BIORATIONAL MANAGEMENT OF MOSQUITO LARVAE IN BANGLADESH

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BIORATIONAL MANAGEMENT OF MOSQUITO LARVAE IN BANGLADESH

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CERTIFICATE

This is to certify that thesis entitled **"BIORATIONAL MANAGEMENT OF MOSQUITO LARVAE IN BANGLADESH"** submitted to the **Faculty of Agriculture,** Sher-e-Bangla Agricultural University (SAU), Dhaka in partial fulfillment of the requirements for the degree of **MASTER OF SCIENCE (MS) IN ENTOMOLOGY**, embodies the result of a piece of bona fide research work carried out by **MD. SAWGAT HOSSAIN, Registration no. 13-05720** under my supervision and guidance. No part of the thesis has been submitted earlier for any other degree or diploma.

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

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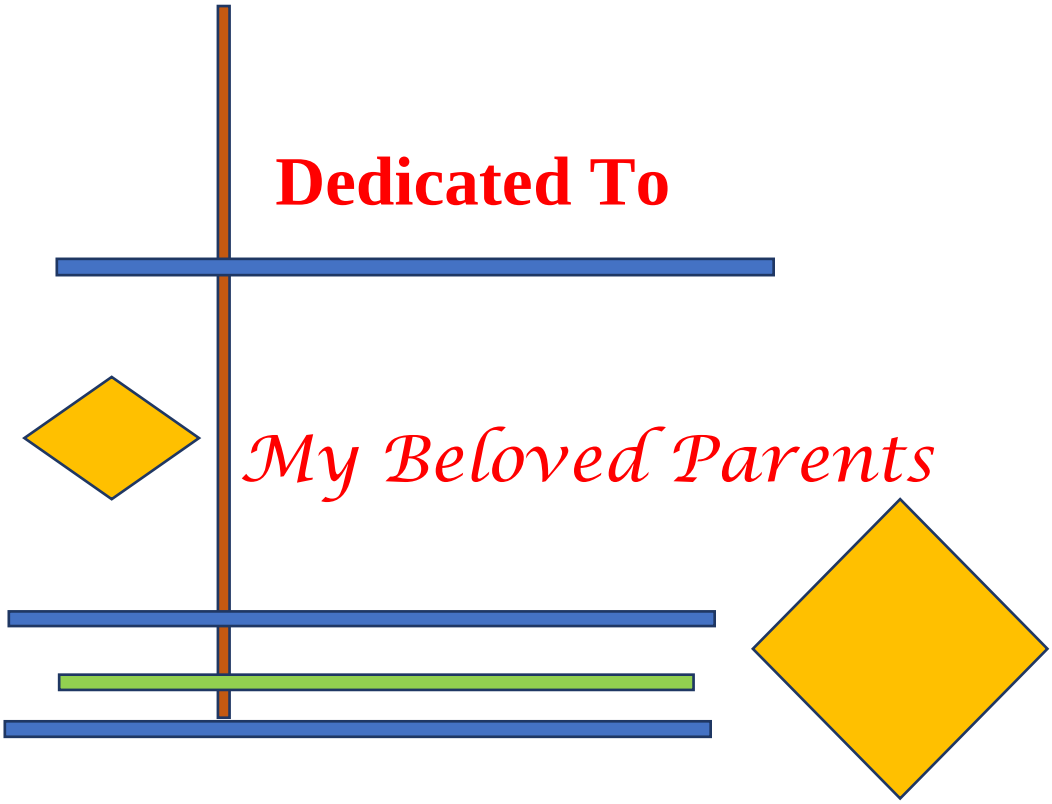
Dated: June 2020
Place: Dhaka, Bangladesh

Dedicated To



My Beloved Parents

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BIORATIONAL MANAGEMENT OF MOSQUITO LARVAE IN BANGLADESH

Abstract

The present study evaluated the efficacy of nine larvicides for bio-rational management of mosquito larvae in Bangladesh from 30 September 2018 to 11 February 2019. Experiment was conducted in the laboratory of SAU and insecticides used in this experiment are spinosad, emamectin-benzoate, pyrazine, buprofezin, cyfluthrin, lambda-cyhalothrin, temephos, neem seed kernel extract, neem leaf extract at different concentration. Dosages used in this experiment were; spinosad (dose:1 ppm, 2 ppm, 5 ppm and 10 pmm), cyfluthrin (dose: 1 ppm, 2 ppm, 5 ppm and 10 pmm), lambda-cyhalothrin (dose:1 ppm, 2 ppm, 5 ppm and 10 pmm), pyrazine (dose: 25 ppm, 40 ppm, 80 ppm, 160 ppm), emamectin-benzoate (dose: 25 ppm, 40 ppm, 80 ppm, 160 ppm), buprofezin (5 ppm, 10 ppm, 20 ppm, 40 ppm) temephos (10 ppm, 5 ppm, 2 ppm, 1 ppm) and two botanical as neem leaf extract(dosages: 30000 ppm, 50000 ppm, 100000 ppm, 200000 ppm) and neem seed kernel extract(dose:1.0 ppm, 2.0 ppm, 5 ppm,10 ppm). Of the nine larvicides tested lambda-cyhalothrin gave the lowest median lethal concentration of LC_{50} 0.000848 thereafter temephos gave second lowest median lethal concentration of LC_{50} 0.00114 after 48 hours and spinosad gave third lowest median lethal concentration of LC_{50} 0.00433. It was followed by cyfluthrin with LC_{50} 1.1067, buprofezin with LC_{50} 1.7650, neem seed kernel with LC_{50} 2.3360, pyrazine with LC_{50} 6.0700, emamectin-benzoate with LC_{50} 7.5823 and neem leaf extract gave median lethal concentration of LC_{50} 10534. From the study it was revealed that order of toxicity of nine larvicides is lambda-cyhalothrin > temephos > spinosad > cyfluthrin > buprofezin > neem seed kernel extract > pyrazine > emamectin -benzoate > neem leaf extract. Mortality percentage also calculated to find out the efficacy of nine larvicides. Lambda-cyhalothrin and temephos gave highest mortality percentage of 100% after 48 hour. From the study we found the order of mortality percentage of nine larvicides is lambda-cyhalothrin > temephos > spinosad > pyrazine > emamectin-benzoate > buprofezin > neem seed kernel extract > neem leaf extract > cyfluthrin. Considering environmental safety temephos and spinosad could be effectively used for mosquito larvae control in stagnant water bodies and lambda-cyhalothrin in small containers retaining water temporarily with appropriate dosages.

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ABBREVIATIONS AND ACRONYMS

%= percentage

CV%=Percent Coefficient of Variation

DMRT= Duncan Multiple Range Test

e.g=exempli gratia(L),for example

et al= And others

etc= etcetera

g=Gram(s)

L=Litre

LSD= Least Significant Difference

M.S= Master of Science

mg=Miligram

SAU=Sher-e-Bangla Agricultural University

USA=United States of America

WHO= World Health Organization

µg=Microgram

µl=Microliter

LC_{50}= Lethal concentration

CHAPTER I

INTRODUCTION

Mosquitoes are insects belonging to the order Diptera, the True Flies. Like all True Flies, they have two wings, but unlike other flies, mosquito wings have scales. Female mosquitoes' mouthparts form a long piercing-sucking proboscis. Males differ from females by having feathery antennae and mouthparts not suitable for piercing skin. A mosquito's principal food is nectar or similar sugar source. Mosquito works as vector for different disease both for human and animal.

Mosquitoes can be an annoying, serious problem in man's domain. They interfere with work and spoil hours of leisure time. Their attacks on farm animals can cause loss of weight and decreased milk production. Some mosquitoes are capable of transmitting diseases such as malaria, yellow fever, dengue, filariasis and encephalitis [St. Louis encephalitis (SLE), Western Equine encephalitis (WEE), LaCrosse encephalitis (LAC), Japanese encephalitis (JE), Eastern Equine encephalitis (EEE) and West Nile virus (WNV) to humans and animals.

There are over 3,000 different species of mosquitoes throughout the world; in Bangladesh Records for 123 species were collected, although some species had only a single record. This is an increase of ten species over the most recent complete list, compiled nearly 30 years ago (Irish *et al*,. 2016).

Mosquito cause more damage than any other vector to human health. It also causes different health hazards to animal. They cause different disease to human such as malaria, Chikungunya, dog heartworm, dengue, yellow fever, westnile virus, zika virus etc.

The *Aedes* mosquitoes are responsible for the transmission of dengue, chikungunya, and zika in our country. This vector is mainly a domestic habitat, highly climate sensitive in the environment, and breeds in a small quantity of water especially in urban environments. (Mustuddy *et al.*, 2019). The escalating dengue situation in Bangladesh has been emerging as a serious public health problem in terms of morbidity and mortality. Results of analysis of 40,476 cases of Bangladesh occurring during 2000–2017 indicated that 49.73% of the dengue cases occurred during the

monsoon season (May–August) and 49.22% during the post-monsoon season (September–December). During 2015–2017, in the pre-monsoon season, the dengue cases were reported to be more than seven times higher compared to the previous 14 years. Despite the efforts to control dengue, based primarily on the vector control and case management, the burden and costs of the disease and similar vector-borne diseases will continue to grow in future in our country. Developing a cost-effective vaccine against all the 4 strains of dengue remains a challenge (Mustuddy *et al*., 2019).

The advent of synthetic insecticides occurred in the 1940s and revolutionized the way that vector control was conducted (World Health Organization, 2014). Throughout the 1950s and 1960s large-scale insecticidal treatments diminished many of the vector-borne diseases (World Health Organization, 2014). Vector control programs began to lapse with the global abatement of many vector-borne diseases in the 1960s (World Health Organization, 2014). Increased international travel and commerce over the last few decades have created more pathways for vectors and their associated diseases to spread to new places.

Mosquito control in the United States has evolved from reliance on insecticide application for control of adult mosquitoes (adulticide) to integrated pest management programs that include surveillance, source reduction, larvicide, and biological control, as well as public relations and education. Adulticides still play a vital role when flooding causes extreme numbers of nuisance mosquitoes or when outbreaks of diseases (Rose, 2001)

Detection of large numbers of immature mosquitoes in areas where source reduction or biological control is not feasible may require larvicide treatment to prevent the emergence of adult mosquitoes. Use of larvicides is less controversial than use of adulticides, although use of larvicides may lead to public concern about their effects on untargeted beneficial aquatic arthropods and vertebrates (Rose, 2001)

Pesticides vary in their toxicity to people and to non-target organisms, and in their potential ecological impact. Pest control materials that are relatively non-toxic to people with few environmental side-effects are called “biorational” pesticides in this Guide. Biorational pesticides mostly include the following categories further defined in this section: biopesticide, organic pesticide, minimum-risk pesticide, *and* biological control (Benelli *et al*,. 2016).

In this work some biorational larvicides are tested to find out their efficacy against mosquito larvae at different dose to control the mosquito. There are 6 biorational pesticides were used to find out their efficacy such as Spinosad, Cyfluthrin, Emamectin Benzoate, Buprofezin, Pyrazine, Lambdacyhalothrin . These are easily find out in our country.

The goal of the experiment is to manage mosquito vectors with the application of bio-rational larvicides with minimal disruptive influence upon the environment and its inhabitants. In order to achieve the goal, the present study is designed with the following objectives:

- To determine the bio-efficacy of several selected bio-rational larvicides against the larvae of common mosquitoes in Bangladesh.
- To determine the effective dosages of the tested bio-rational products against the larvae of common mosquitoes in Bangladesh.

CHAPTER II

REVIEW OF LITERATURE

An update knowledge regarding the current status of 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.

2.1 Background

Aedes, Anopheles and *Culex* species of mosquitoes transmit diseases to humans and animals. They are most prevalent in developing and under developed countries, and spread diseases like malaria, dengue, chikungunya, yellow fever, filaria (Esteva,*et al.*, 2007) Despite decreasing incidence of human mortality, mosquito borne diseases are still the cause of serious health issues to over 214 million people(WHO ,2015) in developing and under developed countries (Atwa *et al.*, 2017).

Mosquitoes are dipteran insects and blood sucking fly pests of man. Mosquitoes are surviving on earth since millions of years. They have always given tough time to men as important carriers of various diseases. People fight globally against mosquitoes and mosquito borne diseases. Malaria, dengue, filaria, Japanese encephalitis, west nile virus and chikungunya are the major diseases spread globally by different mosquito. These diseases challenge the developed and developing countries of the world for irradicaton. Mosquitoes are very well recognized as vectors of protozoan, viruses and other pathogenic organisms, after the discoveries made by Sir Patrick Manson, Sir Ronald Ross and Sir Walter Reed. It is well known also that under the influence of environmental conditions a vector species may show changes in the seasonal distribution in the same area of dominance. The increase in density of a vector species is very much dependent on climatological factors favorable for its breeding, and adult survival (Suresh, 2010).

2.2. Mosquito Ecology

The ecology and distribution of various mosquito species are important in the determination of mosquito vector abundance and associated diseases prevalence. Some aspects of human ecology greatly influence mosquito distribution, species relative abundance and their survival. All mosquitoes breed in water more often quiescent. There are mosquito species groups; subgenus and genus have their own preferred habitat based on locations and conditions of the water body.

Mosquitoes are distributed throughout the world and have occupied many niches including higher altitudes. Thirty four mosquito species of five different genera were recorded within the altitudinal range of 300 to 2000 m from Garhwal region (Pemola and Jauhari, 2004). Effect of natural factors like temperature, humidity and rain fall also have impact on the mosquitoes. Climate has been established as an important determinant in the distribution of vectors and pathogens (Pemola and Jauhari, 2006).

The importance of mosquitoes in human and animal diseases has made them an important target of medical, veterinary and conservation research since Patrick Manson and Sir Ronald Ross first implicated mosquitoes in the transmission of filarial nematodes and malaria in the closing decades of the nineteenth century (D'Antonio and Spielman, 2002). However, the extent to which studies of mosquito ecology have been seen to be central to medical entomology has varied over the years as the focus of disease control has evolved (Charles and Godfry, 2013) in the first half of the twentieth century, ecology, at least descriptive ecology, was accorded high importance as the life cycle and breeding sites of the major vectors were worked out. After the Second World War, the discovery of synthetic insecticides led to optimism that diseases could be eliminated by blanket spraying of the environment with DDT and its successors. This did indeed lead to major successes, for example the end of endemic malaria in Italy and other parts of Europe (Bruce Chwatt and de Zulueta, 1980). But progress in the tropics was much more mixed (Hay *et al*., 2004). In 1969, the Garki Project was launched in Northern Nigeria, a project that used a combination of insecticide spraying and prophylactic drug administration to attempt to eliminate malaria from an area of about 150 villages (Molineaux and Gramiccia, 1980). The intervention failed to interrupt transmission, a disappointment that supported the view that control of mosquitoes (at least away from the human feeding site) was not the best way to reduce disease burden (Charles and Godfry, 2013)

As larvae, mosquitoes make up substantial biomass in aquatic ecosystems globally. They abound in bodies of water ranging from ephemeral ponds to tree holes (Daugherty *et al.,* 2003) to old tyres, and the density of larvae on flooded plains can be so high that their writhing sends out ripples across the surface. They feed on decaying leaves, organic detritus and microorganisms. The question is whether, without mosquitoes, other filter feeders would step in. "Lots of organisms process detritus. Mosquitoes aren't the only ones involved or the most important," says Juliano. "If you pop one rivet out of an airplane's wing, it's unlikely that the plane will cease to fly (Fang, 210).

