BIOEFFICACY, AUTO-DISSEMINATION AND VENEREAL TRANSFER OF MOSQUIRON® 0.12P IN *Aedes albopictus* (Skuse) (DIPTERA: CULICIDAE) POPULATION

NG KIN HOONG

UNIVERSITI SAINS MALAYSIA

2017

BIOEFFICACY, AUTO-DISSEMINATION AND VENEREAL TRANSFER OF MOSQUIRON® 0.12P IN *Aedes albopictus* (Skuse) (DIPTERA: CULICIDAE) POPULATION

by

NG KIN HOONG

Thesis submitted in fulfillment of the requirements

for the Degree of

Master of Science

January 2017

ACKNOWLEDGEMENT

After the effort of eight semesters, the meaningful dissertation for me had been produced. This indicated that the hard work with the help of few people who I respect.

First of all, I would like to wish thousand thanks to my main supervisor, Professor Abu Hassan bin Ahmad with lots of wonderful assistance, guidance, encouragement and advices as giving me this chance to work with such interesting and good future prospectus project as well as his valuable editorial contributions. Many thanks to Professor Abu Hassan that arrange me a suitable laboratory, full equipments and materials to complete my research.

Secondly, I would like to thank to my co-supervisor, Mr Ahmad Ramli bin Saad that gave me ideas and comments about my project. Also, I would like to thank to the members of Vector Control Research Unit, Mr Adanan and Mr Zat that provided me the apparatus in my project.

In addition to all the help that I received from my laboratory mate, Dr Salah Gurbel that assisted me in the statistical analysis of my work that fulfill the experiment design.

Lastly, I would like to thank my parents for giving me a full support and encouragement to motivate me through all my difficult times. Their assistance was greatly appreciated and highly valued.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF ABBREVIATIONS

Ae.	*Aedes*
An.	*Anopheles*
Cx.	*Culex*
SE	Standard error of mean
ppm	parts per million
PPS	Pengawalatur pertumbuhan serangga
IE	Inhibition of emergence
a.i.	active ingredient
IGRs	Insect growth regulators
CDC	Centres for Disease Control
JHA	Juvenile hormone analogs
CFR	Case fatality rate
Bti	*Bacillus thuringiensis israelensis*
ITNs	Insecticide-treated bednets
F1	First filial generation
RNA	Ribonucleic acid
CSIs	Chitin synthesis inhibitors
IR	Incidence rate

BIOEFIKASI, AUTO-PEYEBARAN DAN PEMINDAHAN VENEREAL MOSQUIRON® 0.12P DALAM POPULASI *Aedes albopictus* (Skuse) (DIPTERA: CULICIDAE)

ABSTRAK

Pengawalatur pertumbuhan serangga (PPS) menawarkan alternatif kepada larvisid kimia konvensional yang menunjukkan masalah kerintangan dan keselamatan alam sekitar. Namun, hanya sebilangan terhad PPS yang diluluskan untuk kawalan nyamuk. Dalam kajian terkini ini, keberkesanan Mosquiron® 0.12P yang mengandungi novaluron telah dikaji terhadap nyamuk *Aedes albopictus* di dalam makmal. IE_{50} (kerencatan kemunculan) untuk 24 jam, 48 jam, 72 jam dan 96 jam adalah dalam julat 3.11×10^4 mg a.i./L hingga 3.05×10^2 mg a.i./L untuk larva peringkat pertama manakala IE_{95} untuk 24 jam, 48 jam, 72 jam dan 96 jam adalah dalam julat 2.52×10^8 mg a.i./L hingga 1.48×10^8 mg a.i./L untuk larva peringkat pertama. IE_{50} dan IE_{95} meningkat apabila larva berkembang. Peratus mortaliti larva terhadap Mosquiron® 0.12P semakin meningkat apabila kepekatan Mosquiron® 0.12P meningkat. Bioefikasi Mosquiron® 0.12P menunjukkan > 90% kerencatan kemunculan larva *Ae. albopictus* selepas disimpan selama 5 bulan. Stesen auto-penyebaran rekabentuk B adalah lebih efektif berbanding dengan stesen auto-penyebaran rekabentuk A dalam penumpasan larva peringkat akhir ketiga atau larva peringkat awal keempat. Manakala stesen autodisseminasi yang menggunakan sukrosa sebagai umpan adalah lebih efektif daripada tikus putih dalam mengurangkan nyamuk dewasa. *Aedes albopictus* yang dirawat dengan Mosquiron® 0.12P menunjukkan perbezaan dalam fertiliti, kadar kemunculan pupa, kadar kemunculan nyamuk dewasa, tempoh hidup, kadar kemunculan nyamuk jantan dan betina tetapi tiada perbezaan dalam fekunditi berbanding dengan kawalan bagi

generasi induk. Kesan submaut ke atas *Ae. albopictus* yang dirawat dengan Mosquiron® 0.12P menunjukkan perbezaan dalam kadar kemunculan nyamuk dewasa, tempoh hidup, kadar kemunculan nyamuk jantan dan betina tetapi tiada perbezaan dalam fekunditi, fertiliti dan kadar kemunculan pupa berbanding dengan populasi kawalan. Pemindahan venerel Mosquiron® 0.12P dari nyamuk *Ae. albopictus* jantan dara yang terkontaminasi ke nyamuk betina dara yang tidak terkontaminasi menyebabkan min mortaliti larva peringkat ketiga yang lebih tinggi berbanding dengan pemindahan venerel Mosquiron® 0.12P dari nyamuk *Ae. albopictus* betina dara yang terkontaminasi ke nyamuk jantan dara yang tidak terkontaminasi dalam bioasai insektisid.

