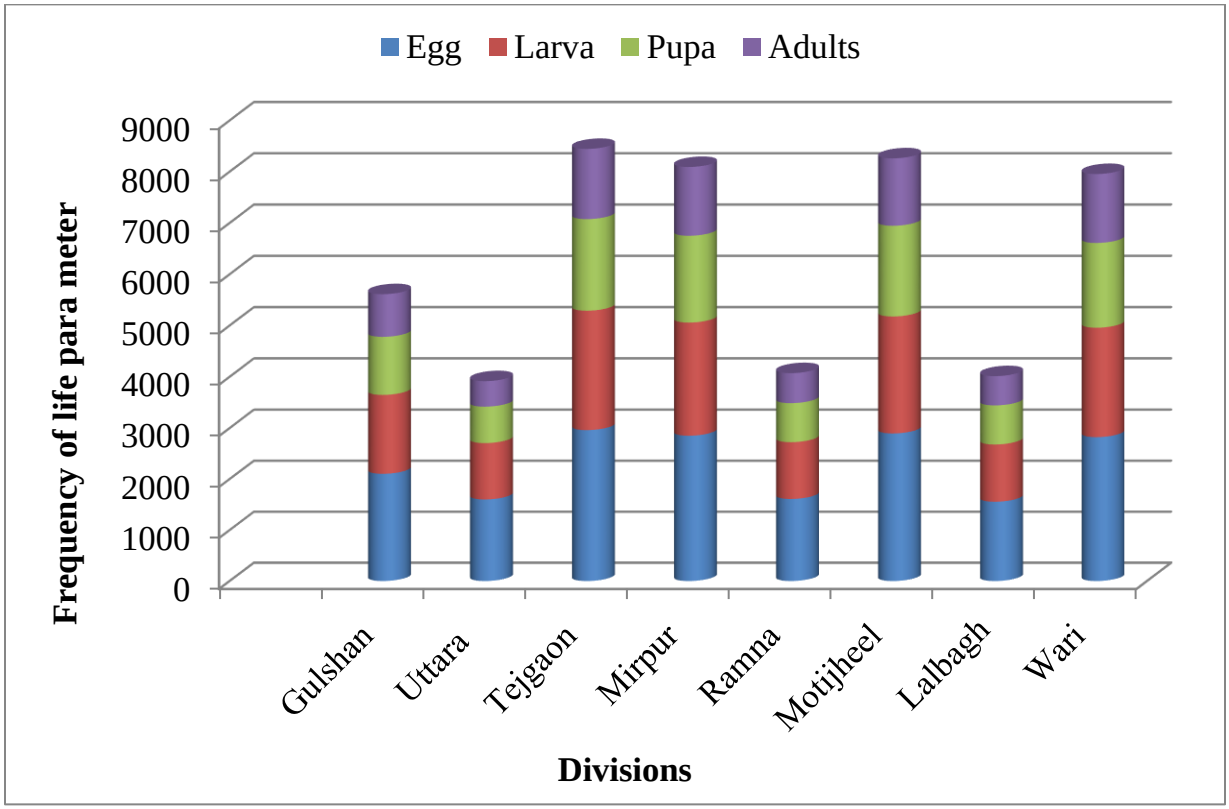


Figure 3.3.15 Proportions of different stages of life cycle of aedes mosquitoes encountered over a year in various divisions of Dhaka city from July,2014 to December, 2015.

3.3.3.4 Egg distribution

The egg density of aedes mosquitoes was widely abundant in Tejgaon division where 2960 ±29.82 eggs per container (Diameter 30cm) encounted following 2895.5 ± 33.23 in Motijheel division and 2850.5 ± 109.60 eggs in Mirpur division and also in Wari division with 2822.66 ± 56.44 eggs per container (Figure 3.3.17). The lowest number of eggs was found in Uttara, Lalbagh and Ramna division ranging from 1600.33 ± 28.54 to 1556 ±51.39. The egg lying condition depended on breeding on a wide variety of resources and climatic conditions. The egg distribution was found in between the above divisions in Gulshan division.

Almost similar findings were found with *Ae. aegypti* showing predominance over *Ae. albopictus* in peri- and intradomiciliary environments (Stickman and Kittayapong 2002). Such results corroborate those recorded by Lim *et al*. 2010 in two Malaysian fishing towns. The eggs distribution of *Ae. aegypti* in this study was higher than that of *Ae. albopictus* in both domiciliary environments. This result was similar to the findings of other authors (Lim *et al*. 2010 and *Dhang et al. 2005*). The superiority of *Ae. aegypti* in urban habitats in general was attributed to its high anthropophily and domesticity (*Braks, et al.2003).*

Chiaravalloti *et al*. (2002) determined the relationship of this species with containers located in peri- and intradomiciliary premises, demonstrating that *Ae. aegypti* is more associated with the vicinity of the house, whereas *Ae. albopictus* occupies natural and disposable breeding grounds in sites farther away from peridomiciliary premises.

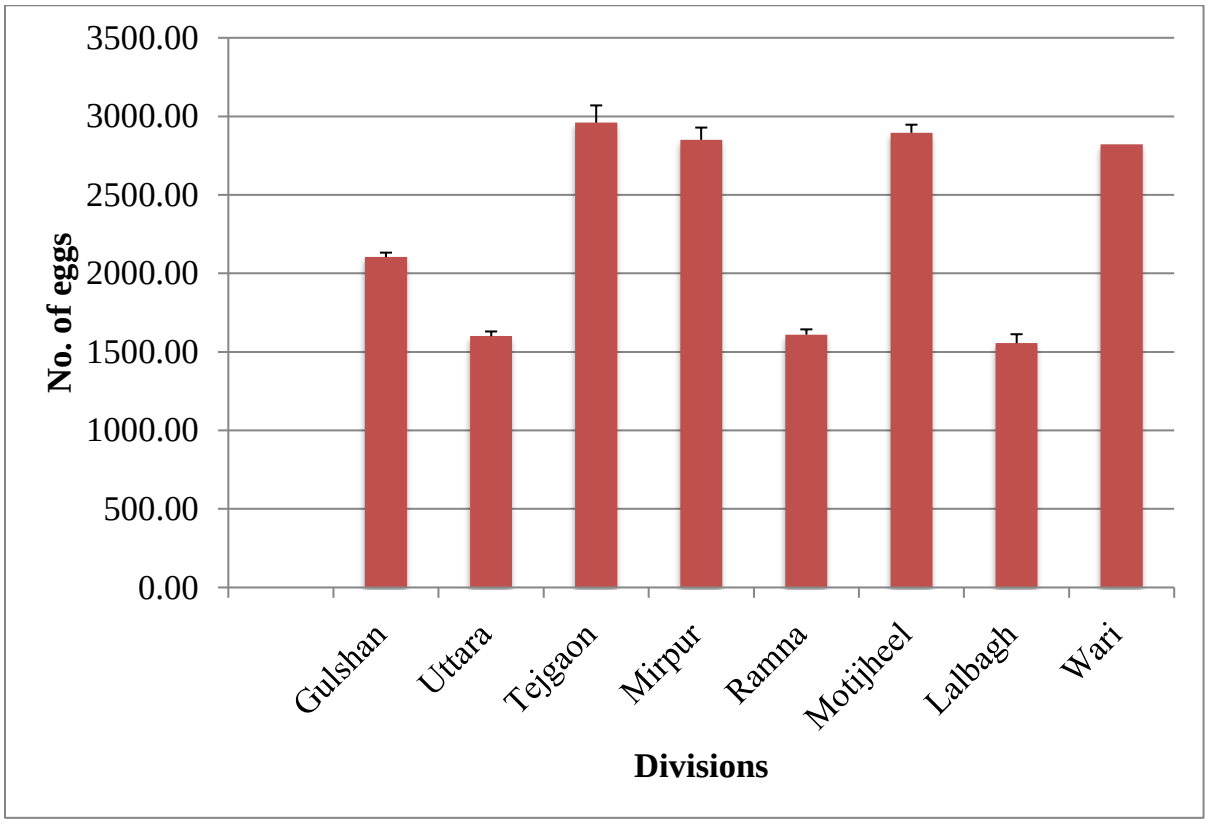


Figure 3.3.16 Distribution of eggs of aedes mosquitoes in different divisions of Dhaka city.

3.3.3.5 Larval distribution

The distribution of larvae of aedes mosquitoes was in the same pattern as in eggs but their number remained lower compared to eggs. The highest number was 2329 ± 4.36 larvae recorded in Tejgaon division and Motijheel, Mirpur and Wari showed little less number than in Tejgaon division. Lalbagh, Uttara and Ramna divisions were almost same and least than other divisions (Figure 3.3.18).

Ahmed *et al.* (1990) found the same result studied *Ae. aegypti* and *Ae. albopictus* in indoor and outdoor containers in different parts of Dhaka city. Nasiruddin (1952) claimed *Ae. Aegyepti* population was more in Tejgaon area in Dhaka city than mirpur. Nasiruddin (1957)gave the same opinion of *Ae. aegypti* larvae in tree holes or in bamboo stumps in Dhaka city. Again, Khan (1980) got the similar findings for both the species prevalence in the Dhaka City. Huang (1974) collected *Ae. aegypti* from artificial containers like tin can, water jar bucket, broken bottle and tyre from urban areas in Thailand.

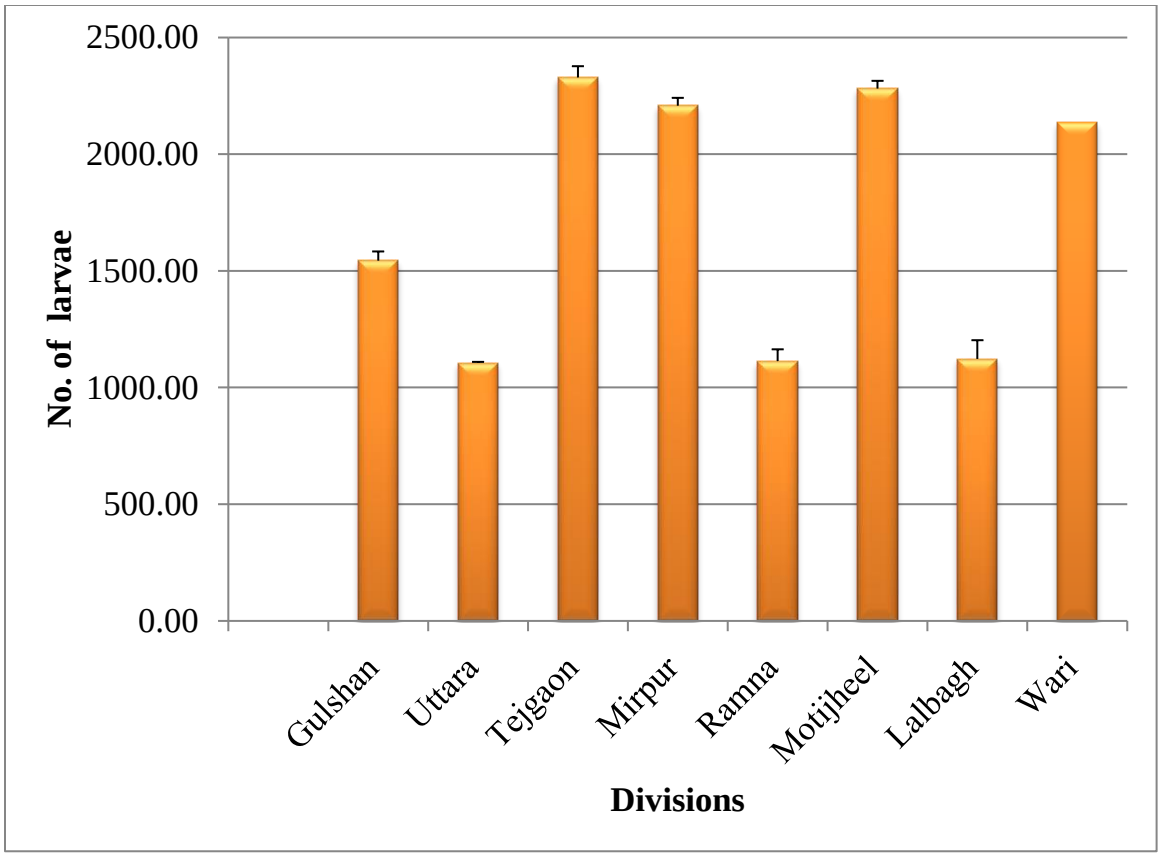


Figure 3.3.17 Distribution density of larvae of aedes mosquitoes in different divisions of Dhaka city.

3.3.3.6. Pupal distribution

Like other stages of aedes mosquitoes pupal density increased in Tejgaon 1786.33 ± 35.92 pupae and also in Motijheel, Mirpur and Wari 1769.5 ± 109.60, 1692.5 ± 36.06 and 1653 ± 95.65 larvae respectively. The latter two divisions showing almost same larval distributin (Figure 3.3.19) The abundancies of pupae of aedes mosquitoes was the lowest in Uttara, Lalbagh and Ramna divisions 712 ± 40.95, 764 ± 34.39 and 762.67± 73.38 pupae respectively.

Present study suggested that larval density influenced pupal, and eventually adult population. Similar view was expressed from the research of Yunus *et al.* (2001) and Akram and Lee (2004). They found that larval density was an important factor in pupal and adult population for *Aedes* and other Culicidae species .

Yunus *et al.* (2001) found higher *Ad. Agypti* density of Tejgaon area than any other part in Dhaka.

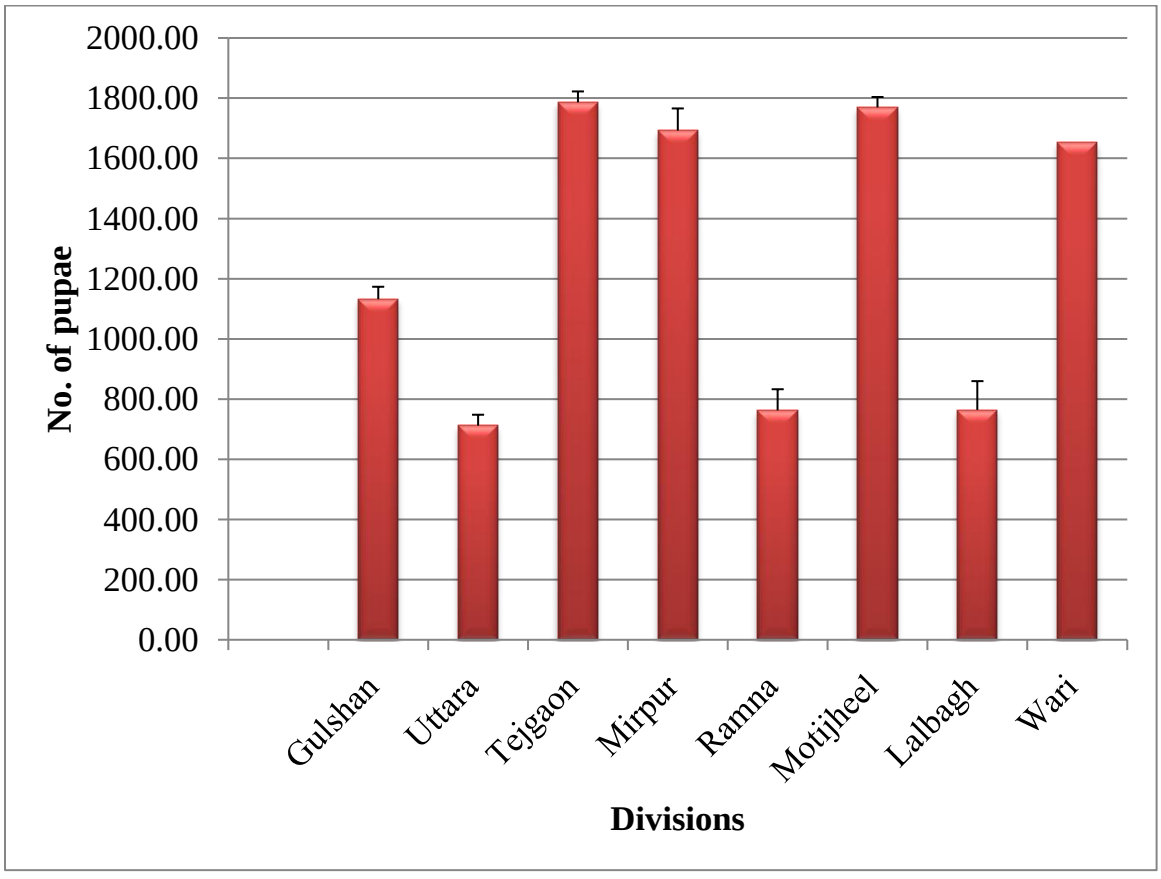


Figure 3.3.18 Distribution density of pupae of aedes mosquitoes in different divisions of Dhaka city.

3.3.3.7 Adult distribution

Adult population density of aedes mosquitoes showed highest in Tejgaon division 1415 ±16.50 adults (Figure 3.3.20). Motijheel was the next division with adult abundance 1369.67± 120.21 adults, Wari 1344.66 ± 32.62 and Mirpur 1342± 38.18 adults.The lowest adult population of aedes mosquitoes was in Uttara division 497.67 ±58.56.The abundances of adult mosquitoes in Lalbagh and Ramna divisions were almost the same and higher than Uttara. It indicated that temporal variation with regard to changes the life stages and pupae population might influence the adult population.

Scott (2000) reported that in smaller mosquitoes, such as those that emerged from manually filled containers or from containers with abundant larvae, should be evaluated as a focus for intervention.

The highest density of aedes mosquitoes inTejgaon, Motijheel, Mirpur and Wari divisions might be due to industrial areas where different classes of people inhabit.