2.2.1 Rainfall

Any time there is rain, there is increased mosquito breeding. Mosquitoes need stagnant water to lay their eggs in, therefore the more puddles there are on the ground, the more of a "playground" for mosquito moms laying eggs, and for those eggs to move through their life cycle to larva, then pupa, then adult. From egg to adult, this can take as little as four days to as long as a month, but generally it takes a little more than a week. Keep in mind, though, that mosquitoes need stagnant water in order for this to happen. But it only takes a bottle cap full of water in order for a female mosquito's raft of nearly 300 eggs to float in, so even if rain puddles dry up on the ground, stagnant water elsewhere make for prime mosquito-breeding areas. Tires seem to be a favorite, as they collect water on the inside and are a protected area for the babies to hatch and grow (Springfield-decatur, 2018). Other studies have shown that a suitable range of humidity stimulating mosquito flight activity is between 44% and 69%, with the most appropriate reaching 65% (Jemal *et al.,* 2018; Khan *et al.,* 2018). During the year of the survey, the annual precipitation was 284 mm in the study area.

2.2.2 Heat and humidity

Mosquitoes are cold-blooded creatures, therefore they can't regulate their body heat and their temperature is essentially the same as their environment. Temperature and mosquito activity goes hand in hand with the insects flourishing in moist, relatively warm environments, functioning best at 80°F. Once the temperature lowers to about 60°F they become lethargic and anything below 50°F they find it hard to function at all. Different species are active at different times, but in general, most mosquitoes are

extra active at dusk and dawn. Warm evening temperatures allow mosquitoes to thrive, since prolonged sun exposure can actually dehydrate them. But depending on the temperature, some species will continue biting throughout the night.

But it can actually get too hot and dry for mosquitoes. The key here is humidity. If it does get too hot and too dry, mosquitoes will not be as active and feeding as they usually are. But once the temperature drops a bit and gets within the tolerable range for mosquitoes, they're more hungry and therefore biting more. Those that are infected with a disease, such as West Nile virus, will be feeding more frequently, which will increase the chances of an outbreak. (Springfield-decatur, 2018). Environmental variables such as temperature, relative humidity and precipitation are known to impact mosquito activity, survival and distribution. Results demonstrate that temperatures significantly affects host searching activity of *Cx. pipiens*, *Ae. detritus* and *Ae. caspius* population. Precisely, temperatures between 15 ^{0}C to 24 ^{0}C seem to be more suitable for their host searching activity. Higher abundance in that period might be direct consequence of the preceding precipitation providing the multiple breeding sites. Temperatures above 28^0 C lead to decrease of the abundance. These results are consistent with previous studies that showed temperatures between 15 ^{0}C to 28 ^{0}C to be more favourable for mosquitoes and that temperatures greater than 30 ^{0}C increase mosquitomortality (Tian *et al.*, 2015; Jemal, and Al-Thukair, 2018; Ciota *et al.*, 2014; Asigau and Parker, 2018). It is reported that high temperatures cause intense metabolic rate leading to low respiration rate and finally to death (Ciota *et al*., 2014; Phanitchat *et al.*, 2017). The Pearson coefficient correlation confirms the negative relationship between temperature and mosquito activity. Relative humidity in the study area ranges between 57% to 79%. Statistical analysis shows a significant moderate positive relationship between monthly relative humidity and number of sampled *Cx. pipiens* and *Ae. detritus*. Significant strong positive relationship was observed for *Ae. caspius*. It has been reported that high humidity increases egg production, larval indices, mosquito activity and influences their activities (de Almeida Costa, *et al.*, 2010; Khan, 2018). Other studies have shown that a suitable range of humidity stimulating mosquito flight activity is between 44%and 69%, with the most appropriate reaching 65% (Khan, 2018; Jemal and Al-Thukair, 2018). During the year of the survey, the annual precipitation was 284 mm in the study area.

2.2.3 Cooler Temperatures

Believe it or not, some species of mosquitoes hibernate. Not all species, but many of them go dormant in the wintertime, finding hiding spots to wait in for warmer weathers. When a female mosquito lays her eggs in the water in the fall, they can lie dormant and eventually freeze. But once the weather warms up a little bit, they can hatch and the life cycle will start all over again, which is why "mosquito season" is generally considered as between spring and fall. Tropical places and where it's hot and humid year-round are unfortunate enough to never have a "season" for mosquitoes it's all the time!

But in the simplest of terms, the recipe for mosquito activity is heat + rainfall = humidity, and this, combined with stagnant water means the perfect soupy combination for mosquito madness (Springfield-decatu,r 2018)

It is well known that environmental variables are interrelated, and for that reason, it is complicated to assess each factor separately. Further, other factors seem to play an important role in mosquito activity and abundance. Ferraguti *et al*. (2016) show that mosquito density and species composition was a_ected by anthropogenetic landscape transformation (Ferraguti *et al.,* 2016). Möhlmann *et al*. (2017) notice specific differences in mosquito abundance and diversity in relationship with di_erent geographical latitudes (Möhlmann *et al.,* 2017).

2.3 Host selection

Host selection by vector mosquitoes is a critical component of virus proliferation, particularly for viruses such as West Nile (WNV) that are transmitted enzootically to a variety of avian hosts, and tangentially to dead-end hosts such as humans. *Culex tarsalis* is a principal vector of WNV in rural areas of western North America. Host selection was likely based both on host availability and differences in utilization (Thiemann *et al*., 2011).

A total of 32 host species were identified for 23 mosquito species, covering 21 mammalian species (including humans) and eleven bird species. Three mosquito species accounted for nearly three quarters of all collected blood-fed mosquitoes: *Aedes vexans* (363 specimens, 46.8 % of all mosquito specimens), *Culex pipienspipiens* form *pipiens* (100, 12.9 %) and *Ochlerotatuscantans* (99, 12.8 %). Non-human mammals dominated the host species (572 specimens, 73.8 % of all

mosquito specimens), followed by humans (152, 19.6 %) and birds (51, 6.6 %). The most common host species were roe deer (*Capreolus capreolus)*; 258 mosquito specimens, 33.3 % of all mosquito specimens, 65 % of all mosquito species), humans (*Homo sapiens*; 152, 19.6 %, 90 %), cattle (*Bos taurus*; 101, 13.0 %, 60 %), and wild boar (*Sus scrofa*; 116, 15.0 %, 50 %). There were no statistically significant differences in the spatial-temporal host-feeding patterns of the three most common mosquito species (Brostler *et al*., 2016).

2.4 Life History

Mosquitoes fall into the Culicidae family of the order Diptera within class Insecta and members of the phylum Arthropod. This family includes two important medical and veterinary *important* disease vectors due to their roles for transmission of various viruses, bacteria, and parasites—Anophelinae and Culicinae. These mosquitoes undergo four stages of transformation during their lifetime: egg, larva, pupa, and adult. These have complete metamorphoses or so called Holometabola. Commonly known as the southern house mosquito, *Culex quinquefasciatus* is a medium-sized brown insect that exists throughout the tropics and the lower altitudes of temperate regions, and a vector of many pathogens of humans as well as both domestic and wild animals. Although an intensified interest in mosquito cytogenetics in the past decade has produced a number of contributions to knowledge on this subject, the available information is still superficial and limited to a few mosquito species only. Therefore, the karyotype of the populations of the mosquito *C. quinquefasciatus* has been studied collected from three provinces: Babylon, Baghdad, and Wasit of Iraq. The study showed that the chromosomes karyotype of this species consisted of three pairs of chromosomes (i.e., 2n = 6). In conclusion, it is stressed that prospects are especially good for evolutionary and genetic studies involving chromosomal polymorphism (Abd, 2020).

2.5 Classification of Mosquito

Kingdom: Animalia

Phylum: Arthropoda

Subphylum: Hexapoda

Class: Insecta

Subclass: Pterygota

Order: Diptera

Suborder: Nematocera

Family: Culicidae

Subfamily: Culicinae (Abd, 2020)

2.6 MOSQUITO LIFE CYCLE

2.6.1 Metamorphosis

Mosquitoes grow to adulthood through four stages. This process is called metamorphosis. Many other insects, including butterflies, moths, dragonflies and beetles, undergo metamorphosis. The four stages in mosquito metamorphosis are egg, larva, pupa and adult.

Female mosquitoes attack humans and animals to obtain the blood necessary to sustain their vital activities and make eggs. They use all warm-blooded animals, causing severe uncomfortable and serious harm due to loss of blood, itching, and allergies as well as transmission of pathogens (Abd, 2020).

All mosquito species go through four stages during their life cycles.

2.6.2 Eggs

Female mosquitoes, depending on the species, may lay eggs on the surface of standing water in groups called rafts, or individually, on dry or moist ground or on vegetation. Female mosquitoes lay eggs about every third day during their lifespan, usually in clumps of 100 to 300 eggs. All mosquito eggs, regardless of species, need water to hatch. Depending on the availability of water, the eggs may hatch within a few minutes or lay dormant for years before they finally emerge as larvae. The eggs, generally white when laid, cannot hatch unless they are in water. One square foot of

salt marsh may contain over 10,000 salt marsh mosquito eggs waiting for a high tide or heavy rain to provide conditions suitable for hatching

2.6.3 Larvae

When the eggs hatch, the larvae emerge. They are called “wigglers” because that’s how they swim. When a mosquito egg hatches, the immature mosquito begins its life in the larval stage. Mosquito larvae, or wrigglers, live only in water. If their habitat dries up before they have developed into adults, they will die. The mosquito larvae are small, worm-like animals with no legs. They have many hairs, especially around their mouthparts. AT the tail there is a tube called the siphon. The larvae stick their siphons out of the water to breathe. Larvae move through the water column by jerking their bodies back and forth. Close observation will reveal their constantly working mouthparts, as they search for small organic particles of food. Mosquito larvae are generally found in shallow water, either fresh or salt, depending on the species, As the larva eats, it grows to the point where it can’t grow further, due to its hard exoskeleton. The larva then sheds, or molts, its exoskeleton, leaving beneath a much softer one that will stretch as it grows. The larva will continue to eat and grow and will molt four times. Each of the four larval stages is called an instar. A mosquito larva goes through four instars, and during the final molt, the pupa emerges, shed their skins four times over about a week, 1^{st} instar (days 4), 2^{nd} instar (days 5),3^{rd} instar (days 6), 4^{th} instar (days 7) and develop into pupae. Larvae are the easiest to kill, using oils that block their breathing or bacteria that poison them.

2.6.4 Pupae

The pupa, or tumbler, resembles a fat comma. It does not feed and has no eyes. This period of time in the mosquito’s development is devoted to growth and change. The pupa normally rests at the surface of the water with its two breathing tubes, ortrumpets, connected to the water’s surface. Occasionally, if danger threatened, the pupa will tumble to the bottom. When the pupa is fully developed, it will come to the water’s surface one last time to emerge into the adult mosquito. It takes about four days for the adult mosquito to emerge.

2.6.5 Adults

When the adult mosquito is ready to emerge, the pupa will rest at the top of the water's surface and straighten out its body. The back of the exoskeleton splits and slowly the adult mosquito emerges. Like a scene from a science fiction movie, a creature with very little resemblance to its former self, emerges out of the pupal skin. The adult mosquito rests briefly on the water's surface, then flies a short distance to some surrounding vegetation to rest and allow time for the newly developed wings to dry. The newly emerged adults climb out of the water to rest and wait for their bodies to dry out. The males will take a day or two to fully develop their reproductive organs, then seek out a female, by the sound of her wingbeats, for mating. They'll live about three to five days after that, feeding on fruit and plant nectar. The females mate once, but continue laying eggs after every blood meal. Under the best conditions, they can live up to a month or two.

Figure 1 Mosquito egg cluster(raft) under microscope

Figure 2 Mosquito egg cluster under microscope

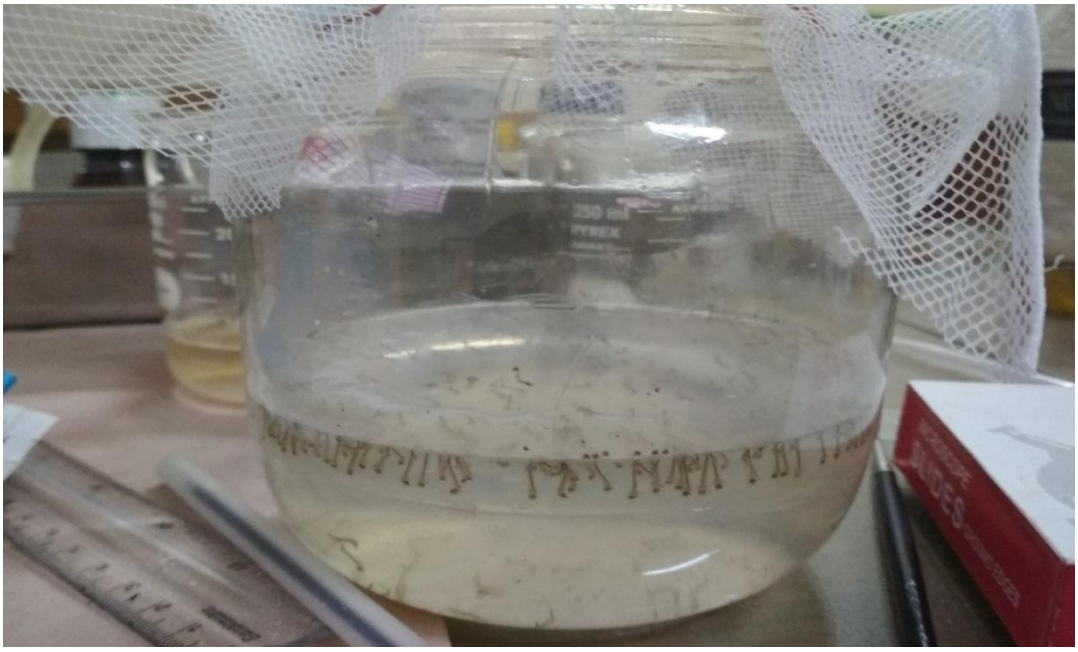

Figure 3 Mosquito larvae of different instar after emerging from egg.