BIOEFFICACY, AUTO-DISSEMINATION AND VENEREAL TRANSFER OF MOSQUIRON® 0.12P IN *Aedes albopictus* (Skuse) (DIPTERA: CULICIDAE) POPULATION

ABSTRACT

Insect growth regulators (IGRs) offer alternatives to conventional chemical larvicides that pose problem of resistance and environmental safety. However, there is only a limited number of IGRs approved for use in mosquito control. In this current study, the effectiveness of Mosquiron® 0.12P (IGR) containing novaluron against *Aedes albopictus* larvae were studied in the laboratory. The IE_{50} (inhibition of emergence) for 24 hours, 48 hours, 72 hours and 96 hours were in the range of 3.11 x 10^4mg a.i./L to 3.05 x 10^2 mg a.i./L for the first-instar larvae. Whereas the IE_{95} for 24 hours, 48 hours, 72 hours and 96 hours were in the range of 2.52 x 10^8mg a.i. /L to 1.48 x 10^8 mg a.i. /L for the first-instar larvae. IE_{50} and IE_{95} increased as larval development progressed. Percentage of larval mortality against Mosquiron® 0.12P gradually increased as the concentration of Mosquiron® 0.12P increased. The bioefficacy of Mosquiron® 0.12P showed > 90% of emergence inhibition against *Ae. albopictus* larvae after being stored for 5 months. Autodissemination station design B was more effective in suppressing late third – instar/ early fourth – larvae compared to autodissemination station design A. Whereas autodissemination station using sucrose as a lure was more effective than that using rat in luring adult mosquitoes. *Aedes albopictus* treated with Mosquiron® 0.12P showed differences in fertility, pupation rate, adult emergence rate, longevity, male and female emergence rate but no difference in fecundity compared to control for the parental generation. Sublethal effect against *Ae. albopictus* that were treated with Mosquiron® 0.12P showed differences in adult emergence rate, longevity, male and female emergence rate but

no difference in fecundity, fertility and pupation rate compared to the control population. Venereal transfer of Mosquiron® 0.12P from contaminated virgin males to uncontaminated virgin females showed that the mean mortality of third-instar larvae were higher than the venereal transfer of Mosquiron® 0.12P from contaminated virgin females to uncontaminated virgin males during insecticide bioassay.

CHAPTER 1

GENERAL INTRODUCTION

1.1 Introduction

Mosquito-borne diseases such as dengue fever (DF) and dengue hemorrhagic fever (DHF) are the most important arthropod borne viral diseases of public health significance (Guha – Sapir and Schimmer, 2005). The incidences have a dramatic increase in recent years, from 2012 to 2013 (21,900 to 43,346 cases) and until 16 weeks of the year 2015, there were more than 32,000 cases have been reported almost three folds compared to the year 2014 (Ong, 2016).

The infection of dengue is due to the dengue virus, which consists of 5 serotypes (DENV-1 to 5) with the filth serotype, DENV-5 was newly identified and announced in 2013 (Normile, 2013). Among these serotypes, four of the serotypes virus (DENV-1 to 4) could be isolated from the infected people in Malaysia at any time; this indicates hyper-endemic of dengue epidemic in the country (Cheah *et al*., 2014). At present, there is yet a specific antiviral drugs for dengue treatment (Simmons *et al*., 2012), although dengue vaccine development is underway, but the vaccine introduction is complicated due to several concerns such as the high risk of severe disease through antibody-dependent enhancement (Webster *et al*., 2009) and the threats of the new serotype, DENV-5 (Normile, 2013).

Aedes albopictus is indigenous in tropical Asia but presently the distribution is worldwide. The abundance of dengue is closely associated with the abundance of the vectors. It was also reported that the abundance of vectors was associated with the environmental factors such as the rainfall, temperature and relative humidity

(Okogun *et al.*, 2003), while the wet seasons are associated with the higher prevalence of mosquito borne diseases.

The indoor-breeding behaviour can potentially increase the biting activity of mosquito vectors that opportunistically bite humans outdoors during the day. This may have important implications to dengue disease transmission. However, despite this epidemiological importance, there have been no previous studies of this issue in dengue vectors (Hamady *et al*., 2011).

Efforts to control dengue have mainly involved insecticide spraying programmes, but this strategy has proven ineffective (WHO, 1999). While a few vaccines actually under development, without immediate prospects for success, vector control remains the only viable method to prevent dengue transmission (Guzman and Kouri, 2002; Pang, 2003; Deen, 2004; Guzman *et al*., 2004). Improved knowledge regarding egg-laying behavior is relevant because it underpins the primary surveillance method, i.e., ovitraping (Ritchie *et al*., 2003). However, the most commonly used ovitrap, the CDC gravid trap, is not appropriate for capturing *Ae. albopictus* (Savage *et al*., 2008).

Auto-dissemination has attracted attention due to its potential to address important gaps with existing mosquito control methods. Auto-dissemination is a method of pesticide 'self-delivery' which is premised upon the use of insects as the delivery agent. Insects carrying small amounts of insecticide can deliver an active ingredient to cryptic larval breeding sites, rather than human applicators and this method using less pesticide relative to broadcasting. For this reason, auto-dissemination approaches have become an important pesticidal method for beetles, termites and moths (Su, 2002).