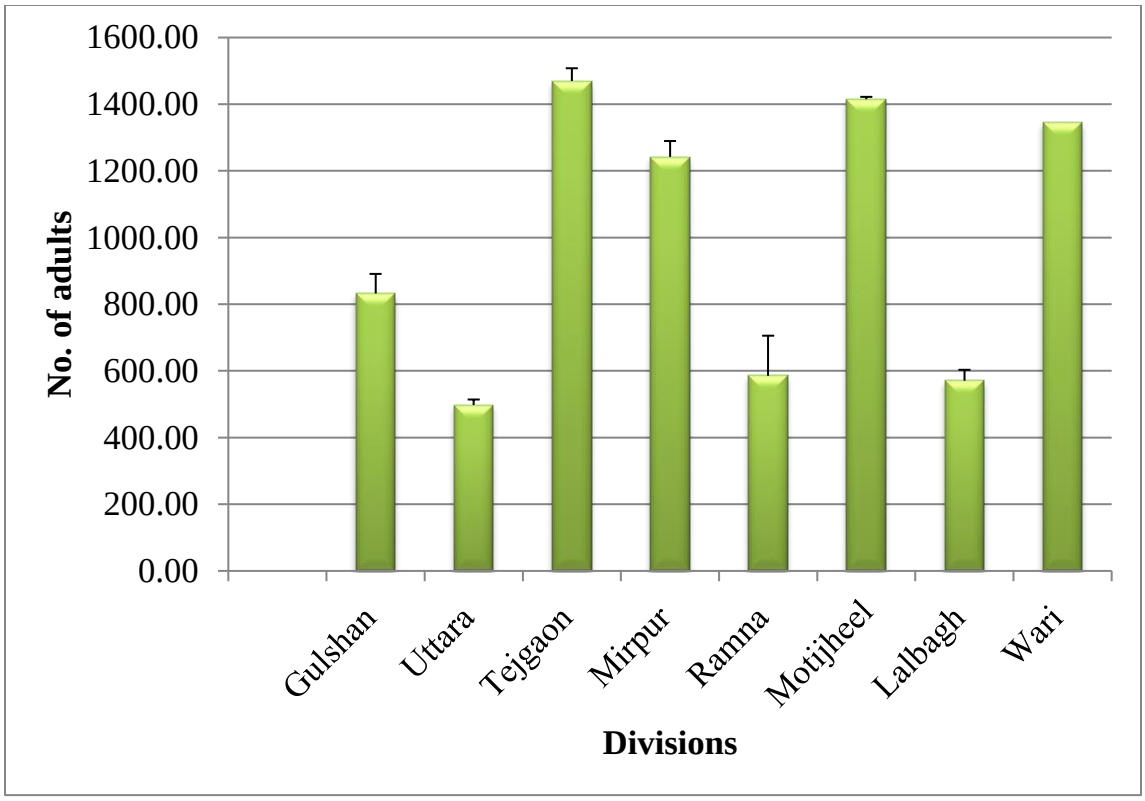


Figure 3.3.19 Distribution density of adults of aedes mosquitoes in different divisions of Dhaka city.

3.3.3.8 Relationship of life stages of aedes mosquitoes with rainfall

The analyses showed that there were highly significant positive relationship between the population density of eggs, larvae, pupae and adults with rainfall with $R^2 = 0.796$, $R^2 = 0.783$, $R^2 = 0.768$ and $R^2 = 0.748$ for eggs, larvae, pupae and adults respectively (Figures 3.3.21, 3.3.22, 3.3.23 and 3.3.24).

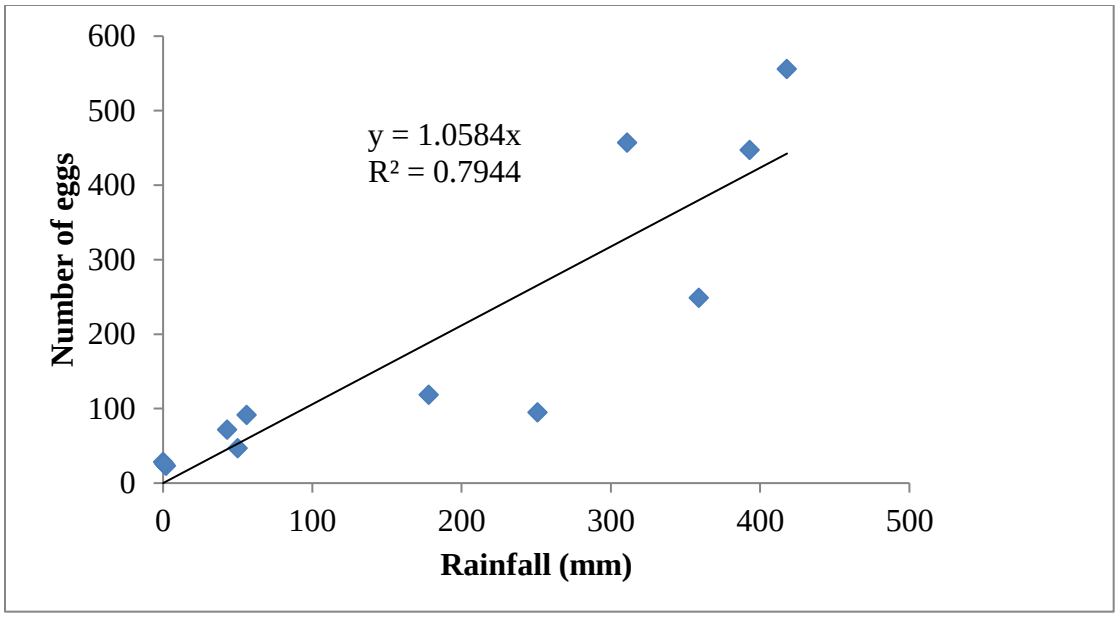


Figure 3.3.20 Relationship between egg density of aedes mosquitoe and rainfall.

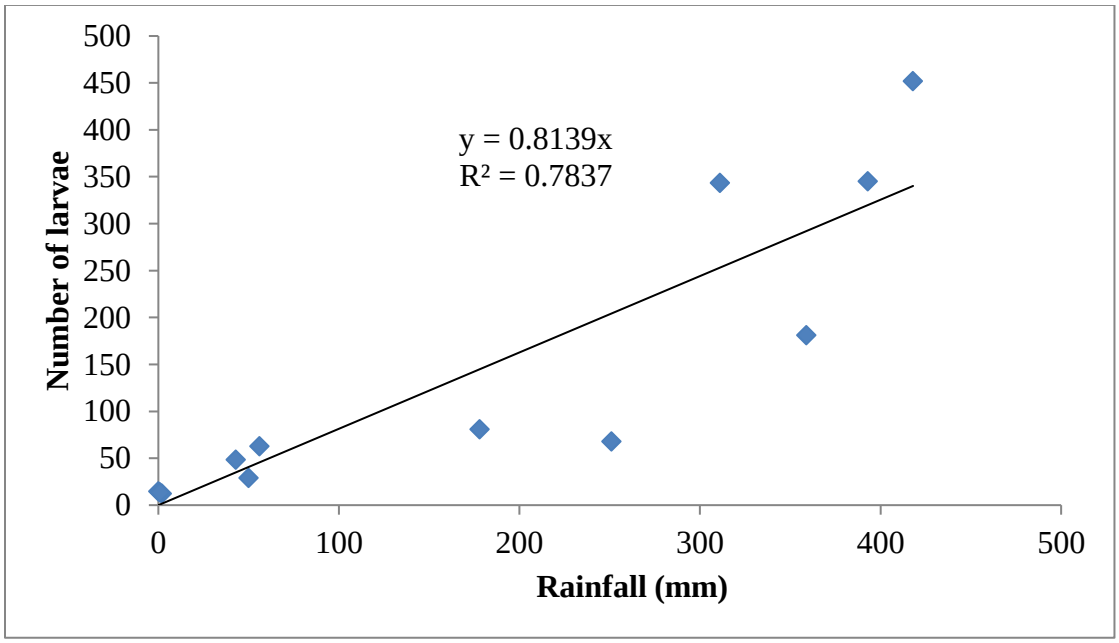


Figure 3.3.21 Relationship between larval population of aedes mosquitoe and rainfall.

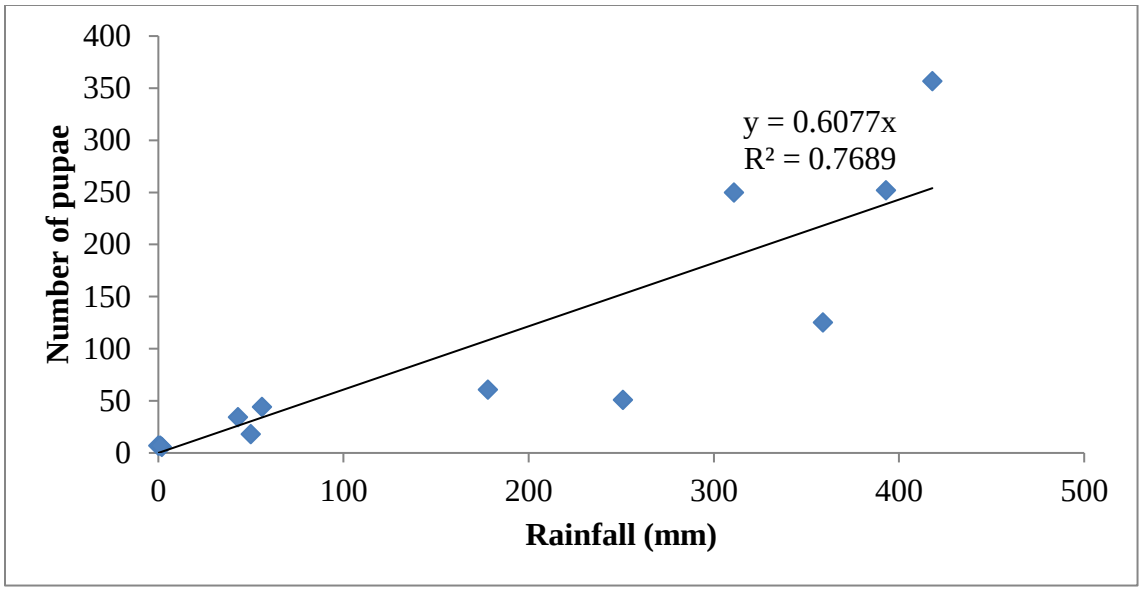


Figure 3.3.22 Relationship between pupal population of aedes mosquitoe and rainfall.

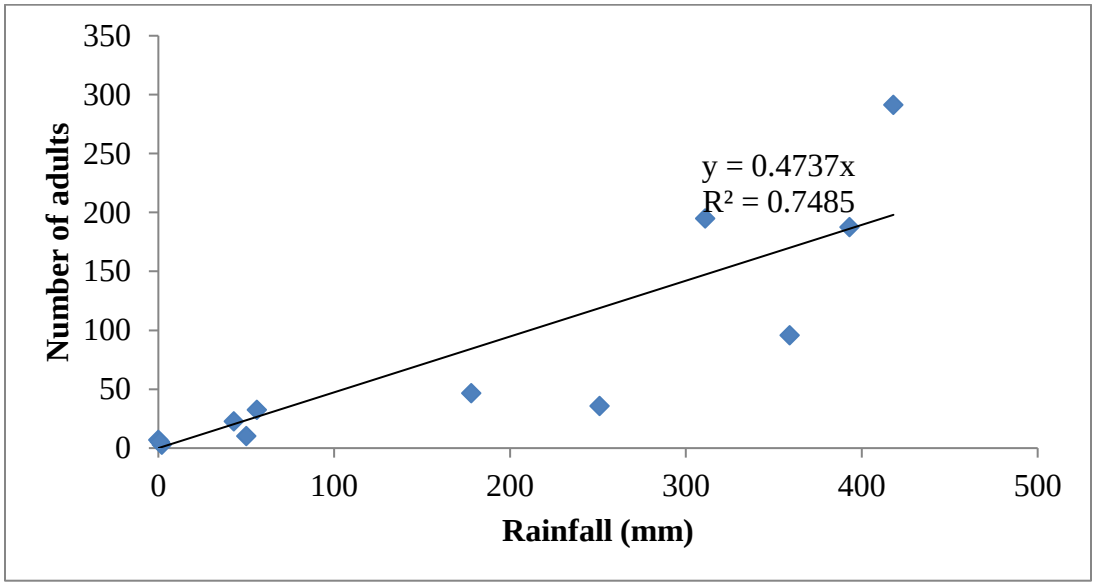


Figure 3.3.23 Relationship between adult population of aedes mosquitoe and rainfall.

Heavy rainfall is associated with mass egg hatching and an increase in the number of mosquitoes (Ndiaye *et al.* 2006). Rainfall has an impact on two factors important for arbovirus transmission: vector density and adult mosquito longevity (Diallo *et al.* 2003). While vector population densities are already high at the beginning of the rainy season, virus amplification occurs primarily at the end of the rainy season (Diallo *et al.* 2003).

A study of *Ae. aegypti* egg numbers in Salta, Argentina found that they remained low during the dry season, increased at the beginning of the rainy season and decreased at the end of the rainy season (Micieli and Campos 2003).

Schaeffer *et al* (2008) found an *Ae. agypti* population increased during the rainy season; however, *Ae. furcifer* population initially increased at the beginning of the rainy season, but then declined. *Ae. furcifer* needs a dry season to mature: eggs laid during the rainy season become mature during the next dry season until new rainfall (Schaeffer *et al.* 2008).

The larvae were most abundant during the wet season, with the largest number of positive containers, the highest larval index and largest number of high density sites (Stickman and Kittayapong 2002).

Rao *et al* (1973) found the average monthly container indices outdoors were related to rainfall. In the present study, we also found the outdoor ovitrap

indices had a greater correlation with rainfall than indoor ovitrap indices. Consequently, *Ae. albopictus* may be more dependent on rainfall than *Ae. aegypti*. Schultz (1993) reported a similar observation in the Philippines.

In Manaus City, Brazil, *Ae. aegypti* reproduction was greater during the rainy season due to less use of water storage vessels, allowing for *Aedes* development (Pinheiro and Tadei 2002).

Rainfall as an early indicator of vector reproduction has obvious advantages over late indicators, such as ovitrap indices, larval density, *Aedes* house indices and Breteau indices (Foo *et al.* 1985).

On the other hand, a negative association between the number of *Aedes* mosquitoes and rainfall was observed in Pulau Ketam. Adnan *et al* (2009) also reported a negative association between ovitrap index and high rainfall on the campus of Universiti Putra Malaysia (UPM), Selangor. The Breteau index (number of positive containers per 100 houses) reached its lowest value at the peak of the rainy season in a study from Jinjang, Kuala Lumpur (Lee and Cheong 1987).

Heavy rain and strong winds may disturb the flight activity of *Aedes* resulting in difficulties in finding hosts and suitable breeding sites (Rozilawati *et al.* 2007). Another reason for the negative impact of heavy rain on the *Aedes* ovitrap index is the larvae were flushed out of the ovitrap

and other potential containers during heavy downpours (Lee and Cheong 1987, Foo *et al.* 1985). Thus, the negative association between outdoor ovitrap index and rainfall was more pronounced than indoors since the rainfall exerted a greater influence on outdoor *Aedes* larvae than indoor *Aedes* larvae. A study from Kolej Mohamed Rasid, Malaysia also showed similar results (Adnan *et al.* 2009).

Thus rainfall had significant effect on outdoor aedes mosquitoes having strong relationship with the life stages.

3.3.3.9 Distribution pattern of dengue diseases in different parts of Dhaka city with respect to seasons

The container breeding of aedes mosquitoes carried dengue virus and caused dengue fever in different parts of Dhaka city dengue cases were not found in all the months of the year. This disease occurred from February to November with a remarkable variation in some (Figure 3.3.25) months. In the month of January dengue was not reported (Table 3.3.1). This disease was found from February with few cases only 3 patients were found in the hospital of Dhaka city. The numbr of the patients were also few up to May where 13 patients were recorded. From June the dengue began to increase in July with number of patients 1393 (Figure 3.3.29). This disease gradually decreased from month of August until November. The dengue cases in

August was half of the number of June. In September to November the dengue patients were negligeable in number with nil in December.

This study recognized the most affected area of Dhaka city was Tejgaon with cases of 569 and next was the Motijheel division with number of dengue patients 456. Mirpur division showed the number of dengue cases almost close to Motijheel. In Ramna and Wari divisions the dengue patients were the same as 342 in number (Table 3.3.3). In other divisions such as Gulshan, Lalbagh and Uttara the reported dengue cases were less ranging from 145 to165. Thus the most vulnerable division of Dhaka city was Tejgaon following Motijheel and Mirpur with intermediate vulnerable divisions of Ramna and Wari but very few cases in other divisions like Gulshan, Lalbagh and Uttara and this can be shown in Figure 3.3.26. There was a linear positive correlation with $R^2 = 0.506$ between monthly distributed pattern of total dengue patients and monthly distribution of aedes mosquito population in Dhaka city (Figure 3.3.28). Figure 3.3.29 showed very strong positive correlation with $R^2 = 0.841$ between monthly distributed pattern of total dengue patients and division wise distribution of aedes mosquito population in Dhaka city.

3.3.3.10 Relationship between incidence of aedes mosquito population and dengue patients in Dhaka city

There was a linear positive relationship (R^2=0.268) between monthly incidence of aedes mosquito population and monthly distributed pattern of total dengue patients in Dhaka city (Figure 3.3.27). Figure 3.3.28 showed very strong positive relationship (with R^2=0.841) between the incidence of aedes mosquitoes population and number of dengue patients in different divisions of Dhaka Metropolitan City in the same area. Figure 3.3.29 showed dengue patients in Square and Ibn Sina hospital in Dhaka city.

Table 3.3.3 Distribution pattern of dengue patients in different divisions of Dhaka city

Divisions	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Total
Tejgaon		1	3	2	14	140	342	40	12	11	4		569
Motijheel		1	2	5	8	79	247	63	21	15	5		456
Mirpur					2	132	231	51	11	3	1		431
Ramna						95	165	65	12	3	2		342
Wari						92	176	65	5	1	3		342
Gulshan		1		1		55	88	19	2	1			165
Lalbagh						41	83	17	5	1	1		147
Uttara				3	3	50	61	23	4	1			145
Total	**0**	**3**	**5**	**11**	**13**	**698**	**1393**	**343**	**72**	**36**	**16**	**0**	**2597**

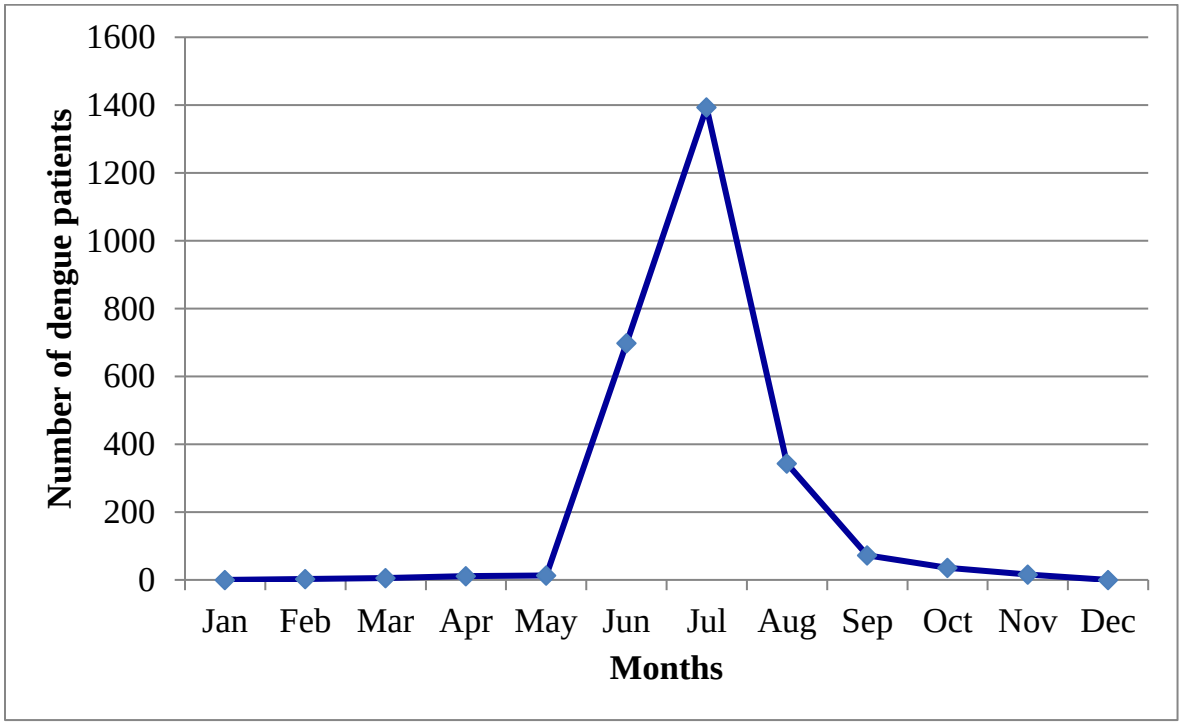


Figure 3.3.24 Distribution pattern of dengue patients in different months in Dhaka city.