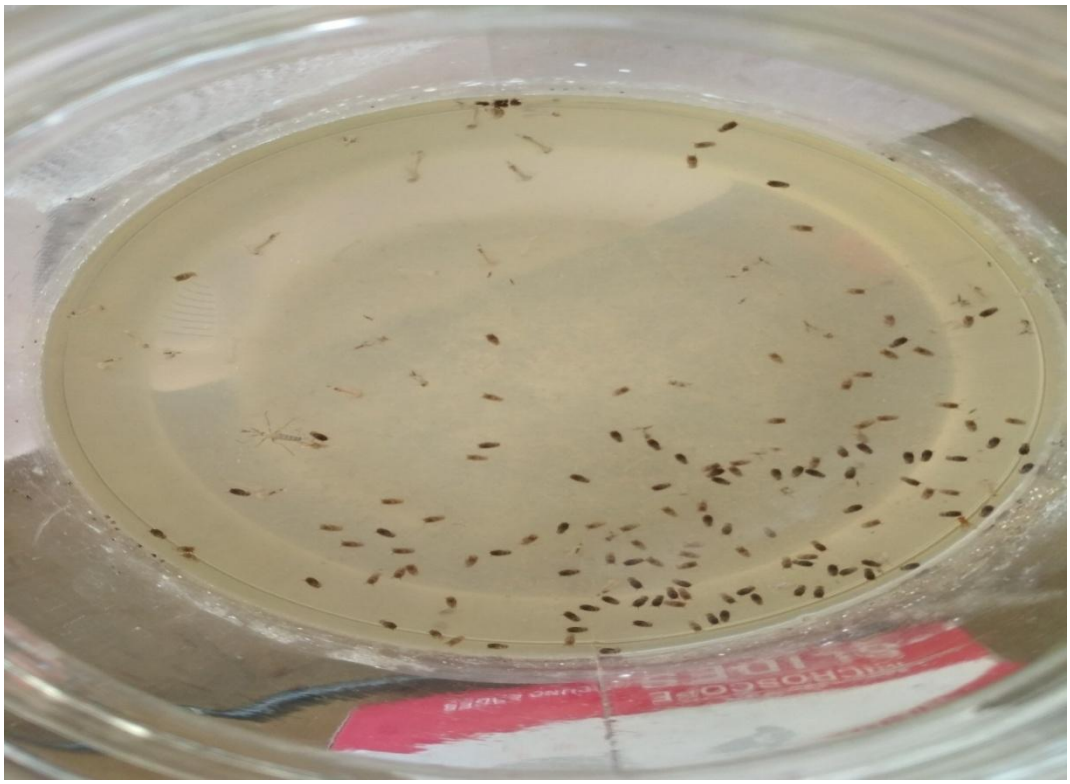

Figure 4 mosquito pupa after emerging from larvae

Figure 5. Adult mosquito after emerged from pupae

2.7 Geographical Distribution and Ecological Importance of Mosquito

Mosquitoes are widespread in all the tropical and subtropical regions of the world, which extend into the Arctic Circle but are absent in Antarctica. The eggs from temperate breeds have more strain than those found in warmer regions. Mosquitoes are found at a height of 550 m and a depth of 1250 m below sea level. The shallow-water marshes containing plants are a preferred environment for the growth and reproduction of mosquitoes. The most important species that prefer these environments are the types of *Culex*, especially *C. pipiens* and *C. Salinarius* Coquillett.

2.7.1 Malaria

Malaria is an ancient disease. In all likelihood originating in Africa, it has been described by the Chinese as far back as 2700BC and the Sumerians from 1700 BC. The malaria parasite (plasmodium) is transmitted by female Anopheles mosquitoes. The term malaria is attributed to Horace Walpole in a letter from Italy in 1740 and is derived from the Italian 'mal-aria" or "bad air" because it was thought to come on the wind from swamps and rivers. Scientists conducted much research on the disease during the 1880s and early 1900s. Approximately 40% of the world's population is susceptible to malaria, mostly in the tropical and sub-tropical areas of the world. It was by and large eradicated in the temperate area of the world during the 20th century with the advent of DDT and other organochlorine and organophosphate mosquito control insecticides. An elevated standard of living, including the use of air conditioners and window screens, along with public health interventions have largely remanded malaria transmission to tropical areas. Nonetheless, it can still be found in northernEurope.

More than one million deaths and 300 - 500 million cases are still reported annually in the world. It is reported that malaria kills one child every 40 seconds (Tolle 2009).

2.7.2 Chikungunya

Chikungunya virus is a pathogen transmitted by mosquitoes, and has established itself in the Caribbean (approximately 350,000 suspected cases in the Western Hemisphere since December 2013).

The mosquito species that transmit this disease are the Asian Tiger Mosquito (*Aedes albopictus*) and the Yellow Fever Mosquito (*Aedes aegypti*). Genetically, it appears

that viral strain currently spreading throughout the Americas is more easily transmitted by *Ae. aegypti*. Both species lay their eggs in containers such as cans, discarded tires and other items that hold water close to human habitation, but *Ae. aegypti* is more geographically confined to the southeastern United States. Traditional mosquito methods of truck-mounted and aerial sprays are ineffective in controlling these mosquitoes. Removal of water-bearing containers and sanitation are key preventive strategies (Tolle 2009).

2.7.3 Dengue

Dengue is a serious arboviral disease of the Americas, Asia and Africa. Although it has a low mortality, dengue has very uncomfortable symptoms and has become more serious, both in frequency and mortality, in recent years. *Aedes aegypti* and *Ae. albopictus* are the vectors of dengue. These mosquitoes prefer to lay their eggs in containers close to human habitations and are not well-controlled by standard spraying techniques. The spread of dengue throughout the world can be directly attributed to the proliferation and adaptation of these mosquitoes. Over the last 16 years dengue has become more common (Tolle, 2009).

2.7.4 West Nile Virus (WNV)

Yellow fever, which has a 400-year history, at present occurs only in tropical areas of Africa and the Americas. It has both an urban and jungle cycle. It is a rare illness of travelers anymore because most countries have regulations and requirements for yellow fever vaccination that must be met prior to entering the country. Every year about 200,000 cases occur with 30,000 deaths in 33 countries. It does not occur in Asia. Over the past decade it has become more prevalent. In 2002 one fatal yellow fever death occurred in the United States in an unvaccinated traveler returning from a fishing trip to the Amazon. In May 2003, 178 cases and 27 deaths caused by yellow fever were reported in southern Sudan. In the Americas 226 cases of jungle yellow fever have been reported with 99 deaths (Tolle, 2009)

2.7.4 Zika virus

Zika virus has emerged from its origins in central Africa and has rapidly spread to the South Pacific and western hemisphere. A Flavivirus related to West Nile, Yellow Fever, St Louis and the equine encephalitides, Zika was first discovered in macaque monkeys in 1947 in the Zika Forest region of Uganda. Since its discovery in 2014 off

the coast of South America, Zika cases have been found in 35 countries in the Americas. Although in rare cases Zika can be spread through sexual contact with an infected person, it is usually transmitted through the bit of an infected *Aedes agypti* or *Aedes albopictus* mosquito. The illness is usually quite mild, with fever, rash, conjunctivitis and joint pain lasting a few days to several weeks or months. Often patients are not sick enough to seek medical treatment so a great many cases are not reported. It is thought that one attack confers immunity. However, cases of microcephaly, a congenital defect of cranium and brain size resulting in profound neurological defects in newborns usually resulting in death have been been positively identified as being caused by Zika infection. An autoimmune condition called Guillain-Barré syndrome, causing damage to nerve cells resulting in muscle weakness and, on occasion, paralysis and death has been linked to Zika infection.

The mosquito vectors of Zika virus are peridomestic, preferring to lay their eggs above the waterline of containers, treeholes, creases in tarpaulins and other vessels that may contain water. *Aedes aegypti*, in particular, will lay eggs in a series of containers after feeding. Both *Aedes agypti* and *Aedes albopictus* will feed day or night when a potential host comes within their limited flight ranges. *Aedes agypti* has more of a tendency to enter and stay within houses if conditions are proper. This species is exceedingly skittish, often leaving its host prior to taking a full blood meal when the host moves. Both mosquitoes also seem to prefer feeding on the host's lower extremities (Tolle 2009).

2.8 Mosquito control

Mosquito control in the United States has evolved from reliance on insecticide application for control of adult mosquitoes (adulticide) to integrated pest management programs that include surveillance, source reduction, larvicide, and biological control, as well as public relations and education. The major principles of integrated mosquito management are available at a new Public Health Pest Control Manual internet website (University of Florida Entomology and Nematology Department). Adulticides still play a vital role when flooding causes extreme numbers of nuisance mosquitoes or when outbreaks of diseases such as SLE occur. Surveillance programs track diseases harbored by wild birds and sentinel chicken flocks; vector-borne pathogens in mosquitoes; adult and larval mosquitoes and larval habitats (by aerial photographs, topographic maps); mosquito traps; biting counts; and follow-up on complaints and

reports by the public. When established mosquito larval and adult threshold populations are exceeded, control activities are initiated. Seasonal records are kept in concurrence with weather data to predict seasonal mosquito larval occurrence and adult flights. Source reduction consists of elimination of larval habitats or rendering of such habitats unsuitable for larval development. Public education is an important component of source reduction. Many county or state mosquito control agencies have public school education programs that teach children what they and their families can do to prevent mosquito proliferation. Other forms of source reduction include open marsh water management, in which mosquito-producing areas on the marsh are connected by shallow ditches to deep water habitats to allow drainage or fish access; and rotational impoundment management, in which the marsh is minimally flooded during summer but is flap-gated to reintegrate impoundments to the estuary for the rest of the year. Biological control includes use of many predators (dragonfly nymphs and other indigenous aquatic invertebrate predators such as *Toxorhynchites* spp. predacious mosquitoes) that eat larvae and pupae; however, the most commonly used biological control adjuncts are mosquito fish, *Gambusia affinis* and *G. holbrooki*. Naturally occurring *Fundulus* spp. and possibly *Rivulus* spp., killifish, also play an important role in mosquito control in open marsh water management and rotational impoundment management. Like many fish, mosquito fish are indiscriminate feeders that may eat tadpoles, zooplankton, aquatic insects, and other fish eggs and fry Courtenay WR, Meffe GK (1989). However, since they are easily reared, they have become the most common supplemental biological control agent used in mosquito control. The entomopathogenic fungus, *Laginidiumgiganteum*, has been registered for mosquito control by EPA under the trade name Liginex, but products have not become readily available. The pathogenic protozoon, *Nosema algerae*, has also not become available for technical reasons. Entomoparasitic nematodes such as *Romanomermis culicivorax* and *R. iyengari* are effective and do not require EPA registration but are not easily produced and have storage viability limitations. A predacious copepod, *Mesocyclopslongisetus*, preys on mosquito larvae and is a candidate for local rearing with *Paramecium* spp. for food. Mosquito traps (such as the New Jersey and the Centers for Disease Control and Prevention designs) have been used for monitoring mosquito populations for years. New designs using mechanical control to capture adult mosquitoes have now become available. These designs use compressed carbon dioxide, burning propane, and octenol to attract

mosquitoes and fans to control air flow. The new technology is expensive: these traps may cost well over $1,000 each. Electric high-voltage insect traps (“bug zappers”) with “black” or ultraviolet light sources do not provide satisfactory adult mosquito control and kill insects indiscriminately (Rose 2001).

2.8.1 Pesticides

Pesticides used by state or local agencies to control nuisance or public health pests have warning labels and directions to minimize risks to human health and the environment. These pesticides are applied by public health employees who are specifically trained to follow proper safety precautions and directions for use. The environmental hazards precautionary statements on many mosquito insecticides labels state that insecticides are toxic to birds, fish, wildlife, aquatic invertebrates, and honeybees. Because of the low rates of application used to control mosquitoes and the special public health pest control training of most applicators, hazard to non targeted organisms is limited. However, honeybees may be killed if exposed when foraging, so proper precautions are warranted. Human exposure in residential areas is also uncommon because of the very low application rates, ultra low-volume methods (ULV), treatment at night when people are indoors, pesticide applicator training, and public prenotification before application. A pesticide residue is the pesticide or its metabolites in or on raw agricultural commodities or processed food and feed. A tolerance is the maximum limit of a pesticide residue considered safe. Tolerances are relevant to adult mosquito control because wind drift may carry the pesticide over agricultural crops where residues subject to legal tolerance requirements may occur. Crop tolerances are listed in the Code of Federal Regulations. Code of Federal Regulations (2000).

2.8.2 Larvicides

Detection of large numbers of immature mosquitoes in areas where source reduction or biological control is not feasible may require larvicide treatment to prevent the emergence of adult mosquitoes. Use of larvicides is less controversial than use of adulticides, although use of larvicides may lead to public concern about their effects on untargeted beneficial aquatic arthropods and vertebrates. Adulticides Effective sustainable integrated mosquito management programs strive to prevent large.

2.8.3 Adulticides

Effective sustainable integrated mosquito management programs strive to prevent large flights or swarms of mosquitoes through all the measures described above, but heavy precipitation, flooding, high tides, environmental constraints, inaccessible larval habitats, missed breeding sites, human disease outbreaks, as well as budget shortfalls, absent employees, or equipment failures, may necessitate use of adulticide. Some local mosquito control programs would use an integrated program if they had adequate resources, but may be so limited in funding and personnel that adulticiding trucks are the only means of mosquito intervention.

Effective adult mosquito control with insecticides requires small droplets that drift through areas where mosquitoes are flying. The droplets that impinge on mosquitoes provide the contact activity necessary to kill them. Large droplets that settle on the ground or vegetation without contacting mosquitoes waste material and may cause undesirable effects on non targeted organisms. To achieve small droplets, special aerial and ground application ULV equipment is used. Insecticides are applied in a concentrated form or technical grade and at very low volumes such as 1 oz (29.6 mL) per acre. Typically, aerial applications produce spray droplets of 30 to 50 microns measured as mass median diameter, with $\leq$2.5% of the droplets exceeding 100 microns. Ground ULV applicators produce droplets of 8 to 30 microns, with none >50 microns mass median diameter. Large droplets of malathion, naled, and fenthion in excess of 50 to 100 microns can damage automotive or similar paint finishes.

Adulticide applications, particularly aerial applications and thermal fogging, are quite visible and contribute to public apprehension. Ground ULV application may be less alarming than aerial application but is not effective over large or inaccessible areas. Preferable air currents for ground applications are 3.2 kph to 12.9 kph and not in excess of 16.1 kph. Excessive wind and updrafts reduce control, but light wind is necessary for drifting spray droplets. Adult mosquitoes are easily controlled with insecticides applied at extremely low rates. For example, malathion is applied at 3 fl oz per acre (219.8 mL/ha) for mosquitoes, while the rate for agriculture is as much as 16 fl oz per acre (1,172 mL/ha).