Auto-dissemination approaches are being explored for mosquito control (Gaugler *et al*., 2011; Devine *et al*., 2009). This method is based on the oviposition of adult mosquitoes and their attraction to breeding sites, including cryptic sites that human operators always fail to find. As current research, auto-dissemination consists of putting artificial adult resting sites (dissemination stations) that are attractive to adult mosquitoes and treated with a persistent juvenile hormone analogue (JHA) (Caputo *et al.,* 2012). Adult mosquitoes become contaminated with JHA when entering the dissemination station, which is not acutely toxic to the adult. The JHA is lethal to immature mosquitoes when their breeding sites become contaminated by the females carrying JHA that arrive to lay eggs and thus introduce the JHA (Mains *et al*., 2015).

Venereal transfer is an auto-dissemination approach that is based on the mating behaviour of adult mosquitoes transferring the IGR from contaminated mosquitoes to uncontaminated mosquitoes. Female mosquitoes contaminated with insect growth regulators (IGR) are released into a cage and mate with uncontaminated male mosquitoes in laboratory. For this study, both female and male mosquitoes are being contaminated with the IGR to mate with uncontaminated male and uncontaminated female mosquitoes (Gaugler *et al.*, 2011).

1.2 Research objectives

The specific objectives of this study are as follows:

1. To determine the larval susceptibility and efficacy of Mosquiron® 0.12P against *Aedes albopictus* larvae.
2. To determine the effectiveness of autodissemination design, type of lures, effects of eggs exposed to Mosquiron® 0.12P on fecundity and fertility, effects of adult exposed to Mosquiron® 0.12P on fecundity and fertility in F1 generation.
3. To study the venereal transfer of Mosquiron® 0.12P from treated males to untreated females and vice versa.

CHAPTER 2

LITERATURE REVIEW

2.1 Dengue Fever (DF)

Dengue virus is an arbovirus that belongs to the family *Flaviviridae,* under the genus *Flavivirus*. In the past, it was classified under the Group B arboviruses. It is a small enveloped virus measuring 50 to 60 nm in size containing a single stranded positive sense RNA genome (MOH, 2003).

Dengue virus is transmitted via the bite of *Aedes* mosquitoes in particular *Ae. aegypti* and *Ae. albopictus*. In human, the cycle of transmission involves man-vector-man. The virus is present in blood in early acute phase only, generally for 1-5 days. The incubation period varies between 3 to 10 days with an average of 4-6 days (WHO, 1997).

There are four serotypes of dengue virus (DEN-1, DEN-2, DEN-3 and DEN-4). They are antigenically very similar to each other but different enough to elicit only transient partial cross-protection after infection by each one of them. Antibodies to one type cross react in tests with other antigens therefore serological antibody tests, in general, do not differentiate between dengue serotypes (MOH, 2003).

The 4 serotypes of the dengue virus may all circulate concurrently in the same season but in different geographical regions. One serotype may predominate over other serotypes depending on the susceptibility or immunity of the population. The trends in circulating dengue viral serotypes in Malaysia have been studied (Ravindran *et al.,* 2001) and found to predominate in a cyclical pattern. In the period from 1990 – 1995, DEN – 3 was the predominant serotype and followed by DEN – 2

between 1997 – 2000. In the year 2001, DEN – 3 reappeared and predominated over DEN – 2 (MOH, 2003).

2.2 Epidemiology of Dengue Fever in Malaysia

Dengue is the most common and widespread arthropod-borne arboviral infection in the world today. The geographical spread, incidence and severity of dengue fever (DF) and dengue haemorrhagic fever (DHF) are increasing in the Americas, South-East Asia, the Eastern Mediterranean and the Western Pacific. Some 2,500 million to 3,000 million people live in areas where dengue viruses can be transmitted. It is estimated that each year 50 million infections occur, with 500,000 cases of DHF and at least 12,000 deaths (WHO, 2002).

The earliest report of a dengue epidemic in the Peninsular Malaysia was from Singapore in 1901 (More, 1904). Skae (1902) described the next epidemic in Penang and has become a major public health problem in Malaysia, especially since the appearance of the first DHF outbreak also in Penang in 1962 (Rudnick *et al*., 1965). Rapid industrial and economic development over the last two decades has brought about massive infrastructure development, creating man-made environment for breeding of *Aedes* mosquito (George, 1992).

The first dengue fever outbreak in Malaysia was recorded in 1962 from Penang with 41 cases and 5 deaths were reported. Subsequently, a series of outbreak reported in 1973 with 1,487 cases with 54 deaths, 1974 with 2,200 cases with 104 deaths and 1982 with 3,006 cases reported including 35 deaths. Since year 2000, the number of dengue cases and incidence rate (IR) continue to increase with the highest ever reported in 2014. A total of 108,698 cases were reported which is equivalent to

IR of 361.1 cases in 100,000 populations in that year. Fifty six percent (56%) of the cases contributed by Klang Valley in which 49% were from Selangor and 7% from Federal Territory of Kuala Lumpur-Putrajaya. In the period of 2000 to 2014, the number of reported dengue cases was between 7,103 and 108,698 cases per year and the annual incidence rate range from 31.6 to 361.1 cases per 100,000 populations. All age group were affected with dengue infection and the most vulnerable were among the productive aged group between 15 to 49 years old. All ethnic groups are also at risk of being infected. The case fatality rate has reduced from 0.62% in the year 2000 to 0.2% in the year 2014. The number of death has increased from 42 in the year 2000 to 215 in the year 2014 (Rose, 2015).

2.3 Distribution of *Aedes albopictus* in Other Countries

Aedes albopictus is native to Southeast Asia. In its tropical range the species is a secondary vector of human arboviruses, such as Dengue, while in temperate regions its impact on public health is mostly due to its aggressive and diurnal biting behaviour (Caputo *et al*., 2012).