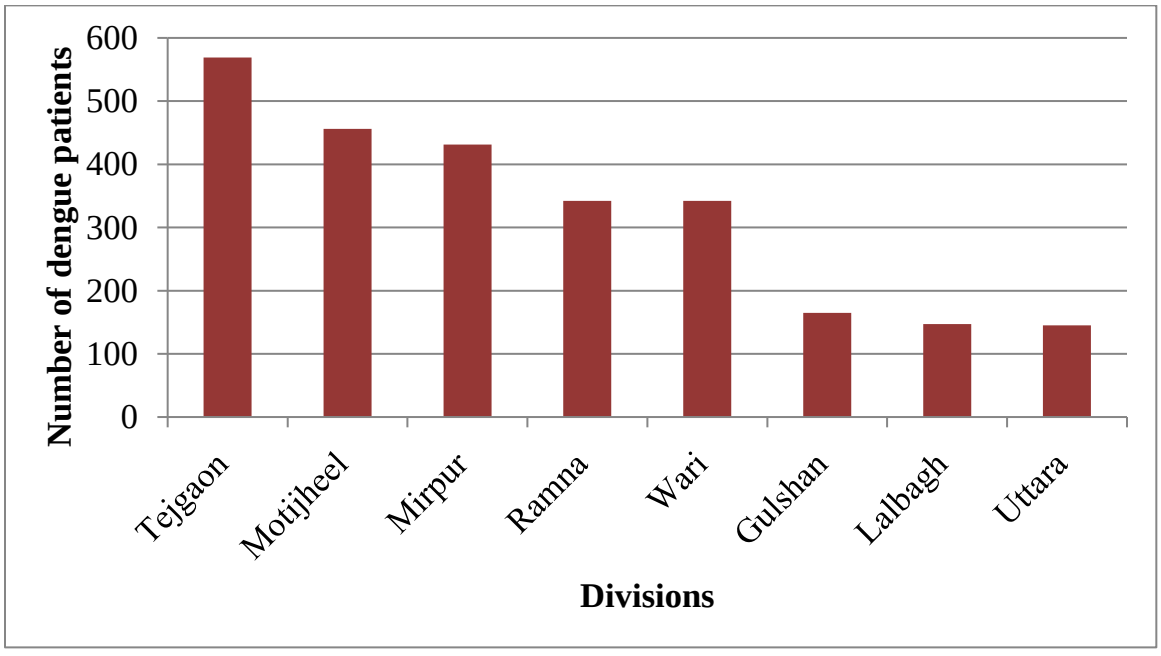


Figure 3.3.25 Distribution pattern of dengue patients in different places in Dhaka city.

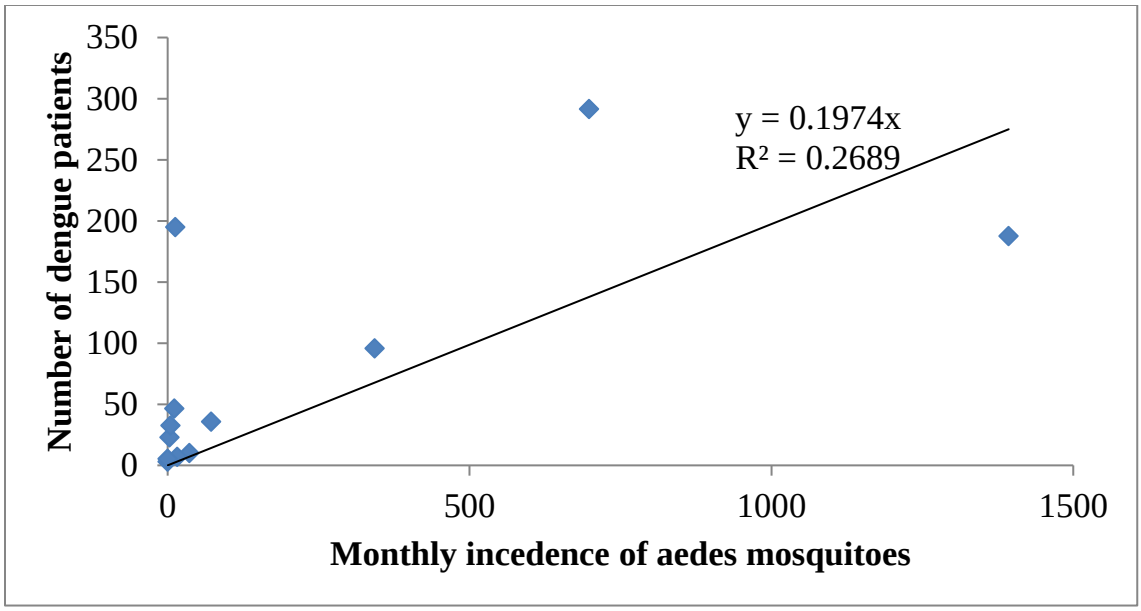


Figure 3.3.27 Relationship between monthly distributed pattern of total dengue patients and monthly distribution of aedes mosquito population in Dhaka city.

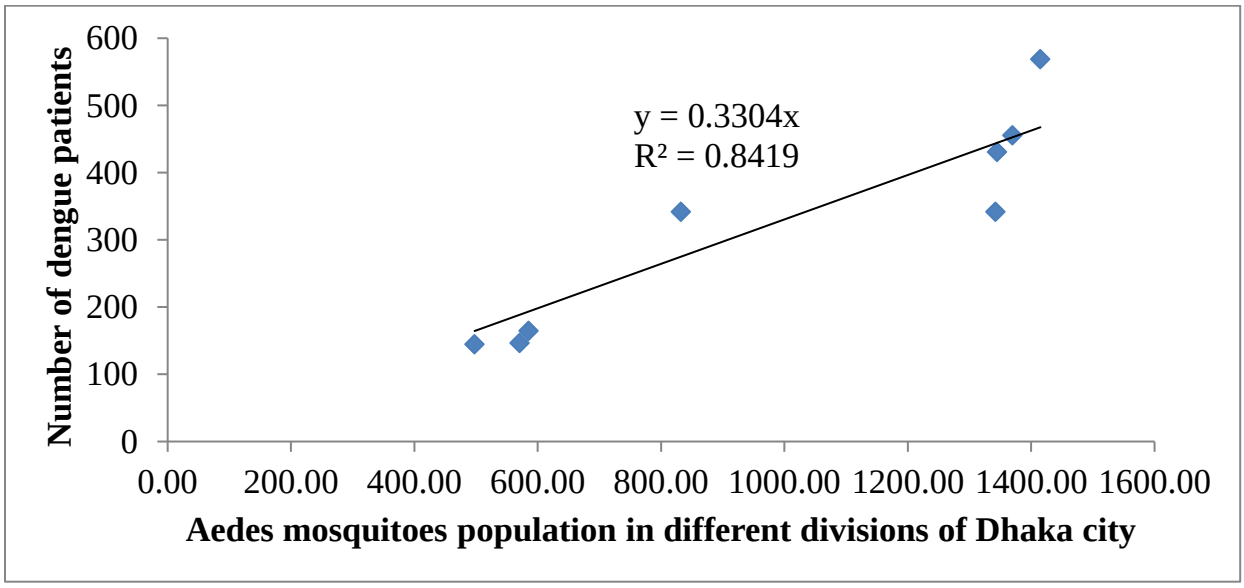


Figure 3.3.28 Relationship between monthly distributed pattern of total dengue patients and division wise distribution of aedes mosquito population in Dhaka city.

Figure 3.3.29 Dengue patients under treatment in Square Hospital (Left) and Ibn Sina (right).

EXPERIMENT 3.4

EVALUATION OF THE EFFICACY OF SOME INSECTICIDES AGAINST LARVAE AND PUPAE OF AEDES MOSQUITOES

EVALUATION OF THE EFFICACY OF SOME INSECTICIDES AGAINST LARVAE AND PUPAE OF AEDES MOSQUITOES

ABSTRACT

This work compared the efficacy of six larvicides, three from synthetic pyrithroids (tetramethrin, deltamethrin and permethrin) at 0.2% W/W one from organophosphate (temephos) and two petroleum oils (kerosene and diesel). Different concentrations as 0.25 ml/L, 0.5 ml/L, 0.75 ml/L, 1.00 ml/L, 1.25 ml/L and 1.50 ml/L were studied against 1^{st}, 2^{nd}, 3^{rd}, 4^{th} instar larvae and pupae of aedes mosquitoes in laboratory. Of the six insecticides the mortality of 4^{th} instars larvae of aedes mosquitoes was the highest in temephos with the lowest LD_{50} value 5.41-9.67 after 6 hours and 1.24-2.74 after 24 hours of treatment. Permethrin and tetramethrin caused the identical mortality and their LD_{50} values were higher the temephos. The petroleum oils such as kerosene and diesel were not so effective in killing aedes mosquitoes larvae with the highest LD_{50} values 11.79-17.42 after 6 hours and 5.6-9.27 after 24 hours of treatment. The toxic action of six insecticides on the pupae of aedes mosquitoes were less than that of larvae with high LD_{50} values. Temephos gave the highest mortality of different instars larvae and pupae after 24 interval followed by permethrin, tetramethrin and deltamethrin.

3.4.1 INTRODUCTION

Aedes mosquitoes (Diptera: Culicidae) are the mosquito responsible for the transmission of dengue in tropical and subtropical regions of the world (Preet and Sneha 2011, Morales *et al.* 2010) including the South Pacific, Southeast Asia, India, Africa and the subtropical zone of America (Suh *et al.* 2007). Breeding sites are essentially artificial: urban (vacant lots, salvage yards, landfills) or domestic (tyres, bottles, open cans or containers of any kind, drinking water, tanks, pots and jars, among others) (Anon. 2011).

Chemical control by using insecticides is the most popular method for the control of household pests and for public health (Hemingway and Ranson 2000, Yap *et al.* 1984). Insecticides approved by the World Health Organization (2006b) for application to drinking water, which are temephos, permethrin, deltamethrin and tetramethrin. These insecticides target mainly aedes mosquitoes such as *Aedes aegypti*, a typical indoor breeder species which often oviposits in fresh water. Temephos is an organophosphate compound which targets the nervous system of mosquito larvae by inactivating the enzyme acetylcholinesterase during nerve transmission. Deltamethrin was another synthetic insecticide used as larvicide. Functioning as insect growth regulator, it prevents the maturation of infected mosquito larvae into adults (McCall and Kittayapong 2007). Deltamethrin

was shown to be more lethal than permethrin, by having a higher knockdown and mortality with lower doses (Chan *et al.* 2011).

Mosquito vector control though the usage of chemicals such as insecticides is an effective control measure because of their fast action, it is toxic to nature (Becker 2003). The available modern chemical insecticides may be more effective to suppress the pest. Initially the insecticides may be tested in the laboratory before using in the field. Although several researchers reported the efficacy of different chemicals separately but information on the efficacy of these chemicals is scanty in Bangladesh. Keeping this view in mind a study was undertaken with the following objectives:

- to evaluate the effectiveness of six chemicals against larvae and pupae of aedes mosquitoes
- to select the best insecticide against aedes mosquitoes

3.4.2 MATERIALS AND METHODS

The experiment was conducted at Central Laboratory of Sher-e-Bangla Agricultural Univerity, Dhaka under room condition from April to December 2016.

3.4.2.1 Selection of insecticides

Six chemicals namely temephos 25EC, tetramethrin 0.2% WP, permethrin 0.2% WP, deltamethrin 0.2% WP and temephos (Figure 3.4.1), kerosene and diesel with different doses were tested against larvae and pupae of aedes mosquitoes. Six doses of each chemical compound such as 0.25 ml/L, 0.5 ml/L, 0.75 ml/L, 1.0 ml/L, 1.25 ml/L, and 1.5 ml/L of water were used in this experiment. The technical information of these chemicals has been presented Table 3.4.1.

Table 3.4.1 Chemicals with mode of action and doses used against aedes mosquitoes

Common Name	Trade Name	Active ingredient	Group	Mode of action	Doses used
Temephos	Temper 50EC	Aquabac 2.86%	Organophosphate	Contact and stomach poison	0.25ml/L 0.5ml/ L 0.75ml/L 1.0ml/L 1.25ml/L 1.50ml/L
Permethrin	Ambush	500g/L Permethrin	Pyrehroids	Contact and stomach poison	0.25ml/L 0.5ml/ L 0.75ml/L 1.0ml/L 1.25ml/L 1.50ml/L
Tetramethrin	Tetramethrin	Methyl Ester	Pyrehroids	Contact and stomach poison	.25ml/L 0.5ml/L 0.75ml/L 1.0ml/ L 1.25ml/ L 1.50ml/L
Deltamethrin	Decis	Acrinathrin and Allethrin	Pyrehroids	Contact and stomach poison	0.25ml/L 0.5ml/L 0.75ml/L 1.0ml/ L 1.25ml/L 1.50ml/L
Kerosene	Jet A1	Octane (C8 H18)	Petroleum oil	Stomach poison	0.25ml/L 0.5ml/L 0.75ml/L 1.0ml/L 1.25ml/L 1.50ml/L
Diesel	Diesel	2,4-Dimethy1-6 tertbutylphenol	Petroleum oil	Stomach poisons	0.25ml/L 0.5ml/L 0.75ml/L 1.0ml/L 1.25ml/L 1.50ml/L

3.4.2.2 Larvae collected from stock culture

Aedes mosquitoe larvae were collected from the stock of mass rearing in the central laboratory of Sher-e-Bangla Agricultural University, Sher-e-Bangla Nagar, Dhaka.

3.4.2.3 Bioassay of selected insecticides

To evaluate the efficacy of six selected chemicals against 1st, 2nd, 3rd and 4th instar larvae and pupae of aedes mosquitoes experiment was set up in the central laboratory at SAU (Figure 3.4.1). Each chemical solution was prepared by mixing with distilled water at different doses. Two liters distilled water was taken in each enamel plate. Exact amount of each insecticide was taken using syringe and mixed well in each plate. Only two liters distilled water was used for untreated control. Before adding insecticides yeast and blood meal were added into water as food for the larvae. After shaking, the volume was made up to the mark by adding more distilled water. Then ten 1st instar larvae of aedes mosquito were placed in each plate. Four replications were made for every dose of each treatment. Same method was followed for the bioassay of 2nd, 3rd, 4th instar larvae and pupae.

Data on dead and alive larvae and pupae were recorded from each treatememt and replication at 6, 12, 18 and 24 hr interval. The larvae and pupae were observed for first 6 hours then at 12th, 18th and 24th hour for

mortality and the moribund larvae and pupae were considered as dead. Finally after 24 hours, the number of dead larvae and pupae was counted in each group. Percentage of mortality was calculated by the following formula.

$$\% \text{ Mortality } = \frac{\text{Number of dead larvae or pupae}}{\text{Total number of larvae or pupae}} \times 100$$

Mortality data were corrected using the Abbot's formula (1925).

$$\% \text{ Corrected mortality } = (1 - \frac{\text{Population in treatment after treatment}}{\text{Population in control after treatment}}) \times 100$$

3.4.2.4 Data analysis

The LD_{50} values were calculated using 'Probit Analysis', which was initially developed by D.J. Finney (1971) and later discussed in detail elsewhere (Robertson *et al.* 2007, Finney 1978). In general, the data from bioassays mortality proportions and corresponding doses are given a S-shape curve. In order to make this curve linear, the proportions are transformed to probits and doses to log10.

Percent mortality data were analyzed using computer based MSTAT-C program for CRD and means separation was done by Dancan's Multiple Range Test (DMRT).

Plate 3.4.1 Treatment of larvae and pupae with larvicides and pupicides.

3.4.3 RESULTS AND DISCUSSION

3.4.3.1 Toxicity of insecticides against 1st instar larvae of aedes mosquitoes

The larval and pupal mortality data were subjected to probit analysis for calculating LD_{50} at 95% fiducial limits of upper confidence limit and lower confidence limit and CRD values were calculated using the MSTAT software. Results with $P < 0.05$ considered to be statistically significant.

Based on the result of the first instar larvae significantly, temephos gave the lowest median lethal dose, LD_{50} of 1.24 after 24 hr in the range of 5.41 – 1.24 from 6 hr to 24 hr interval having LSD 1.18-0.44 at P=0.05 and next permethrin at the LD_{50} of 2.54 (Table 3.4.2). The LD_{50} for tetramethrin was 3.58 after 24 hr. Deltramethin was the medium and scored 4.45 at 24 hr interval. Petroleum oil kerosene and diesel were less effective than organophosphate and pyrethoids. Kerosene having 5.6 LD_{50} value that was higher than the deltamethrin and less than the diesel. Diesel was the least effective insecticide with 8.74 LD_{50} value at 24 hr interval.

The results of the present investigation were almost similar to the findings of Shaalan et al. (2005) who found that the LD_{50} values of tetramethrin and deltamethrin were 2.96 and 4.37, respectively at 24 hr interval. Khater and

Shalaby (2008) observed that LD_{50} value of permethrin was 2.97 which was similar to this study.

Table 3.4.2 LD_{50} values of insecticides against 1st instar larvae of aedes mosquitoes at different intervals

Treatment	LD_{50} values after different interval of data collection			
	6 hr	**12hr**	**18 hr**	**24 hr**
Temephos	5.41d	3.68e	2.36f	1.24f
Permethrin	8.17c	4.35e	3.38e	2.54e
Tetramethrin	8.49c	5.51d	4.56d	3.58d
Deltamethrin	10.71b	6.77c	5.50c	4.45c
Kerosene	11.79ab	8.52b	6.63b	5.61b
Diesel	12.57a	10.65a	9.76a	8.74a
LSD (P=0.05)	**1.18**	**0.69**	**0.44**	**0.82**

3.4.3.2 Toxicity of insecticides against 2nd instar larvae of aedes mosquitoes

Based on the result (Table 3.4.3), temephos gave the lowest median lethal dose, LD_{50} of 2.39 after 24 hr in the range of 9.39 – 2.39 from 6 hr to 24 hr interval having LSD 1.10-0.58 at P=0.05 followed by permethrin at the LD_{50} of 3.12. The LD_{50} for tetramethrin was 4.44 after 24 hr. Deltamethrin was the medium and scored 6.47 at 24 hr interval. Petroleum oil kerosene and diesel were less effective than organophosphate and pyrethoids. Kerosene having 8.47 LD_{50} value that was more than the deltamethrin and less than the diesel. Diesel was the least effective insecticide with 10.46 LD_{50} value at 24 hr interval.