2.8.5 Insecticide Resistance

Vector resistance to certain larvicides and adulticides has occurred periodically. Failure of mosquito control indicating resistance must be verified by laboratory analysis or use of test kits because other factors (improper equipment calibration, dilution, timing and other application errors, off-specification products, climatic factors) can prevent insecticides from providing satisfactory control in the field. Resistance may occur between insecticides within a class or could be passed from immature to adult stages subject to the same insecticidal mode of action. Additionally, different species of mosquitoes may inherently vary in susceptibility to different larvicides and adulticides. Insecticides with different modes of action can be alternated to prevent resistance. Even though source reduction and use of predators such as larvivorous fish are also used for sustainable integrated mosquito management, only two chemical classes of adulticides (organophosphates and pyrethroids) with different modes of action are available. Biological controls (including birds and bats) may be present, but often not in sufficient numbers to provide satisfactory alternative control, particularly in coastal areas where salt-marsh mosquitoes are abundant or when human disease outbreaks occur. Therefore, sustained integrated mosquito management requires alternative use of different classes of insecticides, in conjunction with resistance monitoring, source reduction, biological control, and public education.

2.8.6 Repellents

Insect repellents, primarily N,N-diethylmetatoluamide (DEET), are used to prevent nuisance bites from mosquitoes (as well as ticks, biting flies, and mites) and may aid in lowering disease transmission from these pests. However, they should not be relied upon to prevent disease transmission, particularly where Lyme disease or encephalitis are endemic or malaria, yellow fever, or other vector-borne diseases are prevalent. Repellents, mosquito coils, and permethrin clothing treatment products are subject to EPA pesticide registration performance requirements U.S. Environmental Protection Agency(1999). Citronella and its oil for mosquitoes and 30 other active ingredients are exempted from EPA pesticide registration U.S. Environmental Protection Agency.(2000). However, some of these products may not be efficacious.

2.8.7 Biological control

Vector control strategies have traditionally focused on killing mosquitoes using a variety of insecticides. Environmental management (through reduction or removal of mosquito breeding sites) has often been used alongside chemical or microbiological ovicides, larvicides, and pupicides (Amer and Mehlhorn, 2006; Amer *et al.*,2006; Semmler *et al.*, 2009; Benelli, 2015) in areas where endemic mosquito-borne diseases occur. The use of synthetic insecticides has to be regulated given that the development of insecticide resistance is widespread (Liu, 2015; Ranson and Lissenden, 2016; Strode *et al.*, 2014; Hemingway and Ranson, 2000; Naqqash *et al.*, 2016) and that there is concern regarding the damage to the environment and effects on non-target organisms. The use of insecticides for mosquito control, including organophosphates, carbamates, and pyrethroids, can also have negative effects on human health. Personal protection against mosquito-borne diseases can involve the use of mosquito repellents such as *N*,*N*-diethyl-meta-toluamide (DEET), dimethyl phthalate (DMP), *N*,*N*-diethyl mendelic acid amide (DEM), as well as plant-borne molecules (Mehlorn, H. 2015), light-coloured clothes covering as much of the body as possible, and sleeping under mosquito nets. Insecticide-treated bed nets have played a very important role in the reduction of *Plasmodium falciparum* infection prevalence in malaria endemic Sub-Saharan Africa, which has seen the incidence of clinical disease fall by 40% between 2000 and 2015(Bhatt *et al.*, 2015). However, bed nets are only effective against mosquitoes that bite during the night and concern is growing that insecticide resistance, particularly due to the most commonly used class of pyrethroids, could reverse this trend and lead to rising incidence of malaria and increased fatalities (Hemingway *et al.*, 2016). As insecticide resistance is now widespread in a number of mosquito species (Ranson and Lissenden, 2015; Hemingway and Ranson 2000; Naqqash *et al.*, 2016), there is a growing need for novel, cheap, and reliable mosquito control strategies (Benelli,. 2015; Yakob and Walker, 2016; Jeffries and Walker, 2015). In many countries where mosquito-borne diseases are endemic, the financial burden of insecticide-based vector control programs is also prohibitive to widespread use. Environmentally friendly alternatives have been explored to help reduce the selection pressure for insecticide resistance. These various biocontrol strategies target different stages of the mosquito lifecycle with the aim of being safe for the environment and sustainable. These diverse

biocontrol strategies include natural organisms that kill mosquitoes, exploiting mosquito behaviour to improve mosquito mortality, and releasing mosquitoes that are either sterile or unable to transmit disease.

2.8.8 Plant-Borne Mosquitocides, Repellents, and Oviposition Deterrents

The discovery of the plant-based drug artemisinin for malaria treatment. Tu Y (2011) and the subsequent awarding of the Nobel prize in 2015Callaway E., Cyranoski (2017) highlights the importance of screening plants and fungi as sources of metabolites for parasitological and mosquitocidal properties. Notably, plant-borne molecules are often effective at a few parts per million (ppm) against *Aedes (Ae.)*, *Anopheles (An.)* and *Culex (Cx.)* young larval instars (Benelli and Pavela, 2015) for dedicated reviews on ovicides and larvicides, respectively. Currently, more than 80 plant species have been employed for the successful synthesis of nanomosquitocides, with particular reference to larvicidal purposes. On the other hand, studies on ovicidal and ovideterrentnano formulates are limited (Madhiyazhagan *et al*., 2015). Furthermore, botanicals can also be used as reducing and capping agents for the rapid synthesis of mosquitocidal nano formulations (Benelli, 2016), and can even be employed to prepare cheap repellents with low human toxicity (Semmler *et al*., 2015). Notably, much remains to be discovered about this fast-growing research area, with special reference to the following topics: (i) the chemical characterization and standardization of plant-borne botanicals used for nanobiosynthesis,(Benelli, 2015) (ii) the potential of plant-synthesized nanoparticles as mosquito ovicides and ovideterrents,(Subramaniam *et al.,* 2016) (iii) the utility of industrial by-products of plant origin for biofabrication of nanomosquitocides (e.g., neem cake) (Benelli, 2015), (iv) field evaluation of mosquitocidal properties of green nanoparticles against Culicidae,(Dinesh *et al*., 2015) (v) the non-target effects and environmental fate of plant-synthesized nanoparticles used against mosquito vectors (Benelli *et al*., 2016).

2.8.9 Mosquito predators

Natural enemies feeding on mosquito larvae and pupae in aquatic environments can play an important role in reducing Culicidae populations (Louca *et al.,* 2009; Kumar *et al.,* 2006; Griffin *et al*., 2012). Indeed, mosquito young instars are preyed upon by a large number of aquatic organisms including fish (Chandra *et al.,*2008;Kamareddine

2012; Subramaniam *et al.*, 2015; Subramaniam *et al.*, 2016); amphibians(Brodman *et al*., 2006; Bowatte *et al.*, 2013; Schaper, 1999; Vu *et al.*,1998), odonate young instars (Singh *et al*., 2003), water bugs (Bailey, 1989; Venkatesan *et al.*,2007), and even larvae of other mosquito species (Steffan *et al*., 1981) Focks (Sackett *et al*.,1985). Biological control of mosquitoes using vertebrates has mostly focused on the role of larvivorous fish that consume the aquatic larval stage of mosquitoes (Griffin *et al.*, 2012). Fish predation of mosquito larvae has been recorded in many habitats, from small plastic containers (Connor, 1922) to complex natural ecosystems, including coastal wetland environments (Harrington and Harrington,1982). Larvivorous fish have been demonstrated to be very effective at reducing mosquito larval populations in many parts of the world, and in a variety of habitats (Van Dam and Walton, 2007; Van Dam *et al*., 2008). In particular, larvivorous fish belonging to the genus *Gambusia* and *Poecilia* (Poeciliidae) have been introduced in more than 60 countries for mosquito control purposes (Chandra *et al*., 2008; Kamareddine, 2012; Das and Prasad, 1991; Walton, 2007; Ohba *et al.*, 2010; Chobu *et al.*, 2015; Kweka *et al.*, 2011). However, introduced larvivorous fish are often considered a threat to native aquatic fauna, including amphibians, highlighting the need to carefully consider the ecological cost of introducing predatory species intended to contribute to mosquito control.

From an integrated vector management perspective, it has been recently observed that the employment of ultra-low quantities of botanicals or green-synthesized nanomosquitocides boosts the predation rates of a range of mosquito larvae predators. This has been demonstrated for various species of copepods e.g., *M.edax (*Chandramohan *et al*., 2016); *M. thermocyclopoides (* Mahesh Kumar, 2012), *Megacyclopsformosanus*, *M. aspericornis*, tadpoles (e.g., *Hoplobatrachustigerinus*), fish (e.g., *Gambusia affinis*, *Poecilia reticulata*, *Carassius auratus*, *Aplocheiluslineolatus*), This opportunity should be explored further, since the exploitation of synergies between ultra-low doses of plant-fabricated mosquitocides and biological control agents may represent a further control option readily available in tropical and sub-tropical developing countries worldwide (Benelli, 2013; Benelli *et al.*, 2016).

2.8.10 Bti and entomopathogenic fungi

Naturally occurring organisms that are pathogenic to mosquitoes can also be considered for biocontrol strategies. *Bacillus thuringiensis* var. *israelensis* (Bti) is currently the most common mosquito larvicide employed in European countries. Bti is a gram-positive, spore-forming bacterium that releases insecticidal toxins and virulence factors that selectively target the larval stages of insects (Becker, 1997). Application of Bti has been used to reduce the number of *Ae. aegypti* and *Ae. albopictus* larvae, but longerterm use is subject to the development of resistance to Bti toxins (Georghiou *et al.,* 1997), and the use of Bti in large mosquito breeding sites in urban environments is logistically demanding (Gómez-Dantés *et al*., 2009). Entomopathogenic fungi produce infective spores (conidia) that attach to and penetrate the cuticle of mosquitoes, releasing toxins that result in mosquito death. Several studies have shown the pathogenic effect on malaria mosquito vectorsand on *Ae. aegypti*. As entomopathogenic fungi are mostly targeted towards adult mosquitoes, and because several different toxins produced during fungal infection are lethal to mosquitoes (Cholt *et al.,* 2007), selection pressure for resistance is likely to be less intense when compared to rapid-killing insecticides. Therefore, the evolution of fungus resistance is predicted to be much slower than the evolution of insecticide resistance (Knols *et al.,* 2010). The paucity of studies describing the effects of fungi on mosquito populations indicates further research is needed to determine the viability, infectivity, and persistence of fungal spores in mosquito field populations (Mnyone *et al*., 2010). Clearly to deliver large-scale application of fungal spores into wild mosquito populations, optimal methods need to be determined (Benelli *et al.,* 2016).

2.8.11 Biorational and organic pesticides

Pesticides vary in their toxicity to people and to non-target organisms, and in their potential ecological impact. Pest control materials that are relatively non-toxic to people with few environmental side-effects are called “biorational” pesticides in this Guide. Biorational pesticides mostly include the following categories further defined in this section: *biopesticide, organic pesticide, minimum-risk pesticide,* and *biological control*. Federal law governs pesticide registration through the EPA, and materials derived from living things are defined as “biopesticides”. Organic production is regulated through the USDA National Organic Program which defines what inputs are

allowed for pest management. “Organic” and “biopesticide” are partially overlapping categories, and each is defined by specific criteria that are unique. “Minimum risk pesticide” is another category that is defined by EPA; these are exempt from federal registration. “Biological control” describes living organisms that suppress pests. Some biological controls are naturally occurring, some are insects purchased by farmers for pest control, and some are microbes formulated for sale as biopesticides (Benelli *et al.*, 2016).

2.8.11.1 Types of biorational pesticides

2.8.11.1.1 Botanicals

Botanical are plant-derived materials such as pyrethrin, azadiractin, and extracts of plants such as Chenopodium ambrosioides and Swingleaglutinosa. Plant-derived oils such as neem oil, canola oil, and sesame oil are also included in this group. Botanicals are generally short-lived in the environment, as they are broken down rapidly in the presence of light and air. Products generally have low mammalian toxicity and a broad spectrum of activity. Many botanicals are considered minimum risk pesticides and are exempt from registration by EPA (Benelli, Jeffrier and Walker, 2016).

2.8.11.1.2 Microbial pesticides

Microbial pesticides are formulated from living microorganisms and/or their by-products. Microbial insecticides tend to be selective, so specific pests may be controlled with little or no effect on non-target organisms, while most microbial disease control products have a wider spectrum of activity. Microbial insecticides may be derived from bacteria (e.g. *Bacillus thuringiensis*, spinetoram and spinosad, *Chromobacteriumsubtsugae*), virus (e.g. *nuclear polyhedrosis* virus of corn earworm) or fungi (e.g. *Beauvariabassiana*). Microbial disease control products are living organisms, including beneficial fungi and bacteria. Examples of microbial disease control organisms are the fungus *Trichoderma harzianum* and the bacterium Bacillus subtilis. While these active ingredients are generally approved for organic production (OMRI listed) because of their natural origin, certain formulated products are prohibited because the inert ingredients or procedures used in making the product are prohibited.

2.8.11.1.3 Minerals

Some pesticides made from minerals, mined from the earth and minimally processed, are allowed in organic production. Kaolin clay, copper hydroxide, and iron phosphate are examples.

2.8.11.1.4 Synthetics

Minerals and other natural materials that are heated, chemically reacted, or mixed with surfactants may be considered synthetics. Synthetics also include insect growth regulators (IGR), which interrupt or inhibit the life cycle of a pest. They may also work by strengthening plant defenses. National organic standards include some allowed synthetics.

2.8.11.1.5 Biopesticides

Biopesticides, as defined by EPA, are certain types of pesticides derived from such natural materials as animals, plants, bacteria, and certain minerals. As of April 2016, there are 299 registered biopesticide active ingredients and 1401 active biopesticide product registrations. EPA generally requires less data to register a biopesticide than to register a conventional pesticide, thus the registration process is faster. Categories of biopesticides include:

- Microbial pesticides, in which a microorganism (e.g., a bacterium, fungus, virus or protozoan) is the active ingredient
- Plant-Incorporated-Protectants (PIPs), in which pesticidal substances are produced by crop plants as a result of genetic material being added to the plant (e.g., *Bt* insecticidal protein)
- Biochemical pesticides, which are naturally occurring substances that control pests by non-toxic mechanisms, such as sex pheromones that interfere with mating and scented plant extracts that attract insect pests to traps. With plant-incorporated protectants, the toxin and its genetic material, but not the plant itself, are regulated by EPA.