The distribution of *Ae. albopictus* is subtropical, with a temperate distribution in North America, and in the United States has expanded rapidly over the past few years. This species was first documented in Texas in 1985 (Sprenger and Wuithiranyagool, 1986) and is currently established in 866 counties in 26 states (CDC, 2001). A year later, the Asian tiger mosquito was found in Florida at a tire dump site near Jacksonville (O'Meara, 1997). Since that time, this species has spread rapidly throughout the eastern states, including all of Florida's 67 countries (O' Meara, 1997). The potential public health threat presented by this invasion was

reported with the collection of a female *Ae. albopictus* in Memphis, Tennessee (Reiter and Darsie, 1984). By 1985, a breeding population of *Ae. albopictus* was established in Harris County, Texas (Sprenger and Wuithiranyagool, 1986). *Aedes albopictus* has now successfully colonized much of the eastern and central United States (Rai, 1991). The species container-breeding habit has brought it into close contact with urban populations and sustained the possibility of disease transmission (Mitchell, 1991).

The worldwide distribution includes most of Asia and covers tropical and subtropical regions worldwide with introduction into Caribbean (Morbidity and Mortality Weekly Report 1989). Endemic to Asia and the Pacific Islands, the range has greatly expanded to include North and South America, Africa and Europe (O'Meara, 1997).

Before its recent colonization of various sites worldwide, *Ae. albopictus* was confined to Asia. *Ae. albopictus* occurs in both tropical and temperate Asia. Its temperate Asian distribution includes China, South Korea, and Japan. Temperate *Ae. albopictus* overwinters as a diapausing egg (Hong *et al*., 1971). Tropical *Ae. albopictus* cannot diapause and is less cold hardy than temperate *Ae. albopictus* (Hawley *et al*., 1987).

2.4 Life Cycle of *Aedes albopictus*

Aedes albopictus overwinter in the egg stage in temperate climates (Lyon and Berry, 2000) but are active throughout the year in tropical and subtropical habitats. Eggs are laid singly on the sides of water-holding containers such as tires, animal watering dishes, birdbaths, flowerpots and natural holes in vegetation. They are black

and oval with a length of 0.5 mm. Eggs can withstand desiccation up to one year. Larval emergence occurs after rainfall raises the water level in the containers. The eggs may require several submersions before hatching. Additionally, oxygen tension greatly affects egg hatch. A number of studies have shown low oxygen tension stimulates the hatching of *Ae. albopictus* eggs and is a more important factor than flooding or temperature on inducing egg hatch. Development is temperature dependent, but the larvae usually pupate after five to ten days and the pupa stage lasts two days. Larvae, also called wigglers, are active feeders. They feed on fine particulate organic matter in the water. The larvae use a breathing siphon to acquire oxygen and must periodically come to the surface to do so. The larvae develop through four instars prior to pupation. Unlike many other insects, the pupae of mosquitoes are active and short-lived. They do not feed but can move about (Hawley, 1988).

Pupae will develop until the body of the newly formed adult flying mosquito emerges from the pupal skin and leaves the water. After adult mosquitoes emerge, male mosquitoes feed on nectar from flowers and female mosquitoes feed on humans and animals for blood to produce eggs. After feeding, female mosquitoes will look for water sources to lay more eggs (Hawley, 1988).

Adults are found in shady areas, rests in shrubs near the ground (Hawley, 1988). In a study of urban, suburban and rural areas in Brazil, it was found that adult males and females *Ae. albopictus* were much more common in rural areas (93%) and outdoors (90%), demonstrating their preference to rest in areas with more vegetation. In forested areas, this species is more common at the forest edges than within the interior (Hawley, 1988).

Males are believed to seek mates every day, while females seek hosts only every 3-5 days. Males that attracted to females attempt to mate with the females. Flight sounds of females initiate sexual behaviour of males (Hawley, 1988).

The flight range of adults is limited usually less than 1km and they have not been observed to fly in strong winds. Most adults disperse less than 180m during their lifetime however some have been recorded dispersed greater than 800m within a 6-day period in Brazil. Dispersal will vary depending on availability of shelter, food and breeding sites (Bonnet and Worcester, 1946).

2.5 Description of *Aedes albopictus*

Adult *Ae. albopictus* are easily recognized by the bold black shiny scales and distinct silver white scales on the palpus and tarsi (Hawley, 1988). The scutum (back) is black with a distinguishing white stripe down the center beginning at the dorsal surface of the head and continuing along the thorax. It is a medium-sized mosquito (approximately 2.0 to 10.0 mm, males are on average 20% smaller than females). Differences in morphology between male and female include the antennae of the male are plumous and mouthparts are modified for nectar feeding. The abdominal tergites are covered in dark scales. Legs are black with white basal scales on each tarsal segment. The abdomen narrows into a point characteristic of the genus *Aedes*. Field identification is very easy because of these distinct features.

2.6 Breeding Habitats of *Aedes albopictus*

The breeding habitats of *Ae. albopictus* are extremely variable. This mosquito is a semi-domestic container breeder which has adapted to a wide range of environmental conditions. It exploits a variety of different larval habitats (Hawley, 1988; Ayres *et al.*, 2002) and has the ability to colonise new areas. This species has been shown to have distinct cold tolerant and tropical strains (Knudsen, 1995).

It is usually found within urban, suburban, rural and forested environments, in tropical, subtropical and temperate climatic regions (Hawley, 1988). In densely crowded urban areas which lack vegetation and outdoor breeding sites, or rural areas where the vegetation has been removed, this mosquito may be rare or absent (Hammond, 1966 cited in Hawley, 1988).