The results of the present experiment were similar to the findings of Thomas *et al.* (2004). They found that LD_{50}value of deltamethrin and temephos were 6.50 and 3.10 respectively of 3rd instar larvae after 24 hr. They also found the LD_{50} values of kerosene and diesel were 6.12 and 9.57 respectively of 2nd instar larvae after 24hr. Rahuman et al. (2009) observed that in case of 2nd instar, LD_{50} values of permetrhin and tetramethrin were 3.53 and 4.61.

Table 3.4.3 LD_{50} values of insecticides against 2nd instar larvae of aedes mosquitoes at different intervals

Treatment	**LD_{50} values after different interval of data collection**			
	6 hr	**12hr**	**18 hr**	**24 hr**
Temephos	9.39f	5.14d	3.95f	2.39f
Permethrin	10.93e	5.49e	4.87d	3.12e
Tetramethrin	12.57d	8.41c	7.50d	4.44d
Deltamethrin	13.71c	10.36bc	9.58c	6.47c
Kerosene	15.89b	12.62ab	11.55b	8.47b
Diesel	17.42a	14.31a	12.43a	10.46a
LSD (P=0.05)	**1.10**	**2.65**	**0.27**	**0.58**

3.4.3.3 Toxicity of insecticides against 3rd instar larvae of aedes mosquitoes

The average larval and pupal mortality data were subjected to probit analysis for calculating LD_{50}, at 95% fiducial limits of upper confidence limit and lower confidence limit and RCBD values were calculated using the MSTAT software. Results with $P < 0.05$ were considered to be statistically significant.

Based on the result (Table 3.4.4), temephos gave the lowest median lethal dose, LD_{50} of 3.03 after 24 hr in the range of 6.56 – 3.03 from 6 hr to 24 hr interval having LSD 0.66-0.67 at P=0.05 followed by permethrin at the LD_{50} of 4.49. The LD_{50} for tetramethrin was 5.58 after 24 hr. Deltamethin was the medium and scored 6.62 at 24 hr interval. Petroleum oil kerosene and diesel were less effective than organophosphate and pyrethoids. Kerosene having 8.31 LD_{50} value that was more than the deltamethrin and less than the diesel. Diesel was the least effective insecticide with 9.35 LD_{50} value at 24 hr interval.

The efficacy was statistically contradicted to the experimental findings conducted by Ghosh *et al*. (2012). They observed that the LD_{50} values of temephos at 3rd and 4th instar larvae were 1.01 and 14.45 respectively after 18 hr and 24 hr interval. On the other hand LD_{50} values of permethrin and deltamethrin were 6.12 and 8.57 of 3rd instar larvae of aedes mosquitoes respectively after 18 h interval that were similar to the same stage and interval.

Table 3.4.4 LD_{50} values of insecticides against 3rd instar larvae of aedes mosquitoes at different intervals

Treatment	**LD_{50} values after different interval of data collection**			
	6 hr	**12hr**	**18 hr**	**24 hr**
Temephos	6.56f	5.45d	4.23f	3.03e
Permethrin	7.36e	6.18d	5.58e	4.49d
Tetramethrin	8.68d	7.52c	6.64d	5.58c
Deltamethrin	9.50c	8.17c	7.47c	6.22c
Kerosene	11.36b	10.38b	9.39b	8.31b
Diesel	12.59a	11.44a	10.45a	9.35a
LSD (P=0.05)	**0.66**	**0.95**	**0.41**	**0.67**

3.4.3.4 Toxicity of insecticides against 4th instar larvae of aedes mosquitoes

Based on the result (Table 3.4.5), temephos gave the lowest median lethal dose, LD_{50} of 7.74 after 24 h in the range of 12.57 – 1.24 from 6 hr to 24 hr interval having LSD (P=0.05) followed by permethrin at the LD_{50} of 3.42. The LD_{50} for Tetramethrin was 5.19 after 24 hr. Deltamethrin was the medium and scored 7.31 at 24 hr interval. Petroleum oil kerosene and diesel were less effective than organophosphate and pyrethoids. Kerosene having 7.27a LD_{50} value that was more than the deltamethrin and less than the diesel. Diesel was the least effective insecticide with 9.26 LD_{50} value at 24 hr interval.

The results supported the observation of Lin *et al.* (2008), they observed that the LD_{50} value of deltamethrin was after 24 hr. Rawani *et al.* (2010) found that LD_{50} value of temephos was 2.54 at 24 hr interval 4th instar larvae of aedes mosquitoes.

Table 3.4.5 LD_{50} values of insecticides against 4th instar larvae of aedes mosquitoes

Treatment	LD_{50} values after different interval of data collection			
	6 hr	12hr	18 hr	24 hr
Temephos	9.67f	8.02e	6.02f	2.74d
Permethrin	10.83e	8.98d	7.39e	3.42cd
Tetramethrin	11.57d	10.32c	9.14d	5.19bc
Deltamethrin	13.57c	12.66b	11.33c	7.31ab
Kerosene	14.70b	13.22b	12.31b	9.27a
Diesel	15.70a	14.31a	13.44a	9.26a
LSD (P=0.05)	**0.30**	**0.84**	**0.47**	**2.20**

3.4.3.5 Toxicity of insecticides against Pupae of aedes mosquitoes

The average larval and pupal mortality data were subjected to probit analysis for calculating LD_{50}, at 95% fiducial limits of upper confidence limit and lower confidence limit and RCBD values were calculated using the MSTAT (Statistical Package of Social Sciences) software. Results with $P < 0.05$ were considered to be statistically significant.

Based on the result (Table 3.4.6), Temephos gave the lowest median lethal dose, LD_{50} of 2.44 after 24 hr in the range of 9.62 – 2.44 from 6 hr to 24 hr interval having LSD 0.30, 0.84, 0.47 and 2.20 respectively followed by permethrin at the LD_{50} of 3.52. The LD_{50} for tetramethrin was 5.05 after 24 hr. which was the medium. Petroleum oil kerosene and diesel were less effective than organophosphate and pyrethoids. Kerosene having 7.25b LD_{50} value was more than the deltamethrin and less than the diesel. Diesel was the least effective insecticide with 8.38a LD_{50} value at 24 hr interval.

Mortality count was made after 6 hr, 12 hr, 18 hr and 24 hr intervals where highest mortality was recorded for each test after 24 hr. No survival was observed indicating that pupae were susceptible to temephos, deltamethrin, permethrin, tetramethrin, kerosene and diesel.

The efficacy was statistically contradicted to the study of Anees (2008), they found that LD_{50} values of temephos and kerosene were 14.56 and 21.34

respectively after 24 hr. Bansal et al. (2012) observed that the LD_{50} value of tetramethrin was 21.54 after 24 hr Kennedy and Wightman (2011) observed that the LD_{50} values of permethrin and deltamethrin were 3.21 and 5.98 against pupae after 24 h which are almost similar to this study.

Table 3.4.6 LD_{50} values of insecticides against pupae of aedes mosquitoes after different intervals

Treatment	LD_{50} values after different interval of data collection			
	6 hr	**12hr**	**18 hr**	**24 hr**
Temephos	9.62e	7.69d	4.36e	2.44f
Permethrin	11.20d	9.24cd	6.37d	3.52e
Tetramethrin	13.01c	11.14bc	8.12c	5.05d
Deltamethrin	14.30b	12.59ab	10.18b	6.11c
Kerosene	15.56a	13.39a	11.42a	7.25b
Diesel	16.34a	14.45a	12.40a	8.38a
LSD (P=0.05)	**0.79**	**1.92**	**0.99**	**0.54**

The chemical insecticides used against larvae and pupae resulted higher mortality in organophosphate and pyrethroid classes of insecticides and proved to be more toxic to aedes mosquitoes at immature stages but kerosene and diesel did not show such mortality and not effective against the aedes mosquitoes. The early instar larvae and pupae were more susceptible than the latter ones.

3.4.3.6 Mortality of 1st, 2nd, 3rd, and 4th instar larvae and pupae of aedes mosquitoes in suspension of six selected insecticides

Mortality of larvae and pupae after 24 hr varied significantly in different chemicals. In case of first instar larvae the highest mortality (100%) was observed in temephos followed by permethrin (99.25%) having no significant variation between them. On the other hand the lowest larval mortality (92.50%) was found in diesel having no significant difference with kerosene but significantly different from rest of the chemicals. Permethrin and tetramethrin also gave the similar result against first instar larvae. Similar results were observed against 2nd, 3rd and 4th instar larvae and pupae of aedes mosquitoes. The trend of mortality was highest in first instar larvae and consequently decreased from second instars to pupae except temephos. The above results indicate that all the chemicals tested gave more than 85% mortality of different instars larvae and pupae of aedes mosquitoes. But the highest result was found for temephos followed by permethrin and tetramethrin. The order of effectiveness of six chemicals against four instars larvae and pupae of aedes mosquito was temephos> permethrin> tetramethrin> deltamethrin> kerosene> diesel.

The result on the mortality of larvae under different treatments agree with the the study of Smith *et al.* (2009), they found that percent mortality of temephos, permethrin and deltamethrin were 99.50%, 97% and 93%

respectively after 24 hr of application on 3rd instar larvae. Kennedy and Wightman (2011) observed that the percent mortality of tetramethrin was 92% after 24 hr of treatment against 4th instar larvae that was similar to this study. Ghosh *et al.* (2012) observed that the percent mortality of temephos and deltamethrin were 99% and 91% respectively of pupae after 24 hr which are similar to this study. Dharshini *et al* (2011) found percent mortality of deltamethrin 77% after 24 hr of treatment applied on 3rd instar larvae of aedes mosquitoes that was dissimilar to this study. This dissimilarity was happened due to application method. They sprayed on breeding sites of aedes mosquito where as suspension was used in this study.

Table 3.4.7 Effectiveness of insecticides against 1st instar, 2nd instar, 3rd instar, and 4th instar larvae and pupae of aedes mosquitoes in suspension

Treatments	Percent mortality values after 24 hours				
	1st instar larvae	2nd instar larvae	3rd instar larvae	4th instar larvae	Pupae
Temephos	100.0a	100.00a	100.0a	100.0a	100.0a
Permethrin	99.25ab	97.25b	96.50b	95.75b	95.75b
Tetramethrin	97.00bc	96.25b	94.25bc	91.75c	91.50c
Deltamethrin	95.25cd	93.00c	92.75c	91.50c	90.75cd
Kerosene	93.50de	93.50c	91.75c	90.50c	89.25d
Diesel	92.50e	87.50d	85.25d	85.50d	85.00e
LSD (P=0.05)	**2.63**	**2.86**	**2.62**	**2.51**	**1.53**

In a column means followed same letter(s) are not significantly different at 5% level of probability by DMRT.

CHAPTER IV

SUMMARY AND CONCLUSION

CHAPTER IV

SUMMARY AND CONCLUSION

A comprehensive research was conducted during January, 2013 to December, 2015 was conducted to study the biology, breeding sites, the seasonal distribution and chemical control of aedes mosquitoes in Dhaka city. This research work was done in the central library of Sher-e-Bangla Agricultural University, Sher-e-Bangla Nagar, Dhaka along with 25 thanas of Dhaka city under eight divisions to find out the severity of dengue vector aedes mosquitoes and their chemical control approach. This research was also included a survey of breeding sites of aedes mosquitoes in five types of houses having 11 types of wet containers. The vulnerability of dengue fever accompanied with population density of aedes mosquitoes with seasonal and placement impact was also determined in this study.

The biology of aedes mosquitoes was studied in the central laboratory of Sher-e-bangla Agricultural University, Sher-e-Bangla Nagar, Dhaka from April 2015 to October 2015. Eggs were collected from Sher-e-bangla Agricultural University campus emerged as adults aedes mosquitoes both *Aedes aegypti* and *Aedes albopictus.* The gravid female laid eggs in cluster. Each cluster having 105-129 eggs of mean 117.71 ± 9.12. Each

female laid 3-4 clusters. Initially the colour of the egg was white and gradually turned into black. The incubation period of eggs ranged from 48 to72 h with mean of 60 ± 0.53.

The period of development from the first instar larva to adult stage through pupa for aedes mosquitoes was 8.37 ± 0.18 days for male and 9.5 ± 0.24 days for female respectively. Female aedes mosquitoes fed with blood showed the highest mean survival period which was 26.23 ± 2.17days while male aedes mosquitoes fed with 10% sucrose recorded 19.23 ± 2.21days which was shorter mean survival period. Depending on the gonotrophic cycle for aedes mosquitoes their number of eggs and longevity varied.

Dengue fever (DF), one of the most important emerging arboviral diseases, is transmitted through the bite of water container breeding mosquitoes *Aedes aegypti* and *Aedes albopictus*. A household entomological survey was conducted in Dhaka city from July, 2014 to June, 2015 to inspect left over containers having water in indoor, outdoor, and rooftop locations of five types of houses for aedes mosquitoes larvae for determining mosquito productivity in presence of larvae in each container type and identifying some risk factors such as stagnant water, positision of wet containers etc. of households infested with aedes larvae.

Of 9,222 households inspected, 1,306 (14.2%) were positive for aedes larvae. Of 38,777 wet containers examined, 2,272 (5.8%) were infested with two species of aedes larvae. Out of eleven types of containers used to hold water, such as earthen jars, tanks, and drums were the most common containers for larval breeding. Tyres in outdoor and rooftop locations of the households were also important for larvae for living. Although present in abundance, buckets were of less importance. Factors such as independent household, presence of a water storage system in the house, and fully/partly shaded outdoors werefound significantly associated with household infestation of aedes larvae.

The seasonal prevalence of *Aedes aegypti* and *Aedes albopictus* was studied in Dhaka city divided into eight divisions with 25 thanas from July, 2014 to June, 2015. The abundance of different stages such as eggs, larvae, pupae and adult mosquitoes was noticed throughout the year with variation in different seasons. The outdoor survey using containers for breeding purpose showed that the eggs were the most abundant, and larval, pupal and adult population respectively were less than that of eggs.The peak of the population of all stages occurred in June. When the rainfall was the highest and temperature was high. From September to April population level remained low due to low rainfall and temperature.

The seasonal weather conditions such as rainfall, temperature and humidity influenced significantly on the population abundance resulting positive correlation particularly with rainfall.The dengue disease occurred predominantly in rainy season and almost nil in winter season. This disease was most severe in the month of July and also June and August. The severity of dengue patients was decreasing from September to November.

This work compared the toxic effects of six larvicides, three from synthetic pyrithoids (Pralemethrin, Deltamethrin and Permethrin at 0.2% W/W one from organophosphate (Temephos) and two petroleum oils (Kerosen and Diesel) based on different concentrations such as 0.25 ml/L, 0.5 ml/L, 0.75 ml/L, 1.00 ml/L, 1.25 ml/L and 1.50 ml/L were studied against 1^{st}, 2^{nd}, 3^{rd}, 4^{th} instar larvae and pupae of aedes mosquitoes in laboratory. Of the six insecticides the mortalityof 4^{th} instar larvae of aedes mosquitoes was the highest in Temephos with the lowest LD_{50} value 5.41-9.67 after 6 hours and 1.24-2.74 after 24 hours of treatment. Permethrin and Tetramethrin caused the identical mortality and their LD_{50} values were higher than that of the Temephos. The petroleum oils such as Kerosene and Diesel were not so effective in killing aedes mosquitoes larvae with the highest LD_{50} values 11.79-17.42 after 6 hours

and 5.6-9.27 after 24 hours of treatment. The toxic action of six insecticides on the pupae of aedes mosquitoes were less than that of larvae with high LD_{50} values.

CONCLUSION

Based on findings of the study the following conclusions are drawn

1. Aedes mosquitoes completed their life cycle through four stages like egg, larva, pupa and adult in 19-26 days. The adult mosquitoes were the active stage in the life cycle. All the stages could be recognized easily in the habitat of the household containers which served as breeding place for them.
2. The breeding sites of aedes mosquitoes were identified as different types of household wet containers. The household survey revealed that out of eleven types of wet containers found in indoor, outdoor and rooftop locatios of independent, multistoried, semi-permanent, slum and other houses in Dhaka city; tyre, tanks, flower pots and earthen jars showed potential productivity of larval development and served as breeding sources of aedes mosquitoes.
3. Aedes mosquitoes occurred in defferent parts of Dhaka city like Tejgaon, Motijheel, Mirpur, Wari, Ramna, Gulshan, Lalbagh and Uttara divisions. Among these eight divisions of Dhaka city Tejgaon was the most vulnerable division for dengue fever following Motijheel and Mirpur.

4. The dengue disease occurred predominantly in rainy season and almost nil in winter season. This disease was most severe in the month of July and also June and August. The severity of dengue patients was decreasing from September to November.
5. The abiotic factors such as rainfall and temperature could be the main reasons of high population of aedes mosquitoes and severity of dengue diseases.
6. The organophosphate larvicide as Temephos at 1.5ml/L was the most effective among six chemicals on first instar larvae of aedes mosquitoes with less LD_{50} values and a little higher dose might be required to control mature larvae and pupae of this mosquitoes in the habitat.

RECOMMENDATIONS

1. The density of aedes mosquitoes population should be identified in different areas where different categories of people are living. As the duration of egg to adult emergence is 8-10 days, stagnant water should not be kept more than 8 days.
2. The study recognized the relative risk of some wet containers for breeding places of aedes mosquitoes such as tanks, tyres, flower pots and earthen jars placed in indoor, outdoor and rooftop of different houses should be identified and strict measures must be taken against the breeding of the mosquitoes in these containers.
3. Besides, Dhaka City Corporation should make a plan of action to stop the population build up of aedes mosquitoes outside the houses by well drainage system, cleaning canals, regular spraying of insecticides etc.
4. Temephos was found as the most effective larvicides against aedes mosquitoes, permethrin and tetramethrin also gave almost similar results.
5. The life style of aedes mosquitoes may vary among and within the communities and also among years of the same location and thus further study in several consecutive years in different areas of the

Dhaka city extending the whole country is required to apply appropriate control program.

CHAPTER V

REFERENCES

CHAPTER V

REFERENCES

Abbot, W.S. (1925). A method of computing the effectiveness of an insecticide. *J. Econ. Entomol*. **18**: 265-267.

Abercrombie, J. (2003). Medical entomology studies – IX. The subgenus *Christophersiomyia* of the genus *Aedes* (Diptera: Culicidae). *Contributions of the American Entomological Institute* (*Ann Arbor*), **14**(2): 1–72.

Adames, A.J. and Galindo, P. (2003). Mosquito studies (Diptera, Culicidae) XXX. A new subgenus and species of *Culex* from Colombia. *Contributions of the American Entomological Institute* (*Ann Arbor*), **9**(3): 55–61.