Biopesticides generally fit well into an integrated pest management (IPM) strategy, which relies on monitoring for early detection of pests and emphasizes the use of selective products that protect crops while minimizing negative effects on water, air and soil, and on pollinators and beneficial insects. The purpose of this section is to

bring these types of products together to help growers make decisions about pesticides and biological controls to use on their farm (Benelli *et al.*, 2016).

Vector control remains the only available intervention to prevent and control the transmission of dengue (WHO, 2009).Various vector control strategies aiming at controlling the principal vector of dengue, *Aedes aegypti*, are currently used with the intention of preventing the occurrence of dengue, or controlling outbreaks. These vector control measures often include the application of chemical or biological agents for the control of immature and adult mosquito stages, or environmental control methods that target mosquito breeding sites (WHO, 1982). These vector control measures can be applied as single interventions or in combination (WHO, 2004). However, the efficacy and community-effectiveness of vector control strategies in terms of reductions in dengue transmission remain unclear, as previous systematic reviews have reported regarding the application of single intervention methods such as peridomestic space spraying and the use of *Bacillus thuringiensis israelensis* (Esu *et al.*, 2013).

One of the most commonly employed methods for dengue vector control is the use of the organophosphorous compound temephos (commercial name Abate) as a larvicide. Its use has been documented since 1965. Sztankay-Gulyás (1972) in ponds, marshes and swamps at a dosage of 0.1–0.5 kg/ha for vector control in general, although fewer studies exist in relation to *Ae. aegypti*. Per the WHO Pesticides Evaluation Scheme, temephos can be used safely in potable water when the dosage does not exceed 56–112 g/ha (5.6–11,2 mg/m^2) or 1 mg/l (WHO, 2009) . Moreover, the WHO hazard classification of temephos is "U", meaning it is unlikely to cause acute hazard under conditions of normal usage (WHO, 2008). Temephos is a widely preferred tool for several reasons, including its ease and simplicity of application, selective killing of mosquito larvae and its long lasting effect when compared to traditional oil application methods (Sztankay-Gulyás, 1972). Temephos is commercially available in standardised preparations such as emulsifiable concentrates, dilute solutions, dusts and granules, including slow release formulations. It can be applied in different ways depending on the site and rate of application required. It can be delivered by hand or by injection through drip system devices or power sprayers. Temephos sand granules can be applied to household water storage containers of varying capacity by using a

calibrated plastic spoon in order to administer a consistent dosage of 1ppm(1 ppm = 10^{-6} = 1 parts per million = 0,0001%) (SEARO WHO, 2011).

Temephos has widely been considered a cornerstone for controlling immature forms of *Ae. aegypti* yet while its efficacy has been demonstrated under laboratory conditions, comparable levels of efficacy are not necessarily replicated under field condition (Pinheiro and Tadei, 2002).

Spinosad is highly active in numerous insect species in agriculture, veterinary and public health importance. Spinosad shows variable efficacy among the species and stages and acts both by contact and ingestion. Spinosad was recommended as mosquito larvicide by the United States Environmental Protection Agency and World Health Organization's Pesticide Evaluation Scheme (WHOPES) during 2007 following which 120 suspension concentrate (SC) formulations were registered in Morocco followed by many countries namely, Turkey, Tunisia and Spain with more countries to follow (Hertlein *et al,*. 2010).

Spinosad is a natural pesticide with bacterial origin. It was first isolated from the soil from *Saccharopolyspora spinosa* (Actinomycetales) from an abandoned rum distillery in 1982(Mertz and Yao, 1990). Spinosad contains a mix of two complex organic compounds, spinosyn A, the major component and spinosyn D, the minor component, roughly in 85:15 ratio(Hertlein *et al,*. 2010). It is a white crystalline solid with a unique tetracyclic ring system attached to an amino sugar (D-forosamine) and a neutral sugar (tri-O-methyl-L-rhamnose). Spinosyns are non-volatile, have low water solubility, resistant to hydrolysis up to *p*H 5 that increases slowly beyond this *p*H and show rapid aqueous photolysis at *p*H 7.0 and have a half-life of less than one day (WHO, 2007). These characteristics make it ideal for usage as larvicide.

As per WHO, spinosad as a mosquito larvicide does not pose any threat to the health of users and to the environment. As per WHO Hazard Classification, spinosad is classified as class III compound as slightly hazardous with oral and dermal toxicity (LD_{50} for rat of over 2000 mg/kg body weight) (WHO, 2016).

Cyfluthrin

Cyfluthrin is a synthetic pyrethroid insecticide which is effective against a wide variety of agricultural and public-health pests (WHO, 2003). Its mode of action is characterized by interference with nerve signalling by inhibition of the membrane

sodium channel systems in the target organism. Cyfluthrin is mainly a contact insecticide classified as moderately hazardous (WHO, 2006). It has a very high-knockdown and low excito-repellent effect (Najera and Zaim, 2001). It is also known by the name baythroid (WHO, 2003). The WHO recommends a dosage of 0.02–0.05 $g/m^{2,}$ giving a residual effect lasting three up to 6 months (WHO, 2007). The Indonesian MoH does not recommend this insecticide for IRS as part of its insecticide rotation cycle policy. Several studies have explored the application of cyfluthrin against malaria vectors in Indonesia.

Lambda-cyhalothrin

Lambda-cyhalothrin is a pyrethroid insecticide. Pyrethroids are synthetic chemical analogues of pyrethrins, which are naturally occurring insecticidal compounds produced in the flowers of chrysanthemums (*Chrysanthemum cinerariaefolium*). Insecticidal products containing pyrethroids have been widely used to control insect pests in agriculture, public health, and homes and gardens (Amweg and Weston 2005; Oros and Werner 2005). In agriculture, target crops include cotton, cereals, hops, ornamentals, potatoes, and vegetables, with applications made to control aphid, coleopterous, and lepidopterous pests. Pyrethroids are important tools used in public health management where applications are made to control cockroaches, mosquitoes, ticks, and flies, which may act as disease vectors. Residential use of pyrethroid products has increased because of the suspension of organophosphate products containing chlorpyrifos or diazinon (Oros and Werner, 2005; Weston *et al.,* 2005).

Emamectin-benzoate

Emamectin-benzoate is the 4'-deoxy-4'-epi-methyl-amino benzoate salt of avermectin B1 (abamectin), which is similar structurally to natural fermentation products of Streptomyces avermitilis. Emamectin-benzoate is being developed as a newer broad-spectrum insecticide for vegetables and has a very low application rate. The mechanism of action involves stimulation of high-affinity GABA receptors and a consequent increase in membrane chloride ion permeability. Animal studies indicate a wide margin of safety because mammalian species are much less sensitive due to lower GABA receptor affinities and relative impermeability of the blood-brain barrier. Notably, the literature has not reported human exposure resulting in toxicity (Tzung-Hai Yen and Ja-Liang Lin, 2004).

Buprofezin

Buprofezin, (2-tert-butylimino-5-phenyl-3-propan-2-yl-1,3,5-thiadiazinan-4-one) is a thiadiazine insect regulator, molting inhibitor, and it acts specifically on immature developmental stages of homopteran pests by inhibiting the incorporation of N-acetyl-[D-H3] glucosamine into chitin and interfering with cuticle formation resulting in nymphal mortality during molting (Ishaaya and Horowitz 1998). Hatakoshi M (1992) report thatit also exhibits larvicidal activity against the brown rice planthopper, *Nilaparvata lugens* and the greenhouse whitefly, *Trialeurodes vaporariorum*. Buprofezin exhibits low acute toxicity by oral, dermal or inhalation routes in rat. The oral LD_{50} in rats is reported to be 3847 and 2278 mg/kg body weight (bw) in males and females respectively. Nevertheless, the acute toxicity of buprofezin in aquatic organisms is lacking.

Neem extract

An insecticide containing azadirachtin, a neem tree (Azadirachta indica) extract, was tested against mosquito larvae in the Islamic Republic of Iran under laboratory and field conditions. LC_{50} and LC_{90} values for Neemarin were 0.35 and 1.81 mg/L for Anopheles stephensi, the main local malaria vector, and 0.69 and 3.18 mg/L for Culex quinquefasciatus. The mortality in the pupal stage was significantly higher than the other stages. In field trials, using recommended dosages of 1 and 2 L/hectare, mortality of Anopheles spp. larvae was also higher than Culex spp. Prevention of adult emerged and pupal mortality was the main activity of this compounds. The maximum time of efficacy was 7 days at the highest concentration (2 L/hectare) (Vatandoost and Vaziri, 2004).

CHAPTER III

MATERIALSAND METHODS

3.1 Location of the Experiment

The experiment was conducted at Dr. Wazed Mia central laboratory of Sher-e-Bangla Agricultural University.

3.2 Climate of the experiment area

The experiment was conducted in room temperature at 3rd floor at Dr. Wazed Mia central laboratory of Sher-e-Bangla Agricultural University.

3.3 Selection of insecticides

Nine chemicals namely Spinosad 45 SC, temephos 25 EC, Cyfluthrin2.45 SC, Lambdacyhalothrin 2.5 EC, Pyrazine, Buprofezin 40 SC, Emabectin Benzoate 5 SG, neem seed kernel, neem leaf extract were used against the mosquito larvae of different stage. The technical information of these chemical given in table number 1.

Table 1 Chemical with mode of action and dose used against mosquito larvae

Neem leaf extract	Neem seed kernel extract	Temephos	Buprofezin	Emabectin-benzoate	Pyrazine	Lambda-cyhalothrin	Cyfluthrin	Spinosad	Common name
		Abate 50 EC	Tacoma 40SC Sunfezin	Proclaim 5 SG Arostar 5		Karate 2.5 EC Fighter 0.5 EC	Responcer 2.45 SC	Success,tracer 45 SC	Trade name
azadirachtin	azadirachtin	Aquabac 2.86%	Buprofezin	Emabectin benzoate	pyrazinophthalocyanines	Lambdacyhalothrin	Cyfluthrin	Spinosyn a Spinosyn d	Active ingredie
Bio pesticides	Bio pesticides	Organophosphate	Thiadiazine	Abamectin		pyrethroid	Pyrethroid	Spinosin	Group
stomach	stomach	Contact and stomach poison	Systemic contact	contact	Contact & stomach	Contact & stomach	stomach	Contact & stomach	Mode of action
100ml/lit 136ml/lit 200ml/lit 333ml/lit	0.3125gm/litre 0.625gm/lit 1.25gm/lit 2.5gm/lit	01 µlitre/1liter water 02 µlitre/1liter water 05µlitre/1liter water 10µlitre/1liter water	0.005 ml/liter 0.01 ml/liter 0.02 ml/liter 0.04 ml/liter	0.025 ml/liter 0.040 ml/liter 0.080 ml/liter 0.16 ml/liter	0.10g/400ml water 0.2g/400 ml water 0.4g/400ml water 0.8g/ml water	1 µlitre/1liter water 02 µlitre/1liter water 05 µlitre/1liter water 10 µlitre/1liter water	01 µlitre/1liter water 02 µlitre/1liter water 05 µlitre/1liter water 10µlitre/1liter water	01 µlitre/1liter water 02 µlitre/1liter water 05µlitre/1liter water 10µlitre/1liter water	Doses

3.4 Larvae Culture

We needed different materials to culture larvae, these are:

Adult mosquito, a hen, a bowl with water, a cage to keep the chicken, a net, food for the hen, and food for the mosquito. Following procedure were followed to culture mosquito larvae in laboratory. They are:

3.4.1 Rearing of larvae

First of all adult mosquitoes were collected from different location of SAU by net and taken to the laboratory. Then a hen was kept in the case and cover with mosquito net with a bowl of water inside the net after that some adult mosquito which we collected by net was released into the net .By feeding hen blood the mosquito laid eggs and larvae come out within 7-14 days. Then the larvae were collected from the bowl by a dropper and process continued.

3.5 Bioassay of the selected larvicides

To evaluate the efficacy of nine pesticides against mosquito larvae experiment was done in the central laboratory of SAU to treat the larvae .Solution was made by mixing with water at different doses. Exact amount of pesticides was mixed with 1liter water to make the solution. Pesticides were measure with micro pipette, weigh machine, syringe, and pipette to take accurate amount of pesticides. Then the solution was mix with water thoroughly. Yeast and blood meal was added to the mixture for the food of the larvae. Four replications were made for every treatment.

3.6 Method of the preparation of neem extract

3.6.1.1 Seed collection and preparation

Neem seed collection and handling was carried out according to the method described by Vyas and Mistry (1996). Fully matured fruits were plucked from Neem trees. The collected fruits were washed thoroughly under a running tap and thereafter soaked in a container for 3-days to remove the outer skin of the seed. The seeds were later air-dried under shade for 3 days. The coats covering the seeds were removed by a decortication process by carefully exerting pressure on the seeds using local mortar and pestle to separate the kernel from seeds. The mixture was winnowed and sieved to obtain pure reddish-brown seed kernels. The seed kernel was pulverized using an electric blender.

3.6.1.2 Extraction of neem seed oil

The ground Neem seed kernel was subjected to oil extraction with about 800 ml Analar grade hexane in a Soxhlet apparatus following the method described by Vyas and Mistry (1996). Hexane and ethanol were the only solvents used in the extraction of neem oil because they have been proven to be effective in the extraction of the active ingredient Azadirachtin (Govindarajan *et al.*, 2016). This is followed by ethanol and other solvents such as methanol, water, methyl ethyl ketone but the later solvents have been reported to enhance the degradation of azadirachtin content (Koul, 1996). A rotary vacuum evaporator was used to remove the solvent from the extract.

3.6.1.3 Emulsified neem oil formulation (stock solution)

This was prepared by mixing 2 drops of Tween 80 to 1ml of Neem oil mixed with 10 ml of distilled water in a sample bottle. The solution was shaken vigorously to ensure thorough dissolution of oil in water. This was then made up to 1 L with the addition of more distilled water to obtain a 1000 ppm stock solution.

3.6.2 Preparation of neem leaf extract

Neem leaves were collected from neem tress of SAU. Then the leaves were throuhly washed and dried. The completely dried leaves were coarsely powdered and 50 g was used for successive extraction in 250 ml methanol for three days with periodic shaking. Then, the extract was filtrated and the filtrate was collected. The filtered liquid extracts were subjected to rotary evaporation and subsequently concentrated under reduced pressure.

3.7 Design of the experiment

The experiment was conducted at CRD method

3.8 Data collection and calculation

3.8.1 Mortality calculation

Percent mortality was calculated from the data collected.

$$\text{Percentage of mortality} = \frac{\text{Number of dead larvae}}{\text{Number of larvae introduced}} \times 100$$

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

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

3.8.2 LC_{50} Calculation

The LC_{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 Statistix 10 program for CRD and means separation was done by Dancan's Multiple Range Test (DMRT).