Aedes albopictus is a container breeder which is known for the wide range of container types it inhabits, which vary in size and type of material. This species predominantly breeds in fresh water and documented habitats include artificial containers such as used tyres, tins, bottles, vases, buckets, pot plant saucers, plastic drink cups, cans, rain gutters, ornamental ponds, birds baths, concrete mixers (Alto and Juliano, 2001). It breeds in natural containers including tree-holes, coconut shells, bamboo and fern stumps, leaf axils, rock pools and rock holes (O'Meara, 1997). It has been recorded from subterranean habitats such as underground stormwater drains (Blackmore, 1995). The most typical habitats are man-made containers and tree holes.

2.7 Surveillance and Management of *Aedes albopictus*

Aedes albopictus has proven to be very difficult to suppress or to control due to their remarkable ability to adapt to various environments, their close contact with humans, and their reproductive biology (The Titi Tudorancea Bulletin, 2010).

Efficient monitoring or surveillance is essential to prevent the spread and establishment of the species. In addition to the monitoring of ports, warehouses with imported plants, and stockpiles of tires, rest areas on highways and train stations should be monitored with appropriate methods (The Titi Tudorancea Bulletin, 2010).

The control of the Asian tiger mosquito begins with destroying the places where they lay their eggs, which are never far from where people are being bitten, since the mosquito is a weak flyer, with only about a 200-yard lifetime flying radius. Flower pots, standing flower vases, knotholes and other crevices that can collect water should be filled with sand or fine gravel to prevent mosquitoes from laying their eggs in them. Litter can also hold rain water and should be removed (The Titi Tudorancea Bulletin, 2010).

The standing water in pools and catchment basins that cannot be drained or dumped can be periodically treated with properly labeled insecticides or *Bacillus thuringiensis israelensis* (*Bti*). *Bti* is a bacterium that produces toxins which are effective in killing larvae of mosquitoes and certain other Dipterans, while having almost no effect on other organisms. *Bti* preparations are readily available at farm, garden, and pool suppliers (The Titi Tudorancea Bulletin, 2010).

Insecticide application against adult mosquitoes has only a limited effect. Most mosquito spraying done at night will have little effect on Asian tiger mosquitoes, if the resting places are not treated. Daytime spraying may be a violation

of label directions if foraging bees are present on blossoms in the application area. More importantly, larvae and the dried eggs are a reservoir from which the mosquito population will usually recover rapidly (The Titi Tudorancea Bulletin, 2010).

Management of adult populations is more complicated than for other species due to insecticide tolerance to malathion, temephos and bendiocarb (Morbidity and Mortality Weekly Report 1987). In many suburban areas, complaints to health departments are more frequently due to *Ae. albopictus* was the most commonly reported nuisance mosquito (Morbidity and Mortality Weekly Report 1989). Source reduction is an effective way for people in the community to manage the populations of many mosquitoes, especially container breeding species such as the Asian tiger. The removal of mosquito breeding habitat can be an effective method for mosquito control. The elimination of any standing water in the containers, old tires, and overflow dishes for potted plants should be applied regularly. Personal protection should be used to avoid mosquito bites. Long sleeves and insect repellent such as DEET will reduce exposure to bites. The Asian tiger mosquito is a day biter with feeding peaks in the early morning and late afternoon, so by limiting outdoor activities during crepuscular periods (dawn and dusk) when mosquitoes are generally most active, bites can be avoided (Rios and Maruniak, 2004).

Aerosol space sprays consist of small airborne droplets of insecticide designed to kill adult mosquitoes on contact, but difficulty in reaching indoor adult resting sites can limit their efficacy. Even when space sprays are effective in reducing adult populations, effects are transient due to the continuing emergence of new adults or immigration from untreated areas (Jacklyn *et al*., 2011).

A new paradigm for control should include focused surveillance and strategies that kill adult mosquitoes, and development and testing of products that

appeal to the consumer; this could make national programs more effective and cheaper, and therefore more attractive (Morrison *et al*., 2008).

2.8 Types of insecticides for controlling *Aedes albopictus*

Pesticide treatment has continued to be employed as the principal measure for their control (WHO, 2006). However, resistance of mosquitoes to organochlorides, organophosphate, and pyrethroid pesticides have been reported in various studies (Sawby *et al*., 1992). These pesticides are frequently used for their control as well as in agriculture.

In Malaysia, temephos (Abate® 1% sand granules) has been the larvicide of choice for the control of immature *Ae. aegypti* (Lee and Lim, 1989). It has been documented since 1965 (Gulyas, 1972) in ponds, marshes and swamps at a dosage of 0.1-0.5 kg/ha for vector control even though fewer studies exist in relation to *Ae. aegypti*. According to the WHO Pesticides Evaluation Scheme, temephos can be used safely in pottable water with the dosage not exceeding 56-112g/ha (1 mg/L) (WHO, 2009). However, the susceptibility of this mosquito to temephos is decreasing (Lee and Lim, 1989). In mosquito, temephos resistance has been associated with the alteration of its target site in acetylcholinesterase and also with metabolic mechanisms associated with enzymes involved in the detoxification of xenobiotic compounds (Brogdon and McAllister, 1998; Hemingway *et al*., 2004; Casida and Durkin, 2013). Therefore, the resistance to temephos need other methods of control on mosquitoes.

Controlled semifield studies showed that *Bti* can be effective for about 7-12 weeks (Mulla *et al.,* 2004; Vilarinhos and Monnerat, 2004) in undisturbed conditions.

Although *Bacillus thuringiensis* var. *israelensis (Bti)* can be used to prevent these vectors from breeding, the bacteria cannot self-replicate and as such the residual activity is inadequate. Hence, it is essential to evaluate alternative insecticides that could be used in the event of resistance (Indra *et al*., 2005).