Adnan, R.A, Osman, M., Unyah, N.Z., Abdullah, W.O., Hamat, R.A. and Majid R.A. (2009). Fogging impact on *Aedes* mosquitoes: A preliminary findings on continuous vector surveillance. Kuala Lumpur: 45th Annual Scientific Seminar, Malaysian Society of Parasitology and Tropical Medicine.

Ahmed, F.U., Chowdhury, B.M., Jhulan, D.S., Hoque, S.M., Zaman, R. and Hasan, M.S. (2001). Dengue and Dengue haemorrhagic fever in children during the 2000 outbreak in Chittagong, *Bangladesh Deng. Bull*. **25**: 35-39.

Ahmed, T.U., Joshi, G.P., Ahmed R.U, Dewan, Z.U., Chowdhury, M.S. and Akther, S. (1990). Container habitat mosquitoes of Bangladesh. *J. Zool*. **5**: 7-16.

Ahmed, T.U., Josi, G.P., Ahmed, R.U., Dewan, Z.U., Chowdhury, M.S and Akhter S. (1990). Container habitats mosquitoes in Bangladesh. *Bangladesh J. Zool*. **5**:169-178.

Ahmed, T.U., Rahman, G.M., Bashar, K., Shamsuzzaman, M., Samajpati, S., Sultana, S., Hossain, M.I., Banu, N.N. and Rahman, M.S. (2007). Seasonal prevalence of dengue vector mosquitoes in Dhaka City, Bangladesh. *Bangladesh J. Zool*. **35**(2): 205-212.

Akram, W. and Lee, J.J. (2004). Effect of habitat characteristics on the distribution and behavior of *Aedes albopictus*. *J. Vector Ecol.* **12**: 379-382.

Ali, A., Chowdhury, M.A., Hossain, M.I., Ameen, M., Habiba, D.B. and Aslam, A.F.M. (1999). Laboratory evaluation of selected larvicides and insect growth regulators against field-collected *Culex quinquefasciatus* larvae from urban Dhaka, Bangladesh. *J. American Mosq. Contrl. Assoc*. **15**(1): 43-47.

Ali, M., Wagatsuma, Y. and Emch, M. (2003). Use of a geographic information system for defining spatial risk for dengue transmission in Bangladesh: role for *Aedes* albopictus in an urban outbreak. *American J. Trop. Med. Hyg*. **69**: 634– 640.

Alto, B. W. and Reiskind, M.H. (2008). Size Alters Susceptibility of Vectors to Dengue Virus Infection and Dissemination. **79:** 688-695.

Alto, B.W. and Juliano, S.A. (2001). Precipitation and temperature effects on populations of Aedes albopictus (Diptera: Culicidae): implications for range expansion. *J. Med. Entomol*. **38**: 646-656.

Alto, B.W. and Lounibos, L.P. (2013). Vector competence for arboviruses in relation to the larval environment of mosquitoes. Ecology of parasite-vector interactions. *C. J. M. Koenraadt, Springer***:** 81-101.

Ameen, M. and Moizuddin, M. (1973). Bionomics of the common mosquitoes of Dhaka. *J. Nat. Hist*. **7**: 1-21.

Ameen, M., Chowdhury, M.A. and Hossain, M.I. (1994). Survey of mosquito breeding sites in the city of Dhaka: a report submitted to the Dhaka city corporation. Safeway Pest Control, Banani, Dhaka. 1-78.

Ameen, M., Hossain, M.I. and Khan, M.D.H. (1984). Seaonal prevalence of common mosquitoes of Dhaka City. Dhaka University studies. **32**(2): 79-89.

Anderson, R.T., Rao, P.N. and Rai, K.S. (2010) Genome evolution in the mosquitoes and other closely related members of superfamily Culicoidea. *Hereditas*. **113**: 139–144.

Anees, A.M. 2008. Larvicidal activity of *Ocimum sanctum* Linn. (Labiatae) against *Aedes aegypti* (L.) and *Culex quinquefasciatus* (Say). *Parasitol. Res*. **103**: 1451–1453.

Anonymous. (2009). Dengue and dengue haemorrhagic fever. Factsheet No 117, revised March 2009. Geneva, World Health Organization.

Anonymous. (2005). Communicable Disease Control: Guidelines for Laboratory and Field Testing of Mosquito Larvicides.

Anonymous. (2011). Rights Participation/water_supply_dhaka.pdf. accessed (15 August 2015).

Anonymous. (2012). Centro de Pesquisa Meteorológicas e Climáticas Aplicadas à Agricultura. Accessed 2 December 2012.

Anonymous. (2014). Dengue and severe dengue. Fact sheet N°. 117.

Anonymous. (2007). Dengue hemorrhagic fever -U.S.-Mexico border 2005. *MMWR.* **56**:786-789.

Anonymous. (2012). Dengue and the *Aedes* aegypti mosquito. Available: http://www.cdc.gov/dengue/resources/30Jan2012/aegyptifactsheet .pdf. [accessed 7 August 2014].

Anonymous. (1995). Progress Report of 1994-1995. *World Health Organization.* P. 50. (Control of Tropical Diseases).

Arunachalam, N. Tana, S. and Espino. F.(2010). Eco-bio-social determinants of dengue vector breeding: a multicountry study in urban and periurban Asia. *Bull. World Hlth. Organ.* **88**: 173–184.

Aziz, M.A., Gorham, J.R. and Gregg, M.B. (1967). Dacca fever an outbreak of dengue. *Pakistan J. Med. Res*. **6**: 83-92.

Bansal, S.K., Singh, K.V. Sharma, S. and Sherwani, M.R.K. (2012). Laboratory observations on the larvicidal efficacy of three plant species against mosquito vectors of malaria, dengue/dengue hemorrhagic fever (DF/DHF) and lymphatic filariasis in the semi-arid desert. *J. Environ. Biol.* **33**: 617–621

Barbosa, P.Peters, M.T. and Greenough, N.C, (1972). Overcrowding of mosquito populations: responses of larval *Aedes* aegypti to stress. *Environ. Entomol*. **1**: 89–93

Barraud, P.J. (1934). The fauna of British India, including Ceylon and Burma, Diptera, Vol-5. Taylor and Francis, London 463.

Barrera, R. (1996)**.** Competition and resistance to starvation in larvae of container-inhabiting *Aedes* mosquitoes. *Ecol. Entomol.* **21**: 112-127.

Barrera, R. and Amador, M. (2008). Unusual productivity of *Aedes aegypti* in septic tanks and its implications for dengue control. *Med. Vet. Entomol.* **22:** 62-69.

Barrera, R. and Amador, M. (2006). Ecological Factors Influencing *Aedes aegypti* (Diptera: Culicidae) Productivity in Artificial Containers in Salinas, Puerto Rico. *Pop. Comm. Ecol.* **43**(3): 484-92.

Barret, B.N. and Higgs, V.P. (2007). *Indian Aedes*. Science Publishers, Inc. LebAnon. New Hampshire, USA.

Beebe, N.W., Cooper, R.D., Mottram, P. and Sweeney, A.W. (2009). Australia's dengue risk driven by human adaptation to climate change. *PLoS Negl. Trop. Dis*. **3**(5): e429.

Besansky, D.S. and Fahey, R.A. (2007). Classification of *Culex* subgenus *Culex* in the New World (Diptera: Culicidae). *Proceedings of the United States National Museum*. **120**(3557): 1–122.

Besansky, N.J. and Fahey, G.T. (2015). Utility of the *white* gene in estimating phylogenetic relationships among mosquitoes (Diptera: Culicidae). *Mole. Biol. Evol*. **14**:442–454.

Black, W.C., Rai, K.S, Turco, B.J and Arroyo, D.C. (1989). Laboratory study of competition between United States strains of *Aedes albopictus* and *Aedes aegypti* (Diptera: Culicidae). *J. Med. Entomol.* **26**: 260-271.

Brady, O.J., Golding, N., Pigott, D.M., Kraemer, M.U., Messina, J.P., Reiner Jr, R.C. and Hay, S.I. (2014). Global temperature constraints on *Aedes aegypti* and *Ae. albopictus* persistence and competence for dengue virus transmission. *Parasit Vectors*. **7**(33): 1-4.

Braga, I.A., Lima, J.B.P., Soares, S.S. and Valle, D. (2004). *Aedes aegypti* resistance to temephos during 2001 in several municipalities in the states of Rio de Janeiro, Sergipe and Alagoas, Brazil. *Mem. do Inst. Oswaldo Cruz.* **99**: 199-203.

Braks, M. A. H, Honório, N.A, Lourenço-de-Oliveira, R., Juliano, S.A. and Lounibos P . L (2003). Convergent Habitat Segregation of *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae) in Southeastern Brazil and Florida. *J. Med. Entomol*. **40**: 785-794.

Braks, M.A.H., Hono´rio, N.A., Lounibos, L.P.R. and Juliano, S.A. (2004). InterspeciÞc competition between two invasive species of container mosquitoes, *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae), in Brazil. *Ann. Entomol. Soc. America*. **97**: 130-139.

Bram, R.A. (2013) A redescription of *Culex* (*Acalleomyia*) *obscurus* (Leicester). *Proc. Entomol. Soci. Washington*. **70**: 52–57.

Brogdon, W.G. and McAllister, J.C. (1998). Insecticide resistance and vector control. *Emerg. Infect Dis.* **4:** 605-613.

Brown, R.E., Hogu, R.T and Kitching, I.J. (2011) Reconsideration of anopheline phylogeny (Diptera: Culicidae: Anophelinae) based on morphological data. *Sys. Biod*. **3**: 345–374.

Campos, R.E., and Macia, A. (1996). Observaciones biologicas de una poblacion natural de Aedes aegypti (Dipterea: Culicidae) en la provincial de Buenos Aires, Argentina. *Rev. Soc. Entomol. Argentina*. **55**: 67-72.

Capinha, C., Rocha, J. and Sousa, C.A. (2014). Macroclimate determines the global range limit of *Aedes aegypti*. *EcoHealth.* **11**(3): 420-428.

Chadee, D.D. (1992). Seasonal incidence and horizontal distribution patterns of oviposition by *Aedes aegypti* in an urban environment in Trinidad, West Indies. *J. American Mosq. Contrl. Assoc.* **8**: 281-284.

Chandre, F., Darriet, F., Manguin, S., Brengues, C., Carnevale, P. and Guillet, P.I. (2015). Pyrethroid cross resistance spectrum among populations of *Aedes aegypti* from Cote d'Ivoire. *J. American Mosq. Contrl. Asso.* **15:** 53-59.

Chang, C., Shen, W.K., Wang, T.T., Lin, Y.H., Hsu, E.L. and Dai, S.M. (2009). A novel amino acid substitution in a voltage-gated sodium channel is associated with knockdown resistance to permethrin in Aedes aegypti. *Insect Biochem. Mole. Biol.* **39**: 272-278.

Chaves, L.F. and Kitron, U.D. (2011). Weather variability impacts on oviposition dynamics of the southern house mosquito at intermediate time scales. *Bull. Entomol. Res*. **101**(6): 633-641.

Chen, C. D., Seleena, B., Nazni, W.A., Lee, H.L and Masri, S.M. (2006). Dengue vectors surveillance in endemic areas in Kuala Lumpur city centre and Selangor State, Malaysia. *Deng. Bull.* **30**(1):197.

Chen, C.D., Nazni, W.A., Lee, H.L., Saleena, B. and Mohd, S.A. (2008a) Biochemical detection of temephos resistance in *Aedes* (Stegomyia) *aegypti* (Linnaeus) from Dengue-endemic areas of Selangor State, Malaysia. *Proce. ASEAN Cong. Trop. Med. Parasitol.* **3:** 6-20.

Chen, C.D., Nazni, W.A., Lee, H.L., Saleena, B. and Mohd, S.A. (2008b) Susceptibility of Aedes aegypti and Aedes albopictus to temephos in four study sites in Kuala Lumpur City Center and Selangor State, Malaysia. *Trop. Biomed.* **22:** 207-216.

Chiaravalloti, F.N, Dibo, M.R, Barbosa, A.A.C and Battigaglia, M. (2002). Aedes albopictus (S) na região de São José do Rio Preto, SP: estudo da sua infestação em área já ocupada pelo Aedes aegypti e discussão de seu papel como possível vetor de dengue e febre amarela. *Rev. Soc. Bras. Med. Trop.* **35**: 351-357.

Chistophers, R.S. (1960)**.** *Aedes aegypti.* The yellow fever mosquito: its life history, bionomics, and structure, 1st ed. Cambridge University Press, Cambridge, United Kingdom.

Chistophers, R.S. (2010). *Aedes aegypti (L.)* the yellow fever mosquito. London: Cambridge University Press. 739p.

Chowdhury, M.A., Wagastuma, Y., Hossain, M.I., Ahmed, T.U., Uddin, M.A. and Kittayapong, P. (2000). Entomological assessment during the dengue outbreak in Dhaka city. Abstract: The First International Conference on dengue and dengue haemorrhagic fever, Chiang Mai, Thailand. 110pp.

Chretien, R., Harbach, R.E., Castro, M.G., Motta, M.A. and Peyton, E.L. (2015) *Wyeomyia* (*Prosopolepis*) *confusa* (Lutz): subgeneric validation, species description, and recognition of *Wyeomyia flui* (Bonne-Wepster and Bonne) as the senior synonym of *Wyeomyia kerri* del Ponte and Cerqueira. *J. American Mosq. Contrl. Asso*. **15**: 200–212.

Christophers, S.R. (1960). *Aedes aegypti* (L.) the Yellow Fever Mosquito: its Life History, Bionomics and Structure, Cambridge University Press, New York. 739pp.

Clements, A.N. (1992). The Biology of mosquitoes, Vol. 1. Development, Nutrition and Reproduction. Chapman and Hall, New York, NY. pp. 26-45.

Clements, A.N. (1992). The Biology of Mosquitoes. Chapman and Hall, London.

Clements, A.N. (2000). Studies on the life budget of *Aedes aegypti* in Wat Samphaya, Bangkok, Thailand. *Bull. WHO*. **46**: 211-226.

Clifton, M.E. and Noriega, F.G. (2012). The fate of follicles after a blood meal is dependent on previtellogenic nutrition and juvenile hormone in *Aedes aegypti*. *J. Insect Physio.* **58**(7): 1007-1019.

Cook, C.F., Besansky, N.J., Powell, J.R., Caccone, A., Hamm, D.M., Scott, J.A. and Collins, F.H. (2011). Molecular phylogeny of the *Aedes aegypti* complex suggests genetic introgression between principal malaria vectors. *Proc. National Acad. Scien. USA.* **91**: 6885–6888.

Cratz, S. G. (2014). Critical review of the vector status of *Aedes albopictus*. *Med. and Vet. Entomol.* **18:** 215-227.

Crovello, T.J. and Hacker, C.S. (1972). Evolutionary Strategies in Life Table Characteristics Among Feral and Urban Strains of *Aedes aegypti (*L.). *Evolution.* **26**(2): 185-196.

Devine, G.J., Perea, E.Z., Killeen, G.F., Stancil, J.D., Clark, S.J. and Morrison, A.C. (2009) Using adult mosquitoes to transfer Kerosene and Diesel to *Aedes aegypti* larval habitats. *Proce. National Acad. Scien.* **106:** 11530-11534.

Dhang, C.C., Benjamin, S., Saranum, M.M., Fook, C.Y., Lim, L.H. Ahmad N.W. and Sofian-Azirun, M. (2005). Dengue vector surveillance in urban residential and settlement areas in Selangor, Malaysia. *Trop. Biomed*. **22**: 39-43.

Dharshini, S., Vinobaba, M., Jude, P.J., Karunaratne, S.H. and Surendran, S.N. (2011) Prevalence and insecticide susceptibility of dengue vectors in the district of Batticaloa in eastern Sri Lanka. *Trop. Med. Hlth.* **39**: 47-52.

Diallo, M., Ba, Y. and Sall, A.A. (2003). Amplification of the sylvatic cycle of dengue virus type 2, Senegal, 1999-2000: entomologic findings and epidemiologic considerations. *Emerg. Infect. Dis*. **9**: 362-7.

Dieng, H., Boots, M., Tsuda, Y. and Takagi, M. (2003). A laboratory oviposition study in *Aedes albopictus* (Diptera: Culicidae) with reference to habitat size, leaf litter and their interactions. *Med. Entomol. Zool*. **54**: 43–50.

Dom, N.C., Hassan, A. and Rodziah, I. (2013). Habitat characterization of *Aedes* sp. Breeding in urban hotspot area. *Procedia Social and Behavioral Sciences*. **85**:100-109.

Eisen, L. and Moore, C.G. (2013). *Aedes* (Stegomyia) *aegypti* in the continental United States: a vector at the cool margin of its geographic range. *J. Med. Entomol*. **50**(3): 467-478.

Esteva, L. and Vargas, C. (2003). Coexistence of different serotypes of dengue virus. *J. Math. Biol*. **46**:31-43

Farajollahi, A. and Nelder, M.P. (2009). Aedes moquitoes expansion in New Jersey. *J. Med. Entomol*. **46**: 1220–1224.

Faran, M.E. (2015). Mosquito studies (Diptera, Culicidae) XXXIV. A revision of the Albimanus Section of the subgenus *Nyssorhynchus* of *Aedes*. *Contributions of the American Entomological Institute* (*Ann Arbor*). **15**(7):1–215.

Fien, D.S. (2015). Biology of *Aedes aegypti* (L., 1762) and *Aedes albopictus* (Skuse, 1895) (Diptera: Culicidae). I. Resistance of eggs to low humidity. *Acta Parasitol. Pol*. **23**:395-402.

Foo, L.C., Lim, T.W., Lee, H.L. and Fang, R. (1985). Rainfall, abundance of *Aedes aegypti* and dengue infection in Selangor, Malaysia. *Southeast Asian J. Trop. Med. Pub. Health*. **1**(16): 560-8.

Foster, W.A. (1995). Mosquito sugar feeding and reproductive energetics *Annual Rev. Entomol*. **40**: 443-474.

Foster, W.A. and Walker (2002). Mosquito sugar feeding and reproductive energetic. *Annual Rev. Entomol*. **40**: 443-474.

Gaidamovich, S.Y., Siddiqui, S.M., Haq, F., Klisenko, G.A., Melnikova, E.E. and Obukhova, V.R. (1980). Serological evidence of Dengue fever in the Bangladesh republic. *Acta. Vol*. **2**: 153.

Galliard, H. (1962). Recherches sur la biologie des culicidés à Hanoi (Tonkin, Nord- Vietnam). II. Reproduction et ponte d'*Aedes albopictus*, *A. aegypti* et *Armigeres obturans. Ann. Parasitol. Hum. Comp.* **37**: 348-365.

Getis A., Morrison A.C. and Gray K. (2003). Characteristics of the spatial pattern of the dengue vector, *Aedes aegypti*, in Iquitos, Peru. *American. J. Trop. Med. Hyg.* **69**(5): 494–503.