Figure 6 Set up of mosquito larvae cultivation by putting a chicken inside a mosquito net with bowl of water.

CHAPTER IV

RESULT AND DISCUSSION

4.1 Toxicity of chemicals Against Mosquito Larvae at Different Time Interval

The larvae mortality data was subjected to probit analysis for calculating LC_{50} at 95% fiducial limits of upper confidence limit and lower limit and CRD values were calculated using stastistrix 10 software. Result with $p < 0.05$ considered to be statistically significant.

4.1.1 Toxicity of Cyfluthrin

Toxicity of cyfluthrin against mosquito larvae at four different doses is shown in Table 2. The result indicates that no significant variation was observed among the different doses of cyfulthin up to 12 hours after treatment application. It was also observed that 10.0 ppm dose of cyfluthrin significantly gave the lowest LC_{50} value (median lethal concentration, 1.1067) after 48 hours with the range of 1221.1 -1.1067 from 6 hours to 48 hours interval having LSD value 7786.7-0.4580 at p=0.05. Next dose 5.0 ppm gave median lethal concentration LC_{50} of 1.3607 after 48 hours with the range of 1305.3 - 1.3607 from 6 to 48 hours interval. Then dose 2.0 ppm gave median lethal concentration LC_{50} of 1.4953 from range 6126.6 to 1.4953 and 1.0 ppm gave median lethal concentration LC_{50} of 1.6310 having range 1551.7 -1.6310. Bansal and Singh (2006) find 0.0087, 0.0005 and 0.0004 mg L^{-1} for cyfluthrin against three species of mosquito larvae. LC_{50} and LC_{90} for *Ae. aegypti* fourth instar larvae were 0.002 ppm and 0.007 ppm. The LC_{50} and LC_{90} for *Ae. albopictus* fourth instar larvae were 0.004 ppm and 0.012 ppm, respectively. The order of toxicity of different doses of cyfluthrin was 10.0 ppm > 5.0 ppm > 2.0 ppm > 1.0 ppm. From table 2 we can say that concentration of 10 ppm of cyfluthrin gave the most effective result after 48 hours interval.

Table 2 LC_{50} value of cyfluthrin against mosquito larvae after different time intervals of data collection.

Dose	**LC_{50} value of cyfluthrin at different dosages after different time interval of data collection**				
	6 hours	**12 hours**	**18 hours**	**24 hours**	**48 hours**
1 ppm	1551.7a	1433.1a	16.533a	3.4490a	1.6310a
2 ppm	6126.6a	1411.6a	14.566ab	2.5220b	1.4953ab
5 ppm	1305.3a	1132.3a	13.673ab	1.5577c	1.3607ab
10 ppm	1221.1a	1107.2a	12.243b	1.3347c	1.1067b
LSD	**7786.7**	**403.38**	**2.8617**	**0.7878**	**0.4580**

In a column, means having similar letter(s) are statistically identical and those having different letter(s) are significantly different by DMRT at 5% level of probability.

4.1.2 Toxicity of Buprofezin

Toxicity of buprofezin against mosquito larvae at four different doses is shown in Table 3. The result indicates that no significant variation was observed among the different doses of buprofezin upto 18 hours after treatment application. It was also observed that 40 ppm does of buprofezin gave the lowest LC_{50} value (median lethal concentration 2.2233) after 48 hours with the range of 90.372 to 2.2233 from 6 hours to 48 hours interval having LSD value 142.70 to 1.7650 at p=0.05. Next dose 20ppm gave median lethal concentration LC_{50} 2.5947 after 48 hours in the range of 95.726-2.947 from 6 to 48 hours interval. Then dose 10 ppm gave the median lethal concentration 2.6147 from range 103.85-2.6147 and 5 ppm gave median lethal concentration of 3.742 having range 105.98-3.742. Jahan *et al.* (2011) revealed that laboratory bioassays with buprofezin gave the LC_{50} value range from 0.34 to 2.54 at 48 h post exposure. First 12 hours there was no death of larvae indicating no toxicity of Buprofezin. The order of toxicity of different doses of buprofezin was 40.0 ppm > 20.0 ppm > 10.0 ppm > 5.0 ppm. From table 3 we can say that concentration of 40 ppm of buprofezin gave the most effective result after 48 hours interval.

Table 3 LC_{50} Value after different time interval of data collection for buprofezin

Dose	**LC_{50} value of buprofezin at different dosages after different time interval of data collection**				
	6 hours	12 hours	18 hours	24 hours	48 hours
5 ppm	0	0	105.98a	5.3110a	3.7420a
10 ppm	0	0	103.85a	4.4907ab	2.6147a
20 ppm	0	0	95.726a	3.1317bc	2.5947a
40 ppm	0	0	90.372a	2.2153c	2.2233a
LSD	0	0	142.70	2.0885	1.7650

In a column, means having similar letter(s) are statistically identical and those having different letter(s) are significantly different by DMRT at 5% level of probability

4.1.3 Toxicity of Pyrazine

Toxicity of pyrazine against mosquito larvae at four different doses is shown in Table 4. The result indicates that significant variation was observed among the different doses of pyrazine at 6 hours after treatment application. It was also observed that 160 ppm does of pyrazine gave the lowest LC_{50} value (median lethal concentration 6.070) after 48 hours with the range of 330.51- 6.07 from 6 hours to 48 hours interval having LSD value 5.9380 to 5.1024 at p=0.05. Next dose 80 ppm gave median lethal concentration of LC_{50} 8.1553 after 48 hours in the range 332.25 to 8.1553 from 6 to 48 hours interval. Then dose 40 ppm gave median lethal concentration of 9.6840 from range 342.34 to 9.6840 and 25 ppm gave median lethal concentration of LC_{50} 11.419 having range 353.99 to 11.419. The order of toxicity of different doses of pyrazine was 160.0 ppm > 80.0 ppm > 40.0 ppm > 25.0 ppm. From table 4 we can say that concentration of 160 ppm of pyrazine gave the most effective result after 48 hours interval.

Table 4 LC_{50} value after different time interval of data collections for pyrazine.

Dose	**LC_{50} value of pyrazine at different dosages after different time interval of data collection**				
	6 hours	12 hours	18 hours	24 hours	48 hours
25 ppm	353.99a	133.61a	39.013a	24.463a	11.419a
40 ppm	342.34b	125.68 a	33.997ab	21.973ab	9.6840ab
80 ppm	332.25 c	120.65a	33.373ab	18.680ab	8.1553ab
160 ppm	330.51c	115.77a	29.043b	16.043b	6.0700b
LSD(P=0.05)	5.9380	2.306	8.1198	7.1413	5.1024

In a column, means having similar letter(s) are statistically identical and those having different letter(s) are significantly different by DMRT at 5% level of probability.

4.1.4 Toxicity of Emamectin-benzoate

Toxicity of emamectin-benzoate against mosquito larvae at four different doses is shown in Table 5. The result indicates that no significant variation was observed among the different doses of emamectin-benzoate upto 48 hours after treatment application . It was also observed that 160 ppm does of emamectin-benzoate gave the lowest LC_{50} value(median lethal concentration 7.5823) after 48 hours with the range of 135.65 to 7.5823 from 6 hours to 48 hours interval having LSD value 26.557 to 5.8132 at p=0.05.Next dose 80ppm gave median lethal concentration LC_{50} 8.56 after 48 hours in the range of 139.37-8.56 from 6 to 48 hours interval. Then dose 40ppm gave median lethal concentration of LC_{50} 11.081 from range 143.96-11.081 and 25 ppm gave median lethal concentration of LC_{50} 11.740 having range 148.85 to 11.740. The order of toxicity of different doses of emamectin-benzoate was 160.0 ppm >80.0 ppm >40.0 ppm >25.0 ppm. From table 5 we can say that concentration of 160 ppm of emamectin-benzoate gave the most effective result after 48 hours interval.

Table 5 LC_{50} value after different time interval of data collections for emamectin-benzoate.

Dose	**LC_{50} value of emamectin-benzoate at different dosages after different time interval of data collection**				
	6 hours	12 hours	18 hours	24 hours	48 hours
25 ppm	148.85a	81.387a	37.905a	15.424a	11.740a
40 ppm	143.96a	80.720a	34.572a	12.291a	11.081a
80 ppm	139.37a	78.000a	33.178 a	10.507a	8.5597a
160 ppm	135.65a	74.653a	27.814 a	8.3217 a	7.5823a
LSD	26.557	7.1989	15.121	3.7167a	5.8132

In a column, means having similar letter(s) are statistically identical and those having different letter(s) are significantly different by DMRT at 5% level of probability

4.1.5 Toxicity of Spinosad

Toxicity of spinosad against mosquito larvae at four different doses is shown in Table 6. The result indicates that no significant variation was observed among the different doses of spinosad upto 6 hours after treatment application. It was also observed that 10 ppm does of spinosad gave the lowest LC_{50} value (median lethal concentration 0.00433) after 48 hours with the range of 0.6287 - 0.00433 from 6 hours to 48 hours interval having LSD value 1.7218 to 0.0127 at p=0.05. Next dose 5 ppm gave median lethal concentration of LC_{50} 0.00517 after 48 hours. Then dose 2 ppm gave median lethal concentration of LC_{50} 0.00523 and 1 ppm gave median lethal concentration of LC_{50} 0.00550. Similar result was shown by (Bond, Maria, and Williams, 2004) by laboratory bioassays of a suspension concentrate formulation of Spinosad (Tracer®), the 24h lethal concentration (LC_{50}) against *Aedes aegypti* (L.) third and fourth instars was estimated at 0.025 ppm. Spinosad proved to be highly toxic to larvae of both species of mosquitoes in laboratory bioassays . Spinosad is known to be highly active against Diptera and is registered for control of leafmining dipteran pests of crops in many countries. Due to the favourable United States EPA classification, Spinosad is also used in a bait formulation over very large areas for control of the Mediterranean fruit fly, *Ceratitis capitata*, in Central America (Vargas, Peck, McQuate, Jackson, Stark, and Armstrong, 2001).The order of toxicity of different doses of spinosad was 10.0 ppm >5.0 ppm >3.0 ppm >2.0 ppm>1 ppm. From table 6 we can say that concentration of 10 ppm of spinosad gave the most effective result after 48 hours interval.

Table 6 LC_{50}value after different time interval of data collections for spinosad.

Dose	**LC_{50} value of spinosad at different dosages after different time interval of data collection**				
	6 hours	12 hours	18 hours	24 hours	48hours
1 ppm	1.4537a	0.5540a	0.0917a	0.0240	0.00550a
2 ppm	1.3290a	0.4540b	0.0823a	0.0197	0.00523a
5 ppm	0.8890a	0.3927bc	0.0737a	0.0160	0.00517a
10 ppm	0.6287a	0.3391c	0.0350a	0.0127	0.00433a
LSD(P=0.05)	1.7218	0.0730	0.0631	0.0411	0.0127

In a column, means having similar letter(s) are statistically identical and those having different letter(s) are significantly different by DMRT at 5% level of probability

4.1.6 Toxicity of Lambda-cyhalothrin

Toxicity of Lambdacyhalothrin against mosquito larvae at four different doses is shown in Table 7. The result indicates that no significant variation was observed among the different doses of lambdacyhalothrin upto 12 hours after treatment application. Then dose 2 ppm gave median lethal concentration of LC_{50} 0.00127 from range 0.1657 to 0.00127 .1 ppm gave median lethal concentration of LC_{50} 0.00320 having range 0.2080-0.00320.lambdacyhalothrin and 0.0087, 0.0005 and 0.0004 mg /l Vargas, *et al*.,(2001),(Samal and Kumar, 2018) lambdacyhalothrin LC_{50} 0.0009 recorded. The order of toxicity of different doses of lambdacyhalothrin was 10.0 ppm >5.0 ppm >2.0 ppm>1 ppm. From table 7 we can say that concentration of 10 ppm of lambdacyhalothrin gave the most effective result after 48 hours interval.It was also observed that 10 ppm does of lambdacyhalothrin gave the lowest LC_{50} value (median lethal concentration 0.000848) after 48 hours with the range of 0.1150-0.000848 from 6 hours to 48 hours interval having LSD value 0.1603-0.002054 at p=0.05.Next dose 5ppm gave median lethal concentration of LC_{50} 0.00118 after 48 hours in the range of 0.1217-0.00118 from 6 to 48 hours interval.

Table 7 LC_{50} value after different time interval of data collections for lambda-cyhalothrin.

Dose	**LC_{50} value of lambda-cyhalothrin at different dosages after different time interval of data collection**				
	6 hours	12 hours	18 hours	24 hours	48 hours
1 ppm	0.2080a	0.0420a	0.00320a	0.00320a	0.00320a
2ppm	0.1657a	0.0347a	0.00247ab	0.00127a	0.00127a
5ppm	0.1217a	0.0223a	0.00170ab	0.00118a	0.00118a
10ppm	0.1150a	0.007a	0.00117b	0.000848a	0.000848a
LSD	0.1603	0.0376	0.001534	0.002054	0.002054

In a column, means having similar letter(s) are statistically identical and those having different letter(s) are significantly different by DMRT at 5% level of probability.

4.1.7 Toxicity of Temephos

Toxicity of temephos against mosquito larvae at four different doses is shown in Table8. The result indicates that no significant variation was observed among the different doses of temephos upto 12 hours after treatment application. It was also observed that 10 ppm does of temephos gave the lowest LC_{50} value (median lethal concentration 0.00114) after 48 hours with the range of 0.0489 - 0.00114 from 6 hours to 48 hours interval having LSD value 0.0839-0.0003607 at p=0.05. Next dose 5ppm gave median lethal concentration of LC_{50} 0.00125 after 48 hours in the range of 0.0538 to 0.00125 from 6 to 48 hours interval. Then dose 2 ppm gave median lethal concentration of LC_{50} 0.00134 from range 0.0606 to 0.00134 and 1 ppm gave median lethal concentration of LC_{50} 0.00143 having range 0.0650 to 0.00143 from 6 to 48 hours interval .Similar result was shown by Mohammad, R. A.(2016) as LC_{50}= 0.0523 and LC_{90}=0.3822 and LC_{50} and LC_{90} were calculated as 0.1838 and 0.8505 ppm. The order of toxicity of different doses of temephos was 10.0 ppm >5.0 ppm >3.0 ppm >2.0 ppm>1 ppm. From table 8 we can say that concentration of 10 ppm of temephos gave the most effective result after 48 hours interval.

Table 8 LC_{50} value after different time interval of data collections for temephos.