Due to low mammalian toxicity, speed of action, and high insecticidal activity, pyrethroids (Briet *et* al., 2013) are the only insecticide class recommended by the WHO for use in insecticide-treated bednets (ITNs) (Hougard *et al*., 2003). It is effective on the African vectors *Anopheles gambiae* s.s. and *An. funestus* because these species are endophagic (feed indoors) and endophilic (rest indoors after feeding). Apart from its insecticidal activity, pyrethroids also exert an excito-repellency effect, which can lead to fewer mosquitoes entering house (deterrence) when ITNs are used. It also disrupted blood feeding and premature exit of mosquitoes from the house (induced exophily) (Okumu *et al*., 2013). Because of the excito-repellency property of ITNs, these nets retain their personal protection properties for users even after the nets become holed (Darriet *et al*., 1984).

The insecticide resistance to all four classes of insecticides (pyrethroids, organochlorines, organophosphates, and carbamates) threatens the effectiveness of ITNs and indoor residual house spraying. Recently, 27 countries in sub-Saharan Africa have reported pyrethroid resistance in *Anopheles* vectors (Ranson *et al*., 2011). Because of the pyrethroid dependency, ITNs are especially vulnerable to insecticide resistance, as unlike indoor residual house spraying, there are available alternative insecticides. To prevent amplifying pyrethroid resistance, the WHO recommends that pyrethroid insecticides should not be used for indoor residual house spraying in areas with high long-lasting insecticide-treated bed net coverage (WHO, 2011). Therefore, insect growth regulators should be the alternative ways to control the mosquitoes.

2.9 Insect growth regulators

Insect growth regulators (IGRs) have been found to be highly active against mosquitoes. Most of the compounds evaluated are juvenile hormone analogues (JHA), benzamides, carbamates and urea-type compounds. Some IGRs show ovicidal activities and sterilizing effects and have been designated as third generation insecticides. They induce a variety of morphogenetic aberrations (Mulla, 1991).

In mosquito control programme, most of the IGRs are applied against the aquatic stages of mosquitoes. Mosquito larvae are ideal targets for IGR activity because most IGRs have delayed activity, inducing mortality or morphogenetic abnormalities in stages. IGRs do not induce immediate mortality in treated larvae. Mortality in various stages is dosage dependent, rapid mortality found in the treated stages at higher dosages, but at lower dosages mortality time is delayed and effects materialize in later stages (Mulla, 1991).

Test methods for IGRs in the laboratory take a longer period of action than for the quick-acting larvicides such as organophosphate and synthetic pyrethroid compounds. The IGR evaluation has to follow a treated cohort all the way to adult emergence requiring 4 to 10 days or longer, while the fast-acting compounds produce maximum mortality in 24 to 48 hours (Mulla, 1991).

WHO (1992) have suggested various controlling strategies to control vector transmission at different levels. Among the available vector control methods, chemical control is decisively superior over environmental and biological control strategies that have limited applicability in mitigating sporadic unpredictable outbreaks of vector borne disease. However, *Cx. quniquefasciatus* has also shown resistance to different insecticides used in mosquito control such as organochlorines, organophosphorous, pyrethroids and microbial insecticides throughout the world

(Tikar *et al*., 2008). Among the various synthetic insecticides, insect growth regulators (IGRs) are very specific in their mode of action for insect pests and safer on non-target organisms and the environment (Mulla, 1995). IGRs interrupt the functioning of endocrine mechanism or synthesis of chitin cuticular structures during development of insect (Tunaz and Uygun, 2004). Various synthetic IGRs such as Juvenile Hormone Analogues (methoprene and fenoxycarb) and Chitin Synthesis Inhibitors (diflubenzuron, lufenuron, hexaflumuron, triflumuron, cyromazine and novaluron) have been used against mosquitoes and different insect pests (Cetin *et al*., 2006).

Fenoxycarb was the first JHA compound used to control agricultural pests (Miyamoto *et al*., 1993). Fenoxycarb in which part of the aliphatic chain has been replaced by pyridyl oxyethlene with high insecticidal activities (Riddiford, 1994). Fenoxycarb mimics the action of the juvenile hormones on moulting and reproduction. Fenoxycarb kills eggs and larvae of numerous insect species (Masner *et al*., 1987). The exposure of low concentration of fenoxycarb on mosquito larvae causes the embryonic development to reach halfway, its development is halted and the egg will not hatch. The exposure of high concentration on later instars causes the adult insect to maintain larval characteristics and these insects generally cannot reproduce. Fenoxycarb also disrupted normal reproductive physiology of adults (Sullivan, 2000).

Diflubenzuron and cyromazine induce changes associated with the larvae shedding from the earliest developmental stages (Schmidtmann *et al*.,1989; Miller *et al*., 1991). Larvae cannot shed their old skin and move to the following developmental stage. Those of them that survive have abnormal shapes and some produce deformed pupae. Diflubenzuron administered to adult flies in feed had no

effect on egg laying but caused irreversible changes in hatchability (Kocisova *et al.*, 2000). Under practical conditions, the reduction in the number of flies was recorded 4-5 weeks following the first application, which limits the single use of diflubenzuron and cyromazine (Kocisova *et al*., 2000).

The juvenile hormone analog, methoprene has been used for controlling mosquitoes and other pests belonging to the order Diptera over the past 30 years. In these insects, methoprene induces various morphogenetic and developmental abnormalities resulting in their death during or immediately after metamorphosis (Mulla, 1995). According to Wu *et al*. (2006), the results showed that a majority of *Ae. aegypti* larvae treated with methoprene died during the pupal stage.