Ghosh, A., N. Chowdury, and G. Chandra. (2012). Plant extracts as potential mosquito larvicides. *Indian J. Med. Res.* **135**: 581–598.

Gontenille, D. and Rodhain, F. (2015). Biology and distribution of *Aedes albopictus* and *Aedes aegypti* in Madagascar. *J. American Mosq. Contrl. Assoc.* **5**:219-225.

Grangstrum, D.W., and Chan, E.B. (2011). Interaction of temperature and feeding rate in determing the rate of development of larval *Culex tarsalis* (Diptera, Culicidae). *Ann. Entomol. Soc. America.* **64**:668-71.

Gratz N.G. and Jany, W.C. (1994). What role for insecticides in vector control program *American. J. Trop. Med. Hyg.* **50**: 11-20.

Gubler, D.J. (2004). The changing epidemiology of yellow fever and dengue, 1900 to 2003: full circle. *Com. Immuno. Microbiol. Infect.Dis.* **27**(5): 319-330.

Gubler, D.J., P. Reiter, F. and Rose, W. (2001). Climate variability and change in the United States: potential impacts on vector-and rodent-borne diseases. *Environ. Hlth. Perspec.* **109**(12): 223-233.

Hahn, M. B., Monaghan, A. J., Hayden, M. H., Eisen, R. J., Delorey, M. J., Lindsey, N. P. and Fischer, M. (2015). Meteorological conditions associated with increased incidence of West Nile virus disease in the United States, 2004–2012. *The American J. Trop. Med. Hyg.* **92**(5): 1013-1022.

Hahn, M.B., Eisen, R.J., Eisen, L., Boegler, K.A., Moore, C.G., McAllister, J., Savage, H.M. and Mutebi, J. (2016). Reported Distribution of *Aedes (Stegomyia) aegypti* and *Aedes (Stegomyia) albopictus* in the United States, 1995-2016 (Diptera: Culicidae). *J. Med. Entomol. doi*: 10.1093/jme/tjw072.

Harbach, R.E. (2016). The classification of genus *Aedes* (Diptera: Culicidae): a working hypothesis of phylogenetic relationships. *Bull. Entomol. Res.* **94**: 537–553.

Harbach, R.E. and Howard, T.M. (2016) Index of currently recognized mosquito species (Diptera: Culicidae). *European Mosq. Bull*. **23**: 1–66.

Harizo, T.S., Sakamoto, S., Miyagi, I. (2007). The seasonal appearance of *Aedes aegypti* in Okinawajina, the Ryukyu archipelago, *Japan. Japan Mosq. News.* **42**:179

Harrington, L.C., Scott, K., Coleman, A., Costero, G.G., Clark, J.J. and Jones, S.K. (2005). Dispersal of the Dengue Vector *Aedes aegypti* Within and Between Rural Communities. *American J. Trop. Med. Hyg*. **72**(2): 209-220.

Harris, A.F., Rajatileka, S. and Ranson, H. (2010) Pyrethroid Resistance in Aedes aegypti from Grand Cayman. *American J. Trop.Med. Hyg.* **83**: 277-284.

Hawley, W.A, Reiter, P., Copeland, R.S., Pumpuni, C.B and Craig, G.Jr. (1987). *Aedes albopictus*in North America: probable introduction in used tyres from Northern Asia. *Science.* **36**:1114-1116.

Hawley, W.A. (1985). A high fecundity aedine: factors affecting egg production of thewestern treehole mosquito, *Aedes sierrensis* (Diptera: Culicidae). *J. Med. Entomol*. **22**:220–225.

Hibar, L.J. and Vlach, J. (2004). Mosquito larvae (Culicidae) and other Diptera associated with containers, storm drains, and sewage treatment plants in the Florida Keys, Monroe County, Florida. *Florida Entomol*. **87**(2): 199-203.

Hidayati, H., Sofian-Azirun, M. Nazni, W.A. and lee, H.L. (2005). Insecticide resistance development in Culex quinquefasciatus (Say), *Aedes aegypti* (L.) ande Aedes albopictus (Skuse) larvae againts malathion, permethrin and temephos. *Trop. Biomed.* **22:** 45-52.

Hien, D.S. (1976). Biology of *Aedes aegypti* and *Aedes albopictus* (Skuse, 1895) (Diptera: Culicidae). V. The gonotrophic cycle and oviposition. *Acta Parasitol. Poland.* **24**:37-55.

Hills, S. L., Russell, K., Hennessey, M., Williams, C., Oster, A.M., Fischer, M. and Mead, P. (2016). Transmission of dengue Virus Through Sexual Contact with Travelers to Areas of Ongoing Transmission—Continental United States, 2016. MMWR. Morbidity and Mortality Weekly Report, 65.

Hossain, M.I, Wagatsuma Y and Chowdhury M.A, (2000). Analysis of some Socio-demographic Factors Related to DF/DHF Outbreak in Dhaka City. *Deng. Bull.* **24**: 34–41.

Hotez, P.J. (2011). America's most distressed areas and their neglected infections: the United States Gulf Coast and the District of Columbia. *PLoS Negl. Trop. Dis*. **5**(3): e843.

Islam, M.N., Khan, A.Q. and Khan, A.N. (1982). Viral diseases in Bangladesh, Proceedings of an International Seminar on viral diseases in South-East-Asia and Western Pacific, Feb 8-12, Canbera, Australia. Washing DC, USA: Academic Press, **10**: 205-209.

Jansen, C.C. and Beebe, N.W. (2010). The dengue vector *Aedes aegypti*: what comes next. *Microb. Infect.* **12**(4): 272-279.

Jien, D.S. (2010). Biology of *Aedes aegypti* (L., 1762) and *Aedes albopictus* (Skuse, 1895) (Diptera: Culicidae). V. The gonotrophic cycle and oviposition. *Acta. Parasitol. Poland.* **24**:37-55.

John, D.D. (2009) Review of the systematics and phylogenetic relationships of the Sabethini (Diptera: Culicidae). *Syst. Entomol.* **21**: 129–150.

Joy, T.K., and Arik, A.J. (2010). The impact of larval and adult dietary restriction on lifespan, reproduction and growth in the mosquito *Aedes aegypti*. *Expt. Gerontol*. **45**(9): 685-690.

Judd, D.D. (2008) Review of a bromeliad-ovipositing lineage in *Wyeomyia* and the resurrection of *Hystatomyia* (Diptera:Culicidae). *Ann. Entomol. Soci. America*. **91**: 572–589.

Kalra, N.L., Kaul, S.M. And Rastogi, R.M. (1997). Prevalence of *Aedes aegypti* and *Aedes albopictus,* Vectors of Dengue and Dengue haemorrhagic fever in North, North-East and Central India. *Dengue Bull*. **21**: 84-92.

Kausar, R.M., Faran, M.E. and Linthicum, K.J. (2012). A handbook of the Amazonian species *of Aedes* (*Nyssorhynchus*) (Diptera: Culicidae). *Mosq. Syst.* **13**: 1–81.

Kay, B.H., Nam V.S and Tien T.V. (2002). Control of *Aedes* vectors of dengue in three provinces of Vietnam by use of Mesocyclops (Copepoda) and community-based methods validated by entomologic, clinical, and serological surveillance. *American J. Trop. Med. Hyg.* **66**: 40–48.

Kennedy, D.O., and Wightman, E.L. (2011). Herbal extracts and phytochemicals: plant secondary metabolites and the enhancement of mosquitoe nervous system. *Adv. Nutr*. **1**: 32–50

Khan, A.M. and Ahmed, T.U. (1986). Dengue status in Bangladesh. *Dengue News* **12**: 11.

Khan, A.R. (1980). Studies on the bleeding habitats and seasonal prevalence of larval population of *Aedes aegyti* (L) and *Aedes albopictus* (skuse) in Dhaka city. *Bangladesh Med. Res. Counc. Bull*. **2**:5-52.

Khan, A.R. (1980). Studies on the breeding habitats and seasonal prevalence of larval population of *Aedes aegypti* and *Aedes albopictus* in Dacca City. *Bangladesh. Med. Res. Coun. Bull.* **6**(2): 48-53.

Khan, M. and Ahmed, T.U. (1986). Dengue status in Bangladesh. *Dengue News Letter. WHO*. **11**:12.

Khan, N.I. (1980). Studies on the ecology and seasonal fluctuation of various species of mosquito larvae in Ramna Lake. MSc Thesis, Department of Zoology, University of Dacca. 98.

Khater, H. F., and Shalaby, A.A.S. (2008). Potential of biologically active plant oils to control mosquito larvae (*Culex pipiens*, Diptera: Culicidae) from an Egyptian locality. *Rev. Inst. Med. Trop. São Paulo*. **50**: 107–112.

Khormi, H.M. and Kumar, L. (2014). Climate change and the potential global distribution of *Aedes aegypti*: spatial modelling using GIS and CLIMEX. *Geosp. Hlth*. **8**(2): 405-415.

Kindhauser, M.K. (2003). Dengue: Global Defence Against The Infectious Diseases Threat. *World Health Organization.* Geneva. pp. 140-143.

Kittayapong, P. and Strickman, D. (1993). Distribution of containerinhabiting *Aedes* larvae (Diptera: Culicidae) at a dengue focus in Thailand. *J. Med. Entomol*. **30**: 601–606.

Knudsen, A.B. (1997). An assignment report on Dengue. WHO. 35 pp.

Koenraadt, C.J., Jones J.W. and Sithiprasasna, R. (2007). Standardizing container classification for immature *Aedes* aegypti surveillance in Kamphaeng Phet, Thailand. *J. Med. Entomol.* **44**: 938–944.

Koenraadt, C.J.M., Harrington, L.C. (2008). Flushing effects of rain on container-inhabiting mosquitoes *Aedes aegypti* and Culex pipiens (Diptera: Culicidae). *J. Med. Entomol*. **45**:28

Kropelin, J. (2006). The *Stegomyia* mosquitoes of South Africa and some neighbouring territories. Including chapters on the mosquito-borne virus diseases of the Ethiopian zoogeographical region of Africa. *Mem. Entomol. Soci. Southern Africa*. **4**:1–138.

Lee, H.L. (1991). Comparative *Aedes* ovitrap and larval survey in several suburban communities in Selangor. Malaysia. *Mosq. Borne Dis. Bull.* **9**: 9-15.

Lee, H.L., Cheong, W.H. (1987) A preliminary *Aedes aegypti* larval survey in the suburbs of Kuala Lumpur city. *Trop. Biomed*. **4**: 111-118.

Lee, N., Wong C.K, Lam, W.Y, Wong, A. and Lim, W. (2006). Chikungunya fever, Hong Kong. *Emerg. Infect. Dis*. **12**: 1790

Leisnham, P.T., LaDeau, S.L. and Juliano, S.A. (2014). Spatial and temporal habitat segregation of mosquitoes in urban Florida. *PloS one*. **9**(3): e91655.

Lim, K.W, Sit N.W, Norzahira, R., Sing, K W., Wong, H.M, Chew, H.S., Firdaus, R., Cheryl, J.A., Suria, M., Mahathavan, M., Nazni, W.A, Lee H.L, McKemy, A. and Vasan, S.S. (2010). Dengue vector surveillance in insular settlements of Pulau Ketam, Selangor Malaysia. *Trop. Biomed*. **27**: 185-192.

Lima, A., Lovin, D.D., Hickner, P.V. and Severson, D.W. (2016). Evidence for an Overwintering Population of *Aedes aegypti* in Capitol Hill Neighborhood, Washington, DC. *The American J. Trop. Med. Hyg.* **94**(1): 231-235.

Lin, R.J., Chen, C.Y., and Walis. L.L. (2008). Cytotoxic activity of *Ipomoea cairica*. *Nat. Prod. Res*. **22**: 747–53.

Lindsay, S.W., Birley, M.H. (2016). Review: climate change and malaria transmission. *Ann. Trop. Med. Parasitol.* **90**:573

Linnaeus, Carolus. (1762). Reise nach Palästina in den Jahen van 1749 bis 1752. *L. Salvii Stockholm*. 1-619.

Lloyd, L. S. (2003). Best Practices for Dengue Prevention and Control in the Americas. Environmental Health Project; Strategic report 7.

Lloyd, L.S, Winch, P and Ortega-Canto, J. (1992). Results of a community-based *Aedes* aegypti control program in Merida, Yucatan, Mexico. *American J. Trop. Med. Hyg.* **46**: 635–642.

Loh, B. and Song, R.J. (2001).Modeling dengue cluster size as a function of *Aedes aegypti* population and climate in Singapore. *Dengue Bull.* **25**: 74-8.

Lucia, A., Harburguer, L., Licastro, S., Zerba, E. and Masuh, H. (2009) Efficacy of a new combined larvicidal-adulticidal ultralow volume formulation against Aedes aegypti (Diptera: Culicidae), vector of dengue. *Parasitol. Res.* **104:** 1101-1107.

Lucio, P.S., Degallier, N., Servain, J., Hannart, A., Durand, B., de Souza, R.N. and Ribeiro, Z.M. (2013). A case study of the influence of local weather on *Aedes aegypti* (L.) aging and mortality. *J. Vec. Ecol.* **38**(1): 20-37.

Maciel-de-Freitas, R. Marques W.A and Peres R.C. (2007). Variation in *Aedes* aegypti (Diptera: Culicidae) container productivity in a slum and a suburban district of Rio de Janeiro during dry and wet seasons. *Mem. Inst. Oswaldo Cruz.* **102**: 489–496.

Madi, M., Ahmad, E., Kulaimi, N.A.M., Ali, W.M., Ismail, S. and Lim, L.H. (2012). Climatic influences on Aedes mosquito larvae population. *Malaysia J. Sci.* **31**:30

Mahmood, B. and Mahmood, S. (2011). Emergence of Dengue in Bangladesh a major international public health concern in recent years. *J. Environ. Res. Manage*. **2**: 035–041.

Mahmood, F., CransM, J. and Savur, N.S. (1997). Larval competition in *Aedes triseriatus* (Diptera: Culicidae): effects of density on size, growth, sex ratio, and survival. *J. Vect. Ecol*. **22**: 90–94.

Mattingly, P.F. (2015) Medical entomology studies – XIV. The subgenera *Rachionotomyia*, *Tricholeptomyia* and *Tripteroides* (Mabinii Group) of genus *Aedes* in the Oriental Region (Diptera: Culicidae). Contributions of the American Entomological Institute (*Ann Arbor*). **17**(5): ii + 1–147.

McCarroll, L. and Hemingway, J. (2002). Can insecticide resistance status affect parasite transmission in mosquitoes? *Insect Biochem. Mole. Biol.* **32**(10): 1345-1351.

Merritt, R.W., Dadd, R. H. and Fier, D.F. (1992). Feeding Behavior, Natural Food, and Nutritional Relationships of Larval Mosquitoes. *Med. Vet. Entomol.* **37:** 349-374.

Methen, J.D., Nazni, W.A., Lee, H.L. and Sofian-Azirun, M. (2013) Susceptibility of *Aedes aegypti* and *Aedes albopictus* to temephos in four study sites in Kuala Lumpur City Center and Selangor State, Malaysia. *Trop. Biomed.* **22:** 207-216.

Micieli, M.V. and Campos, R.E. (2003). Oviposition activity and seasonal pattern of a population of *Aedes* (Stegomyia) *aegypti* (L.) (Diptera: Culicidae) in Subtropical Argentina. *Mem. Inst. Oswaldo*. *Cruz.* **98**: 659-663.

Mireji, P.O. (2010). Biological cost of tolerance to heavy metals in the mosquito *Anopheles gambiae. Med. Vet. Entomol.* **24**(1).

Moore, C.G., Cline, B.L., Ruiz-Tiben, E., Lee, D., Romney-Joseph, H. and Rivera-Correa, E. (1978). *Aedes aegypti* in Puerto Rico: environmental determinants of larval abundance and relation to dengue virus transmission. *American. J. Trop. Med. Hyg.* **27:**1225-1231.

Moore, S.M., Eisen, R.J., Monaghan, A. and Mead, P. (2014). Meteorological influences on the seasonality of Lyme disease in the United States. *American J. Trop. Med. Hyg.* **90**(3): 486-496.

Mori, A. and Oda, T. (1981). Studies on the egg diapause and overwintering of *Aedes albopictus* in Nagasaki. *Trop. Med.* **23**(2): 79-90.

Morrison, A.C, Gray K. and Getis, A. (2004). Temporal and geographic patterns of *Aedes* aegypti (Diptera: Culicidae) production in Iquitos, Peru. *J. Med. Entomol*. **41**: 1123–1142.

Musso, I.J. (2014). Phylogeny and classification of the Culicidae (Diptera). *Syst. Entomol*. **23**: 327–370.

Muturi, E. J., Kim, T.N.and Roek, G.H. (2011). Larval environmental stress alters *Aedes aegypti* competence for Sindbis virus. *Trop. Med. Inter. Hlth.* **16**(8): 955-964.

Nasci, R. S.(1986) .The size of emerging and host-seeking *Aedes aegypti* and the relation of size to blood-feeding success in the field. *J. American. Mosq. Contrl. Assoc*. **2**: 61–62.

Nasci, R.S., Hare, S.G. and Willis, F.S. (2009). Interspecific mating between Lousiana strains of *Aedes albopictus* and *Aedes aegypti* in the field and laboratory. *J. American Mosq. Cont. Assoc.* **5**:416-421.

Nasiruddin, M. (1952). A note on the natural infectivity of mosquitoes in East Pakistan. *Pakistan. J. Hlth.* **2**: 113-115.

Nasiruddin, M. (1957). Mosquitoes breeding in tree-holes and bamboo stumps in Dacca (East Pakistan). *Pakistan. J. Hlth.* **2**: 110-112.

Navarro, J.C. and Liria, J. (2000). Phylogenetic relationships among eighteen Neotropical Culicini species. *J. American Mosq. Contrl. Assoc.* **16**:75–85.

Ndiaye, P.I, Bicout, D.J, Mondet B, Sabatier P. (2006). Rainfall triggered dynamics of *Aedes* mosquito aggressiveness. *J. Theor. Bio.* **243**: 222-9.

Nene, G.H. Berlin, O.G.W. and Belkin, J.N. (2005) Mosquito studies (Diptera, Culicidae) XXXVI. Subgenera *Aedinus*, *Tinolestes* and *Anoedioporpa* of *Culex*. *Contributions of the American Entomological Institute (Ann Arbor).* **17**(2): ii + 1–104.

Nguyen, A.T., Williams, A.J, Kitron, U.D and Chaves, L.F. (2012).Seasonal weather, nutrients, and conspecific presence impacts on the southern house mosquito oviposition dynamics in combined sewage overflows. *J. Med. Entomol.* **49**(6): 1328-1338.