Dose	**LC_{50} value of temephos at different dosages after different time interval of data collection**				
	6 hours	12 hours	18 hours	24 hours	48 hours
1 ppm	0.0650 a	0.0554 a	0.00373a	0.00143a	0.00143a
2 ppm	0.0606a	0.0506 a	0.00343a	0.00134a	0.00134a
5 ppm	0.0538a	0.0454a	0.00232b	0.00125a	0.00125a
10 ppm	0.0489a	0.0358a	0.00155c	0.00114a	0.00114a
LSD	0.0839	0.0615	0.0006558	0.0003607	0.0003607

In a column, means having similar letter(s) are statistically identical and those having different letter(s) are significantly different by DMRT at 5% level of probability.

4.1.8 Toxicity of neem seed kernel extract

Toxicity of neem seed kernel extract against mosquito larvae at four different doses is shown in Table9. The result indicates that no significant variation was observed among the different doses of neem seed kernel extract upto 6 hours after treatment application . It was also observed that 10 ppm does of neem seed kernel extract gave the lowest LC_{50} value (median lethal concentration 02.3360) after 48 hours with the range of 22.120-02.3360 from 6 hours to 48 hours interval having LSD value 8.076-01.3990 at p=0.05. Next dose 5ppm gave median lethal concentration of LC_{50} 3.0017 after 48 hours in the range of 22.372 to 3.0017 from 6 to 48 hours interval. Then dose 2 ppm gave median lethal concentration of LC_{50} 3.8900 from range 23.036 to 3.8900 and 1 ppm gave median lethal concentration of LC_{50} 4.8583 having range 27.859 to4.8583. Similar result also found by Virender,k.(2009) as median lethal concentration (LC_{50}) of the formulation against *Anopheles stephensi*, *Culex quinquefasciatus* and *Aedes aegypti* was found to be 1.6, 1.8 and 1.7 ppm respectively. LC_{50} values of the formulation stored at 26°C, 40°C and 45°C for 48 hours against *Ae. aegypti* were 1.7, 1.7, 1.8 ppm.The order of toxicity of different doses of neem seed kernel was 10.0 ppm >5.0 ppm >3.0 ppm >2.0 ppm>1 ppm. From

table 9 we can say that concentration of 10 ppm of neem seed kernel gave the most effective result after 48 hours interval.

Table 9 LC_{50} value after different time interval of data collections for neem seed kernel extract

Dose	**LC_{50} value of neem seed kernel extract at different dosages after different time interval of data collection**				
	6 hours	12 hours	18 hours	24 hours	48 hours
1	27.859a	14.069a	6.0940a	5.3703a	4.8583a
2	23.036a	13.586a	4.5703b	4.3120ab	3.8900ab
5	22.372a	12.516ab	3.8833b	3.3320bc	3.0017bc
10	22.120a	11.103b	2.7687c	2.2870c	2.3360c
LSD	8.076	1.9650	1.0679	1.9156	1.3990

In a column, means having similar letter(s) are statistically identical and those having different letter(s) are significantly different by DMRT at 5% level of probability

4.1.9 Toxicity of neem leaf extracts

Toxicity of neem leaf extract against mosquito larvae at four different doses is shown in Table10. The result indicates that significant variation was observed among the different doses of neem leaf extract at 6 hours after treatment application . It was also observed that 200000 ppm does of neem leaf extract gave the lowest LC_{50} value (median lethal concentration 10534) after 48 hours with the range of 9.10E+06b - 10534 from 6 hours to 48 hours interval having LSD value 622153-7303.6 at p=0.05. Next dose 100000 ppm gave median lethal concentration of LC_{50} 17227 after 48 hours in the range of 9.37E+06 to 17227 from 6 to 48 hours interval. Then dose 50000 ppm gave median lethal concentration of LC_{50} 18645 from range 9.57E+06 to 18645 and 30000 ppm gave median lethal concentration of LC_{50} 20021 having range 9.10E+06 to 20021.The order of toxicity of different doses of neem leaf extract was 200000.0 ppm >100000.0 ppm >50000.0 ppm >30000.0 ppm. From table 10 we can say that concentration of 200000 ppm of neem leaf extract gave the most effective result after 48 hours interval.

Table 10 LC_{50} value after different time interval of data collections for neem leaf extract.

Dose	**LC_{50} value of neem leaf extract at different dosages after different time interval of data collection**				
	6 hours	12 hours	18 hours	24 hours	48 hours
30000	9.83E+06a	141761a	18254a	22371a	20021a
50000	9.57E+06ab	128845ab	17034b	20952a	18645a
100000	9.37E+06ab	116072bc	16909b	19224a	17227ab
200000	9.10E+06b	101978c	16118b	17695a	10534b
LSD	622153	15653	1095.2	5425.8	7303.6

.In a column, means having similar letter(s) are statistically identical and those having different letter(s) are significantly different by DMRT at 5% level of probability

4.2 Percent mortality rate of mosquito larvae against different chemicals

4.2.1 Percent mortality against lambda-cyhalothrin

Mortality of mosquito larva at four different doses of lambda-cyhalothrin is shown in Table 11. Data express that larval mortality was varied at different doses of lambda-cyhalothrin in various times. The highest percent larval mortality was observed at 10.0 ppm dose (T_4) at five different times. The range of percent larval mortality was varied from 81.0 % to 100%. These results were significantly higher than other three doses of lambda-cyhalothrin. The order of effectiveness of different doses of lambda-cyhalothrin was 10.0 ppm > 5.0 ppm > 2.0 ppm > 1.0 ppm at five different times.

Table 11 Larval mortality of mosquito in different doses of lambda-cyhalothrin at five different times after treatment application.

Treatment	**Larval mortality (%)**				
	After 6 hour	After 12 hour	After 18 hour	After 24 hour	After 48 hour
T_1	65.890 c	87.223 b	87.223 b	92.777 a	95.110 b
T_2	67.557 c	87.890 b	93.777 a	93.110 a	95.443 b
T_3	73.110 b	93.777 a	94.110 a	93.443 a	99.667 a
T_4	81.000 a	94.777 a	95.110 a	93.777 a	100.00 a
CV %	2.81	1.07	1.17	1.19	2.07
LSD (p=0.05)	3.8046	1.8388	2.0435	2.0893	3.8019

In a column, means having similar letter(s) are statistically identical and those having different letter(s) are significantly different by DMRT at 5% level of probability.

[Note: T_1= 1ppm, T_2=2 ppm, T_3=5 ppm,T_4=10]

4.2.2 Effect of cyfluthrin on mortality of mosquito larva

Mortality of mosquito larva at four different doses of cyfluthrin is shown in Table 12. Data express that larval mortality was varied at different doses of cyfluthrin in various times. The highest percent larval mortality was observed at 160.0 ppm dose (T_4) at five different times. The range of percent larval mortality was varied from 27.890 % to 81.333 %. These results were significantly higher than other three doses of cyfluthrin. The order of effectiveness of different doses of cyfluthrin was 10.0 ppm >5.0 ppm >2.0 ppm >1.0 ppm at five different times.

Table 12 Larval mortality of mosquito in different doses of cyfluthrin at five different times after treatment application.

Treatment	Larval mortality (%)				
	After 6 hour	After 12 hour	After 18 hour	After 24 hour	After 48 hour
T_1	21.333 b	20.667 b	26.223 c	34.110 d	47.223 d
T_2	22.000 b	21.667 b	27.223 c	41.00 c	54.110 c
T_3	26.890 a	26.890 a	41.333 b	54.443 b	61.333 b
T_4	27.890 a	27.557 a	48.223 a	67.223 a	81.333 a
CV %	5.67	3.75	3.90	1.79	1.61
LSD (p=0.05)	2.6184	1.7083	2.6246	1.6617	1.8478

In a column, means having similar letter(s) are statistically identical and those having different letter(s) are significantly different by DMRT at 5% level of probability.

[Note: T_1= 1ppm, T_2=2 ppm,T_3=5 ppm,T_4=10]

4.2.3 Effect of Emamectin-benzoate on mortality of mosquito larva

Mortality of mosquito larva at four different doses of emamectin-benzoate is shown in Table 13. Data express that larval mortality was varied at different doses of emamectin-benzoate in various times. The highest percent larval mortality was observed at 160.0 ppm dose (T_4) at five different times. The range of percent larval mortality was varied from 54.443 % to 92.110 %. These results were significantly higher than other three doses of emamectin-benzoate. The order of effectiveness of different doses of emamectin-benzoate was 160.0 ppm > 80.0 ppm > 40.0 ppm > 25.0 ppm at five different times.

Table 13 Larval mortality of mosquito in different doses of Emamectin benzoate at five different times after treatment application.

Treatment	Larval mortality (%)				
	After 6hour	After 12 hour	After 18 hour	After 24 hour	After 48 hour
T_1	27.223 c	41.667 c	42.333 d	54.960 d	73.333 b
T_2	34.110 b	47.220 b	54.553 c	67.223 c	77.223 ab
T_3	35.110 b	55.110 a	62.333 b	75.443 b	83.557 ab
T_4	54.443 a	55.777 a	68.190 a	82.667 a	92.110 a
CV %	3.11	3.42	3.14	2.66	10.51
LSD (p=0.05)	2.2062	3.2180	3.661	3.5106	16.135

In a column, means having similar letter(s) are statistically identical and those having different letter(s) are significantly different by DMRT at 5% level of probability.

[Note: T_1= 25ppm, T_2=40 ppm,T_3=80 ppm,T_4=160 ppm]

4.2.4 Effect of buprofezin on mortality of mosquito larva

Mortality of mosquito larva at four different doses of buprofezin is shown in Table 14. Data express that larval mortality was varied at different doses of buprofezin in various times. The highest percent larval mortality was observed at 40.0 ppm dose (T_4) at five different times. The range of percent larval mortality was varied from 29.333 % to 91.667 %. These results were significantly higher than other three doses of buprofezin. The order of effectiveness of different doses of buprofezin was 40.0 ppm > 20.0 ppm > 10.0 ppm > 5.0 ppm at five different times.

Table 14 Larval mortality of mosquito in different doses of buprofezin at five different times after treatment application.

Treatment	Larval mortality (%)				
	After 6 hours	After 12 hours	After 18 hours	After 24 hours	After 48 hours
T_1	0	0	11.000 c	42.000 c	61.667 d
T_2	0	0	21.000 b	72.000 b	72.333 c
T_3	0	0	21.667 b	72.000 b	82.000 b
T_4	0	0	29.333 a	81.667 a	91.667 a
CV %	0	0	13.12	2.83	2.43
LSD (p=0.05)	0	0	5.1277	3.5642	3.5225

In a column, means having similar letter(s) are statistically identical and those having different letter(s) are significantly different by DMRT at 5% level of probability.

[Note: T_1= 5.00 ppm, T_2=10.00 ppm, T_3=20.00 ppm,T_4=40.00 ppm]

4.2.5 Effect of pyrazine on mortality of mosquito larva

Mortality of mosquito larva at four different doses of pyrazine is shown in Table 15. Data express that larval mortality was varied at different doses of pyrazine in various times. The highest percent larval mortality was observed at 160.0 ppm dose (T_4) at five different times. The range of percent larval mortality was varied from 41.667 % to 93.777 %. These results were significantly higher than other three doses of pyrazine. The order of effectiveness of different doses of pyrazine was 160.0 ppm >80.0 ppm >40.0 ppm >25.0 ppm at five different times.

Table 15 Mortality of mosquito larva in different doses of pyrazine at five different times after treatment application

Treatments	Larval mortality (%)				
	After 6 hours	After 12 hours	After 18 hours	After 24 hours	After 48 hours
T_1	21.000 c	21.000 c	41.333 d	54.777 d	67.890 d
T_2	34.110 b	41.000 b	54.777 c	66.890 c	80.667 c
T_3	34.110 b	54.110 a	68.223 b	81.000 b	87.223 b
T_4	41.667 a	54.777 a	81.000 a	93.777 a	93.777 a
CV %	3.33	2.49	2.15	1.17	1.26
LSD (0.05)	2.05	2.00	2.48	1.63	1.96

In a column, means having similar letter(s) are statistically identical and those having different letter(s) are significantly different by DMRT at 5% level of probability.

[T_1= 25.00 ppm, T_2=40.0 ppm,T_3=80.0 ppm,T_4=160.0 ppm].

4.2.6 Effect of spinosad on mortality of mosquito larva

Mortality of mosquito larva at four different doses of spinosad is shown in Table 16. Data express that larval mortality was varied at different doses of spinosad in various times. The highest percent larval mortality was observed at 10.0 ppm dose (T_5) at five different times. The range of percent larval mortality was varied from 32.22% to 99.00%. These results were significantly higher than other four doses of spinosad. The order of effectiveness of different doses of spinosad was 10.0 ppm > 5.0 ppm > 2.0 ppm > 1.0 ppm at five different times.

Table 16 Larval mortality of mosquito in different doses of spinosad at five different times after treatment application

Treatments	Larval mortality (%)				
	After 6 hours	After 12 hours	After 18 hours	After 24 hours	After 48 hours
T_1	13.33c	40.33 c	45.00 c	53.78 b	59.67 c
T_2	26.63 b	41.00 c	45.33 c	54.33 b	61.00 bc
T_3	31.78 a	54.44 b	55.18 b	55.55 b	63.67 b
T_4	32.22 a	65.56 a	82.00 a	98.67 a	99.00 a
CV (%)	**6.78**	**3.74**	**2.96**	**2.05**	**2.50**
LSD (p=0.05)	**3.25**	**3.31**	**2.96**	**2.36**	**3.15**

In a column, means having similar letter(s) are statistically identical and those having different letter(s) are significantly different by DMRT at 5% level of probability.

[T_1= 1.00 ppm, T_2=2.00ppm, T_3=5.00ppm,T_4=10.00 ppm].

4.2.7 Effect of Temephos on mortality of mosquito larva

Mortality of mosquito larva at four different doses of temephos is shown in Table 17. Data express that larval mortality was varied at different doses of temephos in various times. The highest percent larval mortality was observed at 10.0 ppm dose (T_4) at five different times. The range of percent larval mortality was varied from 86.667 % to 100.00%. These results were significantly higher than other three doses of temephos. The order of effectiveness of different doses of temephos was 10.0 ppm >5.0 ppm >2.0 ppm >1.0 ppm at five different times.

Table 17 Larval mortality of mosquito in different doses of temephos at five different times after treatment application

Treatment	**Larval mortality (%)**				
	After 6 hour	After 12 hour	After 18 hour	After 24 hour	After 48 hour
T_1	73.333 c	82.333 c	92.333c	95.333c	96.000 c
T_2	80.000 b	86.667 bc	94.333bc	97.667 b	98.333 b
T_3	83.333 ab	88.333 b	96.000 ab	100.00a	100.00 a
T_4	86.667 a	94.333 a	98.000 a	100.00a	100.00a
CV %	3.09	2.97	1.36	0.42	0.59
LSD (p=0.05)	4.7071	4.9219	2.4307	0.7687	1.0871

In a column, means having similar letter(s) are statistically identical and those having different letter(s) are significantly different by DMRT at 5% level of probability.