The benzoylphenyl ureas, hexaflumuron and lufenuron are insect growth regulators that interfere with chitin synthesis, disrupt hormonal balance with exchanging in molting process and inhibit the growth of insects (Oberlander and Silhacek, 1998). According to Khajepour *et al*. (2011), application of hexaflumuron and lufenuron on the last instar larvae of *Ephestia figulilella* caused mortality in the larval stage, defects in pupal and adult stages, and in some cases produced larval-pupal intermediates. Malformed pupae were not able to form a pupal case and never emerged into adults. Malformed larvae were nor able to feed and eventually died (Khajepour *et al*., 2011).

Under laboratory conditions, triflumuron was effective against *Ae. aegypti* (Martins *et al*., 2008). When larvae are exposed to a sublethal dose, the viability of resulting adults is affected (Belinato *et al*., 2009). A small number of larvae always remained alive for many days, since the primary action of CSIs is not to induce mortality, but to interfere with development.Triflumuron induced adult emergence

inhibition of *Ae. albopictus* and *Cx. quinquefasciatus* in a dose-dependent way (Belinato *et al*., 2013).

There are a limited number of larvicide products available on the market today to control mosquito populations in urban areas. Some of these products offer control for 21-30 days and only one of these products offers control for a period greater than 30 days. Thus, in most cases, breeding sites must be treated 3 to 4 times per season to achieve seasonal control. Therefore, there is a need for more products that offer control throughout an entire season (Shannon, 2004).

In 2003, Mulla *et al.* conducted a lab and field evaluation of novaluron against *Aedes aegypti*. Results of the study indicated that 10µg/L to 20µg/L of Novaluron provided 100% control of *Ae. aegypti* for a period of 2 months (60 days).

In recent years, a new chitin synthesis inhibitor, novaluron, has become available for the control of a variety of insect pests. Novaluron meets this criterion and it has never been evaluated or tested for activity and efficacy against mosquitoes (Mulla *et al*., 2003). In 2002, extensive laboratory and field studies on novaluron were carried out against *Culex* mosquitoes, where novaluron technical material and EC_{10} (emulsifiable concentrate containing 10% active material) exhibited a high level of activity against *Culex* mosquitoes (Su *et al*., 2003), equaling or surpassing that of diflubenzuron and pyriproxyfen, two commercially available IGRs used in mosquito control programme outside the U.S.A. (Mulla, 1974, 1991, 1995).

Novaluron is a chitin synthesis inhibitor (CSI) belonging to the benzoylphenyl urea group of insecticides. This insecticide acts by inhibiting the production of chitin, a major component of the insect exoskeleton. Insects treated with CSIs are unable to synthesise new cuticle, and therefore unable to successfully moult into the next stage (Ijumba *et al*., 2010). Novaluron has low acute, sub-acute

and chronic toxicity to mammals, birds, fish, earthworms, soil microflora and aquatic plants. Novaluron has no evidence of mutagenic or oncogenic potential. It is already registered for use in agriculture in many countries including USA, Switzerland, Israel, India, and etc., where it is used on a wide variety of crops including maize, potato and soya bean (Shannon, 2004).

2.10 Introduction of novaluron

Novaluron is an insecticide of the class diflubenzoylureas. The compound shows a killing effect against various larvae of the genus Lepidoptera, Coleoptera, Hemiptera, and Diptera. Novaluron has been registered as an insecticide for food crop in foreign countries, including South Africa, Argentina, and Australia. United States authorities approved the compound's registration as a pest control drug for floriculture in September 2001. The compound was applied for registration according to the Agricultural Chemicals Regulation Law by S.D.S. Biotec Inc. (hereinafter "The Applicant") in November 2001 (Mulla *et al*., 2003).

2.11 Mode of action of novaluron

Novaluron is an insecticide which inhibits chitin synthesis, affecting the moulting stages of insect development. It acts by ingestion and contact and causes abnormal endocuticular deposition and abortive moulting. It is used in agriculture/horticulture on a wide range of crops including cotton, soya, maize, pome fruit, citrus, potato and vegetables against a wide range of pests. Novaluron is under evaluation by WHOPES as a mosquito larvicide (Rachid *et al.*, 2009). The chemical structure of novaluron is shown in Figure 2.1.

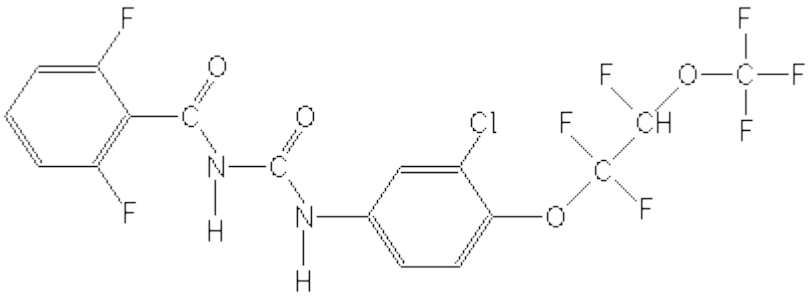

Figure 2.1: *N*-[[[3-chloro-4-[1, 1, 2-trifluoro-2(trifluoromethoxy) ethoxy] phenyl] amino] carbonyl] - 2, 6-difluorobenzamide

2.12 Chemical composition of novaluron

Novaluron N-[[[3-chloro-4 [1, 1, 2-trifluoro-2- (trifluoromethoxy) ethoxy] phenyl] amino] carbonyl] 2, 6- difluorobenzamide, a relatively new CSI (Chitin synthesis inhibitor), that inhibits the chitin formation on larvae of various insects (Lepidoptera, Coleoptera, Homoptera and Diptera). It has a potent insecticidal activity against several important foliage feeding insect pests, and very low toxicity to mammals, birds and earthworms. By inhibiting chitin formation, Novaluron selectively targets immature insect stages, causing abnormal endocuticular deposition abortive moulting (Rachid *et al*., 2009).