Norzahira, R., Hidayatulfathi. O., Wong, H.M, Cheryl, A., Firdaus, R., Chew, H.S., Lim, K. W., Sing, K. W., Mahathavan, M., Nazni, W. A., Lee H.L., Vasan, S.S., McKemey, A. and Lacroix, R. (2011): Ovitrap surveillance of the dengue vectors, *Aedes* (Stegomyia) *aegypti* (L.) and *Aedes* (Stegomyia) *albopictus* (Skuse) in selected areas in Bentong, Pahang, Malaysia. *Trop. Biomed.* **28**: 48-54.

O'Meara, G. F., Evans, L. F. Jr. and Gettman, A. D. (1995). Spread of *Aedes* albopictus and decline of Ae. aegypti (Diptera: Culicidae) in Florida. *J. Med. Entomol*. **32**: 554–562.

Okine, L.K.N., Nyarko, A.K., Armah, G.E., Awumbila, B., Owusu, K., Setsoafia, S. and Ofosuhene, M. (2004). Adverse effects of mosquito coil smoke on lung, liver and metabilishing enzymes in male wistar albino rats. *Ghana Med. J.* **38**(3): 89-95.

Pape, T. (2004) Cladistic analyses of mosquito chromosome data in *Aedes* subgenus *Cellia* (Diptera: Culicidae). *Mosq. Syst*. **24**: 1–11.

Perez, M.H. and F.G. Noriega (2012). *Aedes aegypti* pharate first instar quiescence affects larval fitness and metal tolerance. *J. Insect Physiol.* **58**(6): 824-829.

Phuanukoonnon, S. Mueller, I. and Bryan J.H. (2005). Effectiveness of dengue control practices in household water containers in Northeast Thailand. *Trop. Med. Int. Hlth.* **10**: 755–763.

Pinheiro, V.C.S. and Tadei, W.P. (2002). Frequeney, diversity, and productivity study on the *Aedes aegypti* most preferred containers in the city of Manaus, Amazonas, Brazil. *Rev. Inst. Med. Trop. Sao Paulo.* **44**: 245-50.

Ponçon, N., Toty, C., Ambert, G., Goff, G.L., Brengues, C., Schaffner, F. and Fontenille, D. (2007). Biology and dynamics of potential dengue vectors in Southern France. *Malar. J.* ***6****: 18*

Ponlawat, A., Scott, J.G. and Harrington, L.C. (2005) Insecticide susceptibility of Aedes aegypti and Aedes albopictus across Thailand. *J. Med. Entomol.* **42:** 821-825.

Pratt, N.L. and Moore, W.A. (2014). Classification of the subgenus *Toxorhynchites* (Diptera: Culicidae) II. Revision of the *Toxorhynchites acaudatus* group. *J. Med. Entomol*. **23**: 538–574.

Rao, T.A., Trpis, M., Gillett, J.D. and Teesdale, C. and Tonn, R.J. (1973) Breeding places and seasonal incidence of *Aedes aegypti*, as assessed by the single-larva survey method. *Bull. WHO.* **48**: 615-22.

Rawley, W.A, Reiter, P., Copeland, R.S., Pumpuni, C.B. and Craig, G. Jr. (2008). *Aedes albopictus* in North America: probable introduction in used tyres from Northern Asia. *Science*. **36**:1114-1116.

Regis, L., Monteiro, A.M. and Melo-Santos, M.A.V. (2008). Developing new approaches for detecting and preventing *Aedes aegypti* population outbreaks: basis for surveillance, alert and control system. *Mem. Inst. Oswaldo Cruz.* 103:50

Reinert, J.F. (2000). New classification for the composite genus *Aedes* (Diptera: Culicidae: Aedinii), elevation of subgenus *Ochlerotatus* to generic rank, reclassification of the other subgenera and notes on certain subspecies. *J. America. Mosq. Contrl. Assoc.* **16:** 175-188.

Reinert, J.F. (2001). Revised list of abbreviations for genera and subgenera of Culicidae (Diptera) and notes on generic and subgeneric changes. *J. American. Mosq. Contrl. Assoc.* **17:** 51-55.

Reiskind, M.H. and Lounibos, L.P. (2013). Spatial and temporal patterns of abundance of *Aedes aegypti* L. (*Stegomyia aegypti*) and *Aedes albopictus* (Skuse) in southern Florida. *Med. Vet. Entomol.* **27**(4): 421-429.

Rey, G.Y., Rao, P.N. and Rai, K.S. (2006). Comparative karyotypes and chromosomal evolution in some genera of nematocerous (Diptera: Nematocera) families. *Ann. Entomol. Soc. America*. **80**: 321–332.

Rey, J.R., Nishimura, N., Wagner, B., Braks, M.A., O'Connell, S.M. and Lounibos, L.P. (2012). Habitat segregation of mosquito arbovirus vectors in south Florida. *J. Med. Entomol.* **43**(6): 1134-1141.

Rigau-Perez, J.G., Clark, G.C., Gubler, D.J., Reiter, P., Sanders, E.J., Vorndam, A.V. (1998). Dengue and dengue haemorrhagic fever. *Lancet*. **352**: 971-977.

Rodriguez, M.M., Bisset, J.A., Mohna, D., Lauzan, L. and Soea, A. (2001). Detection of resistance mechanisms in *Aedes aegypti* from Cuba and Venezuala. *J. Med. Entomol.* **38**: 623-628.

Rozilawati. H, Zairi, J. and Adanan, C.R. (2007). Seasonal abundance of *Aedes albopictus* in selected urban and suburban areas in Penang, Malaysia. *Trop. Biomed*. **24**: 83-94.

Rudnick, A. and Lim, T.W. (1986). Dengue fever studies in Malaysia. T.W., Eds., Bull. no. 23, Ins. of Med. Research, Malaysia. 162 pp.

Russell, R.C., Webb, C.E. and Diwer, F.R. (2005). Mark and release recapture study to measure dispersal of the mosquito *Aedes aegypti* in Cairns, Queensland, Australia. *Med. Vet. Entomol.* **19**(4): 451-457.

Samways, M.J. 9(995). Insect Conservation Biology, Chapman and Hall, London, 358 pp.

Saruah, S. and Dutta, P. (2012). Seasonal pattern of abundance of Aedes albopictus in urban and industrial areas of Dibrugarh District Assam. *Asian J. Expt. Biol. Sci.* **3**:559

Schaeffer, B. Mondet, B. and Touzeau, S. (2008). Using a climate-dependent model to predict mosquito abundance: application to *Aedes (Stegomyia) africanus* and *Aedes (Diceromyia) furcifer* (Diptera: Culicidae). *Infect Genet. Evolut*. **8**: 422-32.

Schultz, G.W. (1993). Seasonal abundance of dengue vectors in Manila, Republic of the Philippines. *Southeast Asian J. Trop. Med. Pub. Hlth.* **24**: 369-75.

Scott, T.W, Chow, E. and Strickman, D. (1993). Blood-feedingpatterns of *Aedes* aegypti (Diptera: Culicidae) collected in a rural Thai village. *J. Med. Entomol.* **30**: 922–927.

Scott, T.W., Amerasinghe, P.H., Morrison, A.C., Lorenz, L.H., Clark, G.G., Strickma, D., Kittaypong, P. and Edman, J.D. (2000) . Longitudinal studies of *Aedes aegypti* (Diptera: Culicidae) in Thailand and Puerto Rico: blood feeding frequency. *J. Med. Entomol*. **37**: 89–101.

Scott, T.W., Morrison, A.C., Lorenz, L.H., Clark, G.G., Strickman, D., Kittayapong, P., Zhou, H. and Edman, J.D. (2000). Longitudinal studies of *Aedes aegypti* (Dipterea: Culicidae) in Thailand and Puerto Rico: population dynamics. *J. Med. Entomol.* **37**: 77-88.

Shaalan, E.A., Canyon, M. Wagdy, G. and Younes, F. (2005). Review article A review of botanical phytochemicals with mosquitocidal potential. *Environ. Intl.* **31**: 1149–1166.

Shan, H.H. and Zairi, J. (2016). Permethrin resistance in Aeds albopictus (Diptera: Culicidae) and associated Fitness Costs. *J. Med. Entomol.* **50:** 362-370.

Shepard, A.E., Borkent, A. and Grimaldi, D.A. (2012). The earliest fossil mosquito (Diptera: Culicidae), in mid-Cretaceous amber. *Ann. Entomol. Soci. America*. **97**: 882–888.

Singh, S. (1996). Dengue situation in Malaysia including summary of the global situation past and current trends the reasons for current upsurge. Persidengan Denggi. 9-11 May 1996. Institut Penyelidikan Perubatan, Kuala Lumpur. P.19.

Smith, D. L., Battle, E.R. and Grand, D.C (2009). Ross, Macdonald, and a Theory for the Dynamics and Control of Mosquito-Transmitted Pathogens. *PLoS Pathog.* **8**(4)

Soekiman, S., Machfudz, Subagyo., Adipoetro, S., Yamanishi, H. and Matsumura, T. (1984). Comparative studies on the biology of *Aedes aegypti* (Linnaeus, 1762) and *Aedes albopictus* (Skuse, 1895) in a room condition. In: S. Iwai, ed. *ICMR Annals.* **4**: 143-152.

Soreta, T. and Mogi, M. (2007). Interspecific variation in desiccation survival time of *Aedes* (Stegomyia) mosquito eggs is correlated with habitat and egg size. *Oecologia*. **90**: 354-358.

Stickman, D. (2002). Kittayapong P. Dengue and its vectors in Thailand: Introduction to the study and seasonal distribution of *Aedes* larvae. *American J. Trop. Med. Hyg*. **67**: 247-59.

Stickman, D. Kittayapong, P. (2003). Dengue and its vectors in Thailand: calculated transmission risk from total pupal counts of *Aedes* aegypti and association of wing-length measurements with aspects of the larval habitat. *American J. Trop. Med. Hyg*. **68**: 209–217.

Sucharit, S. and Tumrasvin, W. (1981). Ovipositional attractancy of waters containing larvae of *Aedes aegypti* and *Aedes albopictus*. *Japan J. Sanit Zool.* **32**:261-264.

Taey, W.A. (2013) A high fecundity aedine: factors affecting egg production of the western treehole mosquito, *Aedes sierrensis* (Diptera: Culicidae). *J. Med. Entomol.* **22**:220–225.

Takeda, T., Whitehouse, C.A., Brewer, M., Gettman, A.D and Mather, T.N. (2003). Arbovirus surveillance in Rhode Island: assessing potential ecologic and climatic correlates. *J. American Mosq. Contrl.* **19**:179-89.

Telang, A., Qayum, A. and Qiuta, W.A. (2012). Larval nutritional stress affects vector immune traits in adult yellow fever mosquito *Aedes aegypti* (Stegomyia aegypti). *Med. Vet. Entomol.* **26**(3): 271-281.

Thamise, C.D., Benjamin, S., Saranum, M.M., Chiang, Y.F., Lee, H.L., Nazni, W.A. and Sofian-Azirun, M. (2014). Dengue vector surveillance in urban residential and settlement areas in Selangor, Malaysia. *Trop. Biomed.* **22:** 39-43.

Tilak, R., Gupta, V., Suryam, V. and Yadav, J. (2005). A laboratory investigation into oviposition responses of *Aedes aegypti* to some common household substances and water from conspecific larvae. *Med. J. Armed Forces India.* **61**(3):227-229.

Toekiman, S., Machfudz, Subagyo. G., Adipoetro, S., Yamanishi, H. and Matsumura T. (2008). Comparative studies on the biology of *Aedes aegypti* (Linnaeus, 1762) and *Aedes albopictus* (Skuse, 1895) in a room condition. In: *S. Iwai, ed. ICMR Annals.* **4**:143-152.

Toma, L., Severini. F., Di Luca, M., Bella A, and Romi, R. (2003). Seasonal patterns of oviposition and egg hatching rate of Aedes albopictus in Rome. *J. American Mosq. Contrl. Assoc.* **19**:19–22.

Toma, T.S., Sakamoto, S. and Miyagi, I. (1982). The seasonal appearance of Aedes albopictus in Okinawajima, the Ryukyu archipelago, Japan. *Mosq. News.* **42**: 179-183.

Tristophers, S.R. (2016). *Aedes aegypti (L.)* the yellow fever mosquito: its life history,bionomics and structure. Cambridge: Cambridge University Press. 739p.

Trpis, M. and, Hausermann, W. (1975). Demonstration of differential domesticity of *Aedes aegypti* (L.) (Diptera, Culicidae) in Africa by mark-release-recapture, *Bull. Entomol Res.* **65:** 199-208.

Tun-Lin W, Kay B.H. and Barnes, A. (1995a). The Premise Condition Index: a tool for streamlining surveys of *Aedes* aegypti. *American J. Trop. Med. Hyg*. **53**: 591–594.

Tun-Lin, W., Kay, B.H and Barnes, A. (1995b). Understanding productivity, a key to *Aedes* aegypti surveillance. *American J. Trop. Med. Hyg*. **53**: 595–601.

Urancy, D.B., Moore, C.G. and Eliason, D.A. (2012). Past, present and future of *Aedes albopictus* in the United States. *J. American Mosq. Contrl. Assoc.* **6**:127-132.

Vaughan, A., Chadee, D.D. and French-Constant, R.H. (1998). Biochemical monitoring of organophosphorus and carbamate insecticide resistance in *Aedes aegypti* mosquitoes from Trinidad. *Med. Vet. Entomol.* **12**: 318-321.

Vecieli, F.V. and Gampos, R.E. (2013). Oviposition activity and seasonal pattern of population of *Aedes* (stegomya) *aegypti* (L) (Diptera: Culicidae) in subtropical Argentina. *Mem. Inst. Oswaldo Cruz.* **98**:659.

Vezzani, D., Velazquez, S.M. and Schweigmann, N. (2004). Seasonal pattern of abundance of Aedes aegypti (Dipterea: Culicidae) in Buenos Aires, City, Argentina. *Mem. Inst. Oswaldo*. Cruz. **99**(4): 351-356.

Vezzani, D., Velazquez, S.M. and Schweigmann, N. (2014). Seasonal pattern of abundance of *Aedes aegypti* (Diptera: Culicidae) in Buenos Aires City, Argentina. *Mem. Inst. Oswaldo Cruz.* **99**: 351.

Vitek, C.J, and Livdahl, T.P. (2006). Field and laboratory comparison of laying eggs of Aedes mosquitoes. *J. American Mosq. Contrl. Assoc.* **22**: 609–614.

Weissman, A. and Kindler, S.H. (1962). Effect of Low Temperatures on Development, Hatching and Survival of the Eggs of *Aedes aegypti* (L). *Nature.* **196**(4860): 1231-1232.

Wickerson, A.K., Shankles, P.G., Madhavan, N.M., Hu, D.L. (2015). Mosquitoes survive raindrop collisions by virtue of their low mass. *Proc. Nat. Acad. Sci.* **109**:9822.

Yalliard, H. (2014). Recherches sur la biologie des culicidés à Hanoi (Tonkin, Nord-Vietnam). II. Reproduction et ponte d'*Aedes albopictus*, *A. aegypti* et *Armigeres obturans. Ann. Parasitol. Hum. Comp.* **37**:348-365.

Yang, H.M., Macoris, M.L.G., Galvani, K.C. and Andrighetti, M.T.M. (2011). Follow up estimation of *Aedes aegypti* entomological parameters and mathematical modellings. *BioSyst.* **103**:36

Yang, H.M., Macoris, M.L.G., Galvani, K.C., Andrighetti, M.T.M. and Wanderley, D.M.V. (2009a). Assessing the effects of temperature on the population of *Aedes aegypti*, vector of dengue. *Epidemol. Infect.* **137**:1188

Yang, H.M., Macoris, M.L.G., Galvani, K.C., Andrighetti, M.T.M. and Wanderley, D.M.V. (2009b). Assessing the effects of temperature on dengue transmission. *Epidemol. Infect.* **137**:1179

Yap, H.H., J. Zairi, Y.W. Lee and C.R. Adanan. (2003). Mosquito Control. In: C.Y. Lee, J. Zairi, H.H. Yap and N.L. Chong (eds). *Urban Pest Control: A Malaysian Perspective. 2nd* (end.) Vector Control Research Unit, USM.

Yunus, E.B., Bangali, A.M., Mahmood, M.A.H., Rahman, M.M., Chowdhury, A.R. and Talukder, K.R. (2001). Dengue outbreak 2000 in Bangladesh: From speculation to reality and exercises. *Deng. Bull*. **25**:15-20.