[T_1= 1.00 ppm, T_2=2.00 ppm ,T_3=5.00 ppm,T_4=10.00 ppm].

4.2.8 Effect of neem seed kernel extract on mortality of mosquito larva

Mortality of mosquito larva at four different doses of neem seed kernel extract is shown in Table 18. Data express that larval mortality was varied at different doses of neem seed kernel extract in various times. The highest percent larval mortality was observed at 10.0 ppm dose (T_4) at five different times. The range of percent larval mortality was varied from 37.883% to 90.667 %. These results were significantly higher than other four doses of neem seed kernel extract. The order of effectiveness of different doses of neem seed kernel was 10.0 ppm > 5.0 ppm > 3.0 ppm > 2.0 ppm > 1.0 ppm at five different times.

Table 18 Larval mortality of mosquito in different doses of neem seed kernel extract at five different times after treatment application

Treatment	Larval mortality (%)				
	After 6 hour	After 12hour	After 18 hour	After 24 hour	After 48 hour
T_1	22.000 d	34.110 d	47.223 c	47.557 c	54.443 b
T_2	29.223 c	36.667 c	47.557 c	47.557 c	55.110 b
T_3	33.333 b	41.333 b	53.333 b	56.667 b	56.333 b
T_4	42.000 a	52.333 a	64.667 a	74.000 a	87.333 a
CV %	6.22	2	1.55	2.08	1.93
LSD (p=0.05)	3.7031	1.5487	1.5572	2.2104	2.3007

In a column, means having similar letter(s) are statistically identical and those having different letter(s) are significantly different by DMRT at 5% level of probability.

[$T_{1=}$ 01ppm, $T_{2=}$02 ppm,$T_{3=}$ 05 ppm, $T_{4=}$10 ppm]

4.2.9 Effect of neem leaf extract on mortality of mosquito larva

Mortality of mosquito larva at four different doses of neem leaf extract is shown in Table 19. Data express that larval mortality was varied at different doses of neem leaf extract in various times. The highest percent larval mortality was observed at 200000.0 ppm dose (T_4) at five different times. The range of percent larval mortality was varied from 46.557 % to 90.667 %. These results were significantly higher than other four doses of neem leaf extract. The order of effectiveness of different doses of neem leaf extract was 20000.0 ppm > 10000.0 ppm > 50000.0 ppm > 30000.0 ppm at five different times.

Table 19 Larval mortality of mosquito in different doses of neem leaf extract at five different times after treatment application.

Treatment	**Larval mortality (%)**				
	After 6 hour	After 12 hour	After 18 hour	After 24 hour	After 48 hour
T_1	37.883 b	38.833 c	58.470 d	60.323 c	63.667 d
T_2	38.870 b	56.947 b	62.000 c	63.333 c	68.000 c
T_3	43.673 ab	57.443 b	71.410 b	77.973 b	85.333 b
T_4	46.557 a	63.243 a	78.973 a	86.537 a	90.667 a
CV %	8.82	4.98	2.70	2.67	2.76
LSD (p=0.05)	6.9330	5.0717	3.4473	3.6246	3.9941

In a column, means having similar letter(s) are statistically identical and those having different letter(s) are significantly different by DMRT at 5% level of probability.

[T_1=30000 ppm,T_2=50000 ppm,T_3=100000 ppm,T_4=200000 ppm]

CHAPTER V

SUMMARY AND CONCLUSION

A laboratory experiment was conducted at Dr. M.A Wazed miah central laboratory of Sher-e-Bangla Agricultural University to find out the efficacy of 9 larvicides for bio-rational management of mosquito larvae in Bangladesh.

Nine larvicides as spinosad (dose: 1.00 ppm, 2.00 ppm, 5.00 ppm and 10.00 pmm), cyfluthrin (dose: 1.00 ppm, 2.00 ppm, 5.00 ppm and 10.00 pmm), lambda-cyhalothrin (dose: 1.00 ppm, 2.00 ppm, 5.00 ppm and 10.00 pmm), pyrazine (dose: 25.00 ppm, 40.00 ppm, 80.00 ppm, 160.00 ppm), emamectin-benzoate (dose: 25.00 ppm, 40.00 ppm, 80.00 ppm, 160.00 ppm), Buprofezin (dose: 5.00 ppm, 10.00 ppm, 20.0 ppm, 40.00 ppm) temephos (dose: 10.00 ppm, 5.00 ppm, 2.00 ppm, 1.00 ppm) and two botanical as neem leaf extrac (dosages: 30000 ppm, 50000 ppm, 100000 ppm, 200000 ppm) and neem seed kernel extract(dose:1.0 ppm, 2.0 ppm, 5 ppm,10 ppm). The experiment was laid out in CRD design with three replication of each treatment. Data was collected every 6 hours interval as 6 hours 12 hours 18 hours 24 hours, 48 hours respectively. Dead larvae number and live larvae number was collected. And a sample was set untreated.

Probit analysis was done to find out the LC_{50} of each treatment. This work evaluated the efficacy of nine larvicides and found the mortality percent of larvicides. Of the nine larvicides tested lambda-cyhalothrin gave the lowest median lethal concentration of LC_{50} of 0.000848, after that temephos gave second lowest median lethal concentration LC_{50} of 0.00114 after 48 hours. At third position spinosad gave median lethal concentration of LC_{50} is 0.0043.It was followed by cyfluthrin with median lethal concentration of LC_{50} 1.1067; buprofezin with median lethal concentration of LC_{50} 1.7650 ; pyrazine with median lethal concentration of LC_{50} 6.07; neem seed kernel with median lethal concentration of LC_{50} 2.3360 ; pyrazine with median lethal concentration of LC_{50} 6.0700; emamectin benzoate with median lethal concentration of LC_{50} 7.5823 and neem leaf extract with median lethal concentration of LC_{50} 10534.

From the above result the order of toxicity of nine larvicides is lambda-cyhalothrin > temephos >Spinosad > cyfluthrin > buprofezin > neem seed kernel > pyrazine > emamectin-benzoate > neem leaf extract.

Mortality percentage also calculated to find out the mortality percentage of nine larvicides. Lambda-cyhalothrin and temephos gave highest mortality percentage of 100% after 48 hours, then spinosad gave 99% mortality , it was followed by pyrazine 93.667% mortality; emamectin-benzoate 92.110% mortality ; buprofezin 91.667% mortality; neem seed kernel extract 90.667% mortality; neem leaf extract 87.33% mortality and cyfluthrin 81.333%mortality after 48 hours of treatment.

From the above results,the order of mortality percentage revealed is lambda-yhalothrin > temephos > Spinosad > pyrazine > emamectin-benzoate > buprofezin > neem seed kernel > neem leaf extract > cyfluthrin

CHAPTER VI

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APPENDIX

APPENDIX Ⅰ. Effect of spinosad on mortality of mosquito larva at five different times after treatment application.

Source of Variation	Degree of Freedom	Lc_{50} Values at different time interval				
		6 Hours	12 Hours	18 Hours	24 Hours	48 Hours
Treatment	4	1.00506*	0.08259**	0.00264**	0.001378	0.001524
Error	10	0.34642	0.00656	0.0004843	0.003465	0.003474

**= 1% level of significance *= 5% level of significance

APPENDIX Ⅱ . Effect of temephos on mortality of mosquito larva at five different times after treatment application.

Source of Variation	Degree of Freedom	LC_{50} values at different time interval				
		6 Hours	12 Hours	18 Hours	24 Hours	48 Hours
Treatment	3	97.2222**	74.0833**	17.444**	14.9722**	10.75**
Error	8	6.250	6.83333	1.6667	0.1667	0.3333

**= 1% level of significance *= 5% level of significance

APPENDIX Ⅲ. Effect of lambda-cyhalothrin on mortality of mosquito larva at five different times after treatment application.

Source of Variation	Degree of Freedom	LC_{50} values at different time interval				
		6 Hours	12 Hours	18 Hours	24 Hours	48 Hours

Treatment	3	0.00150*	7.046E-*04	1332808*	2.371E-06*	4.705E-04*
Error	8	0.01780	3.987E-04	714669	6.642E-07	6.654E-04

**= 1% level of significance *= 5% level of significance

APPENDIX Ⅳ. Effect of neem seed kernel extract on mortality of mosquito larva at five different times after treatment application.

SOURCE OF VARIATION	DEGREE OF FREEDOM	LC_{50} values different time interval				
		6 Hours	12 Hours	18 Hours	24 Hours	48 Hours
TREATMENT	3	21.9078*	5.18872*	5.80673**	5.23372*	3.59855**
ERROR	8	18.3992	1.08918	0.32167	1.03508	0.55210

**= 1% level of significance *= 5% level of significance

APPENDIX Ⅴ. Effect of neem leaf extract on mortality of mosquito larva at five different times after treatment application.

Source of Variation	Degree of Freedom	Lc_{50} Values at different time interval				
		6 Hours	12 Hours	18 Hours	24 Hours	48 Hours
Treatment	3	2.541e+11*	8.140e+08*	1332808*	1.095e+07*	3.389e+07*
Error	8	1.191e+11	9.132e+07	714669	8857024	2.224e+07

**= 1% level of significance *= 5% level of significance

APPENDIX Ⅵ. Effect of emamectin-benzoate on mortality of mosquito larva at five different times after treatment application.

Source of Variation	Degree of Freedom	LC_{50} values at different time interval				
		6 Hours	12 Hours	18 Hours	24 Hours	48 Hours

Treatment	3	97.916	28.1637	52.9162	27.0356*	11.8448*
Error	8	198.946	77.7362	64.4953	20.7210	9.5325

**= 1% level of significance *= 5% level of significance

APPENDIX Ⅶ. Effect of buprofezin on mortality of mosquito larva at five different times after treatment application.

Source of Variation	Degree of Freedom	LC_{50} values at different time interval				
		6 Hours	12 Hours	18 Hours	24 Hours	48 Hours
Treatment	3	0	0	157.36	5.71732*	1.229629*
Error	8	0	0	5744.22	1.23041	0.87877

**= 1% level of significance *= 5% level of significance

APPENDIX Ⅷ. Effect of cyfluthrin on mortality of mosquito larva at five different times after treatment application.

Source of Variation	Degree of Freedom	LC_{50} values at different time interval				
		6 Hours	12 Hours	18 Hours	24 Hours	48 Hours
Treatment	3	1.710E+07	84936.5*	9.66941*	2.82408**	0.15003*
Error	8	1.710E+07	34920.4	2.31001	0.17497	0.05918

**= 1% level of significance *= 5% level of significance

APPENDIX Ⅸ. Effect of pyrazine on mortality of mosquito larva at five different times after treatment application.

Source of Variation	Degree of Freedom	LC_{50} values at different time interval				
		6 Hours	12Hours	18 Hours	24 Hours	48 Hours
Treatment	3	351.061**	174.13*	50.0126*	40.8766*	15.5033*

Error	8	9.946	38.2837	18.5978	14.3855	7.3437

**= 1% level of significance *= 5% level of significance

APPENDIX X. Larval mortality of mosquito in different doses of lambda-cyhalothrinat five different times after treatment application.

Source of Variation	Degree of Freedom	Larval mortality %				
		6 Hours	12 Hours	18 Hours	24 Hours	48 Hours
Treatment	3	139.258**	45.8806**	38.8652**	0.55556*	20.8743**
Error	8	4.083	0.9538	1.1780	1.23130	4.0773

*= 5% level of significance **= 1 % level of significance

APPENDIX XI. Larval mortality of mosquito in different doses of cyfluthrin at five different times after treatment application.

Source of Variation	Degree of Freedom	Larval mortality %				
		6 Hours	12 Hours	18 Hours	24 Hours	48 Hours
Treatment	3	1.710E+7	84936.5*	9.66941**	2.82408**	0.15003
Error	8	1.710E+7	34920.4	2.31001	0.17497	0.05918

*= 5% level of significance **= 1 % level of significance

APPENDIX XII. Larval mortality of mosquito in different doses of emamectin-benzoateat at five different times after treatment application.

Source of Variation	Degree of Freedom	Larval mortality %				
		6 Hours	12 Hours	18 Hours	24 Hours	48 Hours
Treatment	3	409.694**	136.642**	374.671**	423.964**	201.774**
Error	8	1.373	2.921	3.196	3.476	73.437

*= 5% level of significance **= 1 % level of significance

APPENDIX XIII. Larval mortality of mosquito in different doses of buprofezinat five different times after treatment application.

Source of Variation	Degree of Freedom	Larval mortality %				
		6 Hours	12 Hours	18 Hours	24 Hours	48 Hours
Treatment	3	0.00000	0.0000	169.639**	890.083**	496.972**

Error	6	0.0000	0.0000	7.417	3.583	3.500

*= 5% level of significance **= 1 % level of significance

APPENDIX XIV. Larval mortality of mosquito in different doses of spinosad at five different times after treatment application.

Source of Variation	Degree of Freedom	Larval mortality %				
		6 Hours	12 Hours	18 Hours	24 Hours	48 Hours
Treatment	4	179.086**	371.881**	735.939**	1169.76**	839.600**
Error	10	3.193	3.305	2.644	1.68	3.000

*= 5% level of significance **= 1 % level of significance

APPENDIX XV. Larval mortality of mosquito in different doses of pyrazine at five different times after treatment application.

Source of Variation	Degree of Freedom	Larval mortality %				
		6 Hours	12 Hours	18 Hours	24 Hours	48 Hours
Treatment	3	221.265**	749.812**	877.240**	860.156**	366.237**
Error	8	1.186	1.131	1.741	0.751	1.082

*= 5% level of significance **= 1 % level of significance

APPENDIX XVI. Larval mortality of mosquito in different doses of neem seed kernel extract at five different times after treatment application.

Source of Variation	Degree of Freedom	Larval mortality %				
		6 Hours	12 Hours	18 Hours	24 Hours	48 Hours
Treatment	3	21.9078*	5.18872*	5.80673**	5.23372**	3.59855**
Error	8	18.3992	1.08918	0.32167	1.03508	0.55210

*= 5% level of significance **= 1 % level of significance

APPENDIX XVIII. Larval mortality of mosquito in different doses of neem leaf extract at five different times after treatment application.

Source of Variation	Degree of Freedom	Larval mortality %				
		6 Hours	12 Hours	18 Hours	24 Hours	48 Hours
Treatment	3	50.4368*	335.952**	258.534**	458.444**	514.972**
Error	8	13.5584	7.256	3.352	3.706	4.500

*= 5% level of significance **= 1 % level of significance