2.13 Autodissemination station

Autodissemination is a pest management method in which insects contaminated with a biological or chemical insecticide transfer lethal concentrations horizontally or vertically to others insect via mating, oviposition, aggregation and other behaviours. Autodissemination is a ‘pull’ (attraction and transfer) and ‘push’ (dispersal and transfer to target habitats) technology. This approach offers the

potential for economic (savings in product and labour) and environmental (less active ingredient/ha as a result of accurate targeting afforded by exploiting insect behaviour) benefits relative to broadcast spray applications (Gaugler *et al*., 2011).

Autodissemination is particularly well suited to be used with insect pathogens because of the small size and host replication ability that are characteristic of infectious microbes and, accordingly, insect pathologists have pioneered this area of investigation. Most studies have examined the dissemination of entomopathogenic fungi and baculoviruses (Soper, 1978; Yu and Brown, 1997; Klein and Lacey, 1999), but even nematode autodissemination has been considered (Lacey *et al*., 1993). These strategies tend to centre on the use of a pheromone lure to bring insects into a contamination station in which there are topically contaminated with a pathogen before they exit the station. The autoinoculated insects serve as carriers to disperse the agent to other habitats (Gaugler *et al*., 2011).

According to Geden and Devine (2012), the evaluation of the potential for autodissemination by female flies to larval development sites is effective. Adult female house flies exposed to filter paper (3.75% active ingredient) or sugar treated with pyriproxyfen (0.01-0.1%) produced significantly fewer F1 pupae than untreated flies. The treatment of larval rearing medium with 0.35ml/cm^2 of a 12mg pyriproxyfen/liter preparation had no effect on the number of pupae developing from eggs but markedly inhibited adult emergence from those pupae (Geden and Devine, 2012).

Less consideration has been given to deploying chemical insecticides in autodissemination. Chemicals would seem to be poorly suited to autodissemination because, in addition to being unable to replicate, they tend to kill or incapacitate

contaminated individuals, which greatly restricts dispersal. Itoh (1994), however, recognized a unique opportunity to use the insect growth regulator (IGR), pyriproxyfen, in an autodissemination strategy against mosquitoes. He noted that pyriproxyfen is a mosquito larvicide that does not impair adult activity (Kawada *et al*., 1993), is active at extraordinarily low concentrations (LC_{50} in *Ae. aegypti* is 0.023 ppb, which is equivalent to 200 times the activity of temephos), and that females might serve as vehicles for transferring insecticides to other larval habitats (Schlein and Pener, 1990). Itoh (1994) exposed blood-fed *Ae. aegypti* in the laboratory to surfaces treated with pyriproxyfen and then allowed the females to oviposit in cups of water seeded with larvae, after which he recorded pyriproxyfen transfer from the females to the water inhibited adult emergence (Gaugler *et al*., 2011).

Devine *et al*. (2009) extended Itoh's (1994) concept to the field, conclusively demonstrating that gravid female *Ae. aegypti* contaminated with pyriproxyfen transferred lethal concentrations to larval habitats. In this experiment, dissemination stations are constructed from plastic pots holding 200 mL of water. The cloths are dusted with pyriproxyfen granules that had been crushed into a fine powder. The powder adhered to the cloth lining, providing a treated surface for the transfer of the IGR powder to visiting females. Sentinel stations (ovicups of water serving as oviposition sites) holding larvae are placed near the dissemination stations. Nearly all of the sentinel stations are visited by contaminated adults and subsequent adult emergence is reduced by 42-98% in replicated experiments. Thus, high coverage of larval habitats is achieved by treating an extremely small area. In short, Devine *et al*. (2009) exploited female oviposition behaviour to demonstrate pyriproxyfen

autodissemination as a possible new approach to the management of container-dwelling mosquitoes (Gaugler *et al*., 2011).

A prototype device for the autodissemination of pyriproxyfen in container-dwelling mosquitoes that is standardized, inexpensive, biodegradable, and free of maintenance requirements, offers extended toxicant activity and maximizes the tropical transfer of the toxicant by manipulating the search behaviour of gravid females. The test subject is the Asian tiger mosquito, *Ae. albopictus*, which, like *Ae. aegypti* is a highly urbanized container-dwelling species, colonizes small-volume crytic larval habitats, and is a skip ovipositor that distributes eggs among multiple water-holding container (Trexler *et al*., 1998). These are highly favourable traits for supporting autodissemination to mosquito oviposition sites (Gaugler *et al*., 2011).

As a possible method of application to larval microcosms of these species that oviposit in water containers and phytotelmata, the horizontal transfer of pyriproxyfen to larval microcosms by adult mosquitoes is evaluated under laboratory conditions. Gravid females are forced to walk on surfaces treated with pyriproxyfen (tarsal contact exposure) and then allowed to oviposit in larval microcosms. Using replicate bioassay cages, each with an oviposition container, and a factorial experimental design, the effects of (i) pyriproxyfen concentration (0.2, 0.3 and 0.4 mg/cm^2) contacted by gravid females, and (ii) the number of treated gravid females added to bioassay cages (one, three or five females/cage) were assessed and the mortality of larvae in oviposition containers were determined (Dell and Apperson, 2003).

The use of IGR-treated oviposition containers to achieve horizontal transfer of pyriproxyfen to mosquito oviposition sites could be a field management technique based on mosquito biology and behaviour. In binary choice tests with *Ae. albopictus*,