Yuore, C.G., Cline, B.L., Ruiz-Tiben, E., Lee, D., Romney-Joseph, H. and Rivera-Correa, E. (2006). *Aedes aegypti* in Puerto Rico: environmental determinants of larva abundance and relation to dengue virus transmission. *American J. Trop. Med. Hyg.* **27**:1225

APPENDICES

APPENDICES

Appendix I Distribution of average temperature (^{0}c), relative humidity (%) and rainfall (mm) during July, 2014 to June, 2015

Months	Temperature(^{0}c)	Rainfall (mm)	Humidity (%)
January	18.7	2	66
February	21.7	43	62
March	26.4	56	61
April	29.3	178	68
May	29.9	311	70
June	30	418	80
July	29.9	393	78
Auguest	29	359	80
September	29.1	251	80
October	27.7	50	80
November	24.4	0	70
December	19.7	1	74

Appendix II. Number of wet and positive container inspected with percent in three locations

Types of containers	Number of container inspected				Number of positive containers			Total	% of positive containers			Positive % of each container		
	Indoor	Outdoor	Rooftop	Total	Indoor	Outdoor	Rooftop		Indoor	Outdoor	Rooftop	Indoor	Outdoor	Rooftop
Bucket	3753	2342	485	6580	34	79	10	123	1.50	3.48	0.44	27.64	64.23	8.13
Flower pot	2734	2546	786	6066	74	103	65	242	3.26	4.53	2.86	30.58	42.56	26.86
Can and bottle	765	3133	1136	5034	54	206	3	263	2.38	9.07	0.13	20.53	78.33	1.14
Earthen jar	876	3641	501	5018	50	378	24	452	2.20	16.64	1.06	11.06	83.63	5.31
Drum	1532	1211	202	2945	88	123	13	224	3.87	5.41	0.57	39.29	54.91	5.80
Tank	654	1655	366	2675	210	61	11	282	9.24	2.68	0.48	74.47	21.63	3.90
Coconut shell	2	2623	135	2760	1	90	7	98	0.04	3.96	0.31	1.02	91.84	7.14
Plastic bowl	1305	1511	182	2998	20	89	6	115	0.88	3.92	0.26	17.39	77.39	5.22
Discarded appliances	127	757	270	1154	1	14	1	16	0.04	0.62	0.04	6.25	87.5	6.25
Tyre	159	851	277	1287	16	529	14	559	0.70	23.28	0.62	2.86	94.63	2.50
Others	592	1532	136	2260	16	79	2	97	0.70	3.48	0.09	16.49	81.44	2.06
Total	12499	21902	4376	38777	34	79	10	2471	0.91	3.37	2.06	8.85	4.61	5.75

Appendix III. Percentage of each positive container for relative risk of wet containers

Types of container	Indoor	Outdoor	Rooftop
Bucket	27.64	64.23	5.75
Flower pot	30.58	42.56	8.62
Can and bottle	20.53	78.33	1.72
Earthen jar	11.06	83.63	13.79
Drum	39.29	54.91	7.47
Tank	74.47	21.63	6.32
Coconut shell	1.02	91.84	4.02
Plastic bowl	17.39	77.39	3.45
Discarded appliances	6.25	87.50	0.57
Tyre	2.86	94.63	8.05
Others	16.49	81.44	1.15

Appendix IV. Two-dimensional presentation for relative risk of wet containers in indoors,indoor, outdoor and rooftop placement

Types of container	Indoor	Outdoor	Rooftop
Bucket	1.50	3.48	0.44
Flower pot	3.26	4.53	2.86
Can and bottle	2.38	9.07	0.13
Earthen jar	2.20	16.64	1.06
Drum	3.87	5.41	0.57
Tank	9.24	2.68	0.48
Coconut shell	0.04	3.96	0.31
Plastic bowl	0.88	3.92	0.26
Discarded appliances	0.04	0.62	0.04
Tyre	0.70	23.28	0.62
Polythene sheet	2.7	3.48	1.5

Appendix V. Mean number of aedes mosquitoes in relation to rainfall (mm) and temperature (°C) against the monthly sample collection

Months	Temp ^{0}c	Rainfall (mm)	Eggs	Larve	Pupae	Adults
Jan	29.9	2	23.28 ± 14.19	12.36 ± 10.33	5.92 ± 7.11	3 ± 4.79
Feb	32.2	43	71.88 ± 32.84	48.28 ± 27.43	34.36 ± 24.86	22.84 ± 20.88
Mar	36.4	56	91.68 ± 39.24	62.56 ± 30.32	44.28 ± 27.42	32.52 ± 22.51
Apr	35.5	178	118.68 ± 37.08	80.68 ± 26.96	60.64 ± 25.21	46.6 ± 22.76
May	36.4	311	457.12 ± 112.92	343.44 ± 118.32	249.88 ± 110.50	195.04 ± 82.39
Jun	36.5	418	556 ± 103.94	451.76 ± 103.42	356.72 ± 102.06	291.44 ± 91.85
Jul	35.8	393	447.36 ± 113.67	345.2 ±106.41	252.08 ± 102.68	187.48 ± 96.65
Aug	34.4	359	248.72 ± 97.70	180.92 ± 89.45	125.08 ± 58.20	95.84 ± 41.62
Sep	34.8	251	95.16 ± 42.28	67.68 ± 32.92	50.8 ± 28.98	35.84 ± 24.35
Oct	36.0	50	46.8 ± 22.09	28.88 ± 17.88	17.96 ± 13.79	10.12 ± 8.76
Nov	33.8	0	28.28 ± 15.78	14.52 ± 9.63	7.04 ± 5.91	7.08 ± 6.59
Dec	29.2	1	25.64 ± 18.74	13.68 ± 12.99	6.96 ± 7.89	5.32 ± 7.03

Appendix VI. Mean number with SD as bar of different stages of life cycle of aedes mosquitoes recorded in eight divisions of Dhaka city July, 2014 to June, 2015

July-2014

Divisions	No. of Eggs Mean ± Sd	No. of Larvae Mean ± Sd	No. of Pupae Mean ± Sd	No. of adults Mean ± Sd
Gulshan	442.00 ± 10.54	327.00 ± 11.27	235.33 ± 6.66	172.00 ± 26.23
Uttara	382.00 ± 7.21	281.67 ± 27.68	172.33 ± 51.98	92.67 ± 74.22
Tejgaon	586.67 ± 7.77	470.00 ± 20.66	363.33 ± 23.86	286.67 ± 11.02
Mirpur	561.50 ± 36.06	461.00 ± 41.01	354.50 ± 33.23	299.50 ± 17.68
Ramna	322.33 ± 10.50	229.00 ± 7.21	140.00 ± 14.73	95.67 ± 8.39
Motijheel	566.00 ± 1.41	453.00 ± 2.83	365.00 ± 4.24	289.00 ± 46.67
Lalbagh	304.00 ± 6.08	218.67 ± 13.65	147.67 ± 12.86	100.67 ± 4.04
Wari	558.00 ± 33.05	454.00 ± 31.19	367.33 ± 19.63	294.00 ± 4.58

August 2014

Divisions	No. of Eggs Mean ± Sd	No. of Larvae Mean ± Sd	No. of Pupae Mean ± Sd	No. of adults Mean ± Sd
Gulshan	214.33 ± 11.55	152.67 ± 25.17	112.33 ± 15.50	89.33 ± 17.67
Uttara	170.33 ± 12.66	101.67 ± 5.51	79.33 ± 6.66	62.67 ± 4.04
Tejgaon	381.33 ± 5.51	315.67 ± 61.78	219.33 ± 57.74	141.67 ± 9.07
Mirpur	350.50 ± 24.75	254.00 ± 31.11	160.00 ± 22.63	124.50 ± 0.71
Ramna	139.00 ± 15.52	93.67 ± 6.81	70.33 ± 14.15	58.00 ± 20.07
Motijheel	344.00 ± 1.41	291.50 ± 70.00	195.50 ± 65.76	166.50 ± 62.93
Lalbagh	156.00 ± 9.54	107.67 ± 1.73	82.00 ± 22.87	61.33 ± 14.22
Wari	348.00 ± 1.00	241.00 ±28.15	153.67 ± 6.51	126.33 ± 5.51

September-2014

Divisions	No. of Eggs Mean ± Sd	No. of Larvae Mean ± Sd	No. of Pupae Mean ± Sd	No. of adults Mean ± Sd
Gulshan	58.33 ± 3.06	33.33 ± 2.31	19.67 ± 1.53	11.00 ± 1.00
Uttara	33.67 ± 2.08	21.33 ± 1.53	9.67 ± 1.53	4.33 ± 1.53
Tejgaon	160.50 ± 19.09	117.00 ± 8.49	92.50 ± 6.36	71.50 ± 6.36
Mirpur	135.00 ± 24.98	103.67 ± 22.68	82.67 ± 26.58	62.33 ± 24.58
Ramna	74.33 ± 6.66	51.33 ± 5.69	40.00 ± 8.54	24.33 ± 7.64
Motijheel	156.50 ± 30.41	109.00 ± 16.97	86.50 ± 13.44	67.00 ± 15.56
Lalbagh	82.67 ± 4.51	59.00 ± 7.00	42.33 ± 5.86	28.00 ± 3.46
Wari	107.67 ± 2.31	79.33 ± 5.77	60.33 ± 4.16	43.00 ± 7.00

October-2014

Divisions	No. of Eggs Mean ± Sd	No. of Larvae Mean ± Sd	No. of Pupae Mean ± Sd	No. of adults Mean ± Sd
Gulshan	35.33 ± 5.13	15.67 ± 3.06	8.00 ± 1.00	2.67 ± 0.58
Uttara	16.00 ± 3.00	8.33 ± 2.52	3.33 ± 2.08	1.33 ± 1.15
Tejgaon	71.50 ± 4.95	46.00 ± 14.14	28.50 ± 14.85	14.50 ± 9.19
Mirpur	67.33 ± 10.50	44.00 ± 7.55	31.00 ± 7.55	17.67 ± 5.77
Ramna	26.67 ± 5.51	11.00 ± 1.00	4.33 ± 0.58	2.33 ± 0.58
Motijheel	68.00 ± 1.41	43.50 ± 10.61	30.50 ± 10.61	16.50 ± 6.36
Lalbagh	45.00 ± 4.00	30.67 ± 13.61	17.33 ± 10.12	12.00 ± 6.93
Wari	43.00 ± 7.00	52.33 ± 1.53	36.33 ± 1.53	23.00 ± 4.36

November-2014

Divisions	No. of Eggs Mean ± Sd	No. of Larvae Mean ± Sd	No. of Pupae Mean ± Sd	No. of adults Mean ± Sd
Gulshan	23.00 ±3.61	11.33 ± 0.58	5.00 ± 1.00	1.67 ± 2.08
Uttara	18.67 ± 5.86	9.00 ± 3.00	2.33 ± 0.58	1.67 ± 0.58
Tejgaon	46.67 ± 10.97	27.33 ± 7.57	15.67 ± 5.03	15.00 ±4.58
Mirpur	43.00 ± 7.07	22.50 ± 2.12	14.00 ± 2.83	14.00 ± 2.83
Ramna	13.33 ± 2.52	4.00 ± 2.65	1.33 ± 0.58	1.33 ± 0.58
Motijheel	43.50 ± 6.36	25.00 ± 2.83	12.00 ± 1.41	9.00 ± 7.07
Lalbagh	10.00 ± 1.00	4.33 ± 1.53	1.33 ± 0.58	7.33 ± 8.74
Wari	42.33 ± 3.21	22.33 ± 3.21	10.67 ± 1.53	13.33 ± 4.04

December-2014

Divisions	No. of Eggs Mean ± Sd	No. of Larvae Mean ± Sd	No. of Pupae Mean ± Sd	No. of adults Mean ± Sd
Gulshan	23.00 ± 2.00	9.00 ± 1.00	4.00 ± 1.73	1.33 ± 1.53
Uttara	15.67 ± 4.04	8.33 ± 0.58	3.67 ± 1.15	2.67 ± 2.52
Tejgaon	51.00 ± 24.58	35.33 ± 21.22	21.33 ± 12.22	16.33 ± 13.58
Mirpur	43.00 ± 5.66	22.00 ± 4.24	10.00 ± 1.41	5.50 ± 4.95
Ramna	8.67 ± 1.53	3.67 ± 3.51	1.00 ± 1.00	0.67 ± 1.15
Motijheel	44.00 ± 1.41	23.00 ± 2.83	12.50 ± 6.36	10.00 ± 2.83
Lalbagh	3.33 ± 1.53	1.00 ± 1.00	0.00 ± 0.00	0.00 ± 0.00
Wari	40.33 ± 4.51	21.00 ± 3.46	10.67 ± 2.52	10.67 ± 2.52

January-2015

Divisions	No. of Eggs Mean ± Sd	No. of Larvae Mean ± Sd	No. of Pupae Mean ± Sd	No. of adults Mean ± Sd
Gulshan	21.67 ± 3.79	10.67 ± 1.53	4.67 ± 1.15	1.33 ± 0.58
Uttara	14.67 ± 2.52	8.00 ± 1.00	2.67 ± 0.58	0.33 ± 0.58
Tejgaon	46.33 ± 3.06	26.33 ± 6.66	13.67 ± 6.43	5.67 ± 4.73
Mirpur	35.00 ± 1.41	21.00 ± 0.00	12.00 ± 0.00	8.50 ± 2.12
Ramna	13.33 ± 2.52	6.33 ± 2.08	2.67 ± 1.53	0.67 ± 0.58
Motijheel	23.00 ± 2.83	11.00 ± 1.41	4.50 ± 0.71	1.50 ± 0.71
Lalbagh	7.33 ± 1.53	1.67 ± 0.58	0.00 ± 0.00	0.00 ± 0.00
Wari	23.33 ± 2.08	10.33 ± 1.15	3.33 ± 1.15	3.00 ± 1.00

February 2015

Divisions	No. of Eggs Mean ± Sd	No. of Larvae Mean ± Sd	No. of Pupae Mean ± Sd	No. of adults Mean ± Sd
Gulshan	52.66 ± 6.66	34.00 ± 11.00	18.67 ± 5.86	9.33 ± 4.62
Uttara	34.33 ± 3.06	20.33 ± 1.15	11.33 ± 0.58	5.67 ± 0.58
Tejgaon	108.67 ± 15.31	72.67 ± 21.50	53.00 ± 25.87	38.67 ± 27.54
Mirpur	86.5 ± 12.02	74.50 ± 28.99	57.50 ± 28.99	37.50 ± 26.16
Ramna	41.67 ± 3.08	21.67 ± 1.15	13.00 ± 2.00	6.67 ± 1.53
Motijheel	123.5 ± 12.02	70.33 ± 27.32	66.50 ± 13.44	49.50 ± 16.26
Lalbagh	59 ± 2.65	49.00 ± 26.06	20.67 ± 2.52	10.33 ± 2.08
Wari	88 ± 6.56	70.00 ± 7.00	61.00 ± 5.29	44.33 ± 3.06

March 2015

Divisions	No. of Eggs Mean ± Sd	No. of Larvae Mean ± Sd	No. of Pupae Mean ± Sd	No. of adults Mean ± Sd
Gulshan	89.67 ± 9.29	68.67 ± 11.37	48.67 ± 12.74	31.33 ± 6.43
Uttara	39.67 ± 6.81	26.00 ± 7.00	13.00 ± 6.93	9.33 ± 5.86
Tejgaon	143.67 ± 11.24	103.00 ± 7.94	80.33 ± 6.81	61.33 ± 6.81
Mirpur	117.50 ± 19.09	77.00 ± 14.14	57.00 ± 15.56	45.00 ± 12.73
Ramna	56.33 ± 1.15	33.33 ± 3.21	18.33 ± 1.53	11.33 ± 0.58
Motijheel	130.50 ± 4.95	91.00 ± 5.66	71.00 ± 8.49	57.50 ± 4.95
Lalbagh	61.00 ± 6.56	41.33 ± 2.89	22.67 ± 2.52	12.33 ± 2.89
Wari	110.00 ± 4.00	92.00 ± 3.61	71.67 ± 3.51	53.67 ± 6.11

April-2015

Divisions	No. of Eggs Mean ± Sd	No. of Larvae Mean ± Sd	No. of Pupae Mean ± Sd	No. of adults Mean ± Sd
Gulshan	109.3 ± 4.73	92.33 ± 5.13	65.33 ± 8.62	52.00 ± 1.00
Uttara	75.67 ± 4.16	55.00 ± 2.65	31.67± 7.77	20.00 ± 9.54
Tejgaon	188.00 ± 29.46	96.67 ± 23.25	74.67 ± 12.42	56.33 ± 9.71
Mirpur	155.00 ± 2.83	103.50 ± 12.02	83.50 ± 13.44	48.50 ± 10.61
Ramna	92.33 ± 5.51	71.00 ± 4.00	53.67 ± 4.93	38.00 ± 11.27
Motijheel	161.00 ± 5.66	113.00 ± 0.00	90.50 ± 0.71	53.00 ± 35.36
Lalbagh	77.33 ± 8.08	55.33 ± 1.15	37.00 ± 1.00	42.67 ± 31.50

May-2015

Divisions	No. of Eggs Mean ± Sd	No. of Larvae Mean ± Sd	No. of Pupae Mean ± Sd	No. of adults Mean ± Sd
Gulshan	467.00 ± 9.85	344.67 ± 5.03	251.33 ± 5.51	183.33 ± 23.67
Uttara	352.67 ± 26.63	216.33 ± 46.05	132.67 ± 10.02	108.67 ± 13.50
Tejgaon	562.67 ± 21.08	464.00 ± 13.86	358.00 ± 6.08	108.67 ± 5.86
Mirpur	561.50 ± 51.62	445.50 ± 16.26	355.00 ± 14.14	264.50 ± 16.26
Ramna	340.33 ± 17.47	209.33 ± 47.50	138.67 ± 9.87	115.00 ± 7.00
Motijheel	573.00 ± 7.07	461.50 ± 9.19	362.00 ± 25.46	270.50 ± 23.33
Lalbagh	325.33 ± 16.65	237.00 ± 5.29	141.33 ± 5.69	98 ± 3.21
Wari	464.67 ± 12.50	354.33 ± 3.06	248.33 ± 7.09	184.33 ± 4.73

June-2015

Divisions	No. of Eggs Mean ± Sd	No. of Larvae Mean ± Sd	No. of Pupae Mean ± Sd	No. of adults Mean ± Sd
Gulshan	667± 6.35	533.67± 28.73	421.33± 12.42	383.33± 17.32
Uttara	552.67± 8.19	465.32± 5.51	321.67± 7.94	308.67± 10.02
Tejgaon	892.67± 30.89	644.11± 31.47	512.31± 11.85	494.33± 5.51
Mirpur	711.55± 36.77	645.53± 11.31	521.12± 18.38	464.57± 6.36
Ramna	540.33± 69.22	409.33 ± 62.95	238.67± 58.97	115.34± 50.27
Motijheel	798.45± 4.95	631.51± 13.44	462.51 ± 0.00	370.53± 2.12
Lalbagh	525.33± 1 1.36	427.31± 12.17	241.33± 16.92	118.67± 3.79
Wari	723.67± 10.58	614.33± 19.70	448.33± 25.32	384.33 ± 6.11

Appendix VII. Relationship between aedes mosquito population and rainfall

Month	Temp ^{0}c	Rainfall (mm)	Eggs	Larve	Pupae	Adult
Jan	29.9	2	23.28 ± 14.19	12.36 ± 10.33	5.92 ± 7.11	3 ± 4.79
Feb	32.2	43	71.88 ± 32.84	48.28 ± 27.43	34.36 ± 24.86	22.84 ± 20.88
Mar	36.4	56	91.68 ± 39.24	62.56 ± 30.32	44.28 ± 27.42	32.52 ± 22.51
Apr	35.5	178	118.68 ± 37.08	80.68 ± 26.96	60.64 ± 25.21	46.6 ± 22.76
May	36.4	311	457.12 ± 112.92	343.44 ± 118.32	249.88 ± 110.50	195.04 ± 82.39
Jun	36.5	418	556 ± 103.94	451.76 ± 103.42	356.72 ± 102.06	291.44 ± 91.85
Jul	35.8	393	447.36 ± 113.67	345.2 ±106.41	252.08 ± 102.68	187.48 ± 96.65
Aug	34.4	359	248.72 ± 97.70	180.92 ± 89.45	125.08 ± 58.20	95.84 ± 41.62
Sep	34.8	251	95.16 ± 42.28	67.68 ± 32.92	50.8 ± 28.98	35.84 ± 24.35
Oct	36.0	50	46.8 ± 22.09	28.88 ± 17.88	17.96 ± 13.79	10.12 ± 8.76
Nov	33.8	0	28.28 ± 15.78	14.52 ± 9.63	7.04 ± 5.91	7.08 ± 6.59
Dec	29.2	1	25.64 ± 18.74	13.68 ± 12.99	6.96 ± 7.89	5.32 ± 7.03

Appendix VIII. Relationship between monthly and division wise distributed pattern of total dengue patients and incidence of aedes mosquito population in Dhaka city

Incedence of aedes mosquitoes (Monthly)	Pattern of dengue patients (Month wise)	Incedence of aedes mosquitoes (Division wise)	Pattern of dengue patients (Division wise)
3	0	1415.00	569
22.84	3	1369.67	456
32.52	5	1344.67	431
46.6	11	1342.00	342
195.04	13	832.33	342
291.44	698	585.33	165
187.48	1393	570.67	147
95.84	343	497.67	145
35.84	72	1415.00	569
10.12	36	1369.67	456
7.08	16	1344.67	431
5.32	0	1342.00	342