ACUTE AND SUBACUTE TOXICITY OF SPINOSAD AND SPINETORAM DELIVERED IN SUGAR SOLUTION TO ADULT *AEDES AEGYPTI* AND *AEDES ALBOPICTUS* (DIPTERA: CULICIDAE)

By

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ABSTRACT

ACUTE AND SUBACUTE TOXICITY OF SPINOSAD AND SPINETORAM DELIVERED IN SUGAR SOLUTION TO ADULT *AEDES AEGYPTI* AND *AEDES ALBOPICTUS* (DIPTERA: CULICIDAE)

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Mosquito-borne pathogens is one of the significant sources of human mortality and morbidity around the world, in particular dengue fever whose principal vectors are the mosquitoes *Aedes aegypti* (Linnaeus) and *Aedes albopictus* (Skuse). A new method of vector control takes advantage of sugar feeding by mosquitoes and involves toxins incorporated into sugar meals presented in bait formulations. Spinosyns comprise a family of bacterial secondary metabolites with a unique mode of action against the insect nervous system, an appealing environmental safety profile, and potential for incorporation in sugar baits. This research evaluated acute and subacute toxicity of spinosad and spinetoram (combinations of certain spinosyns and derivates) in sugar solution as an oral toxin against adult *Ae. aegypti* and *Ae. albopictus*. Spinosad and spinetoram delivered in sugar solution were toxic to males and females in bioassays. Toxicity as measured by an acute exposure doubled from 24 to 48 hours of assessment, revealing a relatively slow action. Spinetoram tended to be more acutely toxic than spinosad. Subacute exposure to these products in sugar solution the exposure significantly reduced the survivorship of males and females of *Ae. aegypti* and *Ae. albopictus* as revealed by longitudinal Kaplan-Meier analysis. Fecundity was not significantly affected for *Ae. aegypti* but was higher in exposed compared to nonexposed females, whereas it significantly increased for *Ae. albopictus*, possibly due to a hormesis effect. Fertility, on the other hand, was significantly

reduced following the exposure to either spinosad or spinetoram in sugar solution for both *Aedes* species, suggesting *in vivo* toxicity to eggs in those females surviving subacute exposures.

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KEY TO ABBREVIATIONS

WHO	world health organization
CDC	centers for disease control and prevention
LC	lethal concentration
ppm	parts per million
S.E.	standard error
IRS	indoor residual spraying
Bti	*Bacillus thuringiensis* serotype *israelensis*
Bt.H-14	*Bacillus thuringiensis* serotype H-14
DENV	dengue virus
IGR	insect growth regulator
ULV	ultra-low volume
Cx	*Culex*
DDT	dichlorodiphenyltrichloroethane
nAChRs	nicotinic acetylcholine receptors
IVM	integrated vector management
DF	dengue Fever
DHF	dengue Hemorrhagic Fever
ITN	insecticide-treated net

DSS	dengue Shock Syndrome
ATSB	attractive toxic sugar bait
WG	water dispersible granule
SC	suspension concentrate.
AI	active ingredient
LC50	median lethal concentration
χ^2	chi square
C.I.	confident interval
DI	distilled water
Ae.	*Aedes*
Bs	*Bacillus sphaericus*
An.	*Anopheles*

CHAPTER 1: LITERATURE REVIEW

INTRODUCTION

Arthropod-borne viruses ("arboviruses") collectively remain one of the major infectious disease scourges of mankind. Classified typically into three families across four genera (the Flaviviridae, genus *Flavivirus*; Togaviridae, genus *Alphavirus*; and Bunyaviridae, genera *Orthobunyavirus* and *Phlebovirus*), they represent a diverse array of viruses with RNA genomes, exhibit a wide range of pathology during the course of infection in humans, and great flexibility across host ranges including both vertebrates and invertebrates. They often exist in zoonotic transmission cycles and few persist with humans as the sole vertebrate host, although certain ones commonly do so such as yellow fever, dengue, zika, and chikungunya viruses. Many show properties of epidemic behavior and emergence from obscurity to widespread geographic range and importance.

Important examples of arboviruses are yellow fever, Japanese encephalitis, dengue, West Nile, Powassan, and tick-borne encephalitis (all flaviviruses); Venezuelan equine encephalitis, eastern equine encephalitis, chikungunya, o'nyong-nyong, Ross River, and Semliki Forest (all alphaviruses); La Crosse, Jamestown Canyon, and Crimean-Congo hemorrhagic fever (all orthobunyaviruses); and sand fly fever (a phlebovirus). Most of the arboviruses are associated with biting flies (mainly mosquitoes, but also biting midges and sand flies). Some are transmitted by ticks.

Many of the mosquito-borne arboviruses are associated with transmission by *Aedes* species with strong affinities for the human living environment. *Aedes (Stegomyia) aegypti* as a single species has been responsible for major epidemics of viruses spanning centuries, including

Yellow fever, Dengue, Chikungunya, and Zika. Many other *Aedes* species in the subgenus *Stegomyia* are also important in transmission of these and other viruses. In the following paragraphs, the biology and epidemiology of dengue viruses is reviewed as a prime example of these *Aedes*-associated viruses, noting that many of them have nearly identical transmission cycles.

DENGUE FEVER

Dengue viruses, consisting of four major serotypes, are associated with the disease known as dengue fever (DF) and a severe form called Dengue Hemorrhagic Fever (DHF). These are very important arboviral infections and disease manifestations in terms of morbidity and mortality and are persistent and geographically expanding diseases around the world (Fig. 2). Dengue viruses are spread primarily by *Aedes aegypti* and *Aedes albopictus* , although other *Aedes* species of more restricted distribution can be important locally.

Dengue is internationally a major public health problem, occurring in more than 100 sub-tropical and tropical regions around the world. Globally, around fifty million causes of dengue infection occur each year and over 2.5 billion of people are threaten by dengue fever (DF) with either dengue hemorrhagic fever (DHF) or dengue shock syndrome (DSS) which are the severe forms of dengue. When dengue fever spreads to new areas the outbreaks frequency is increasing as well as the disease epidemiology. Generally, dengue epidemics are regular occurrences in some temperate, and many tropical and subtropical regions in the world. In 1635, the first dengue epidemic was recorded in the French West Indies (Howe 1977). In Philippines, DHF was recorded and confirmed as first DHF epidemiology was between 1953-1954 and in Thailand and India in 1958 and 1963 respectively. Also, some other countries have reported DHF outbreaks, such as Myanmar, Indonesia, Sri Lanka, and Maldives (WHO 2011).

Outbreak of dengue fever first occurred in the Middle East and East Africa in the 1990s, where major epidemics occurred in Jeddah, Saudi Arabia, in 1994 and in Djibouti in 1991. These outbreaks were the first time in those places in over 50 years (Gubler 1998; Al-Shami et al. 2014; WHO 2011). Dengue fever is still serous public health problem in Saudi Arabia, where it has caused more illness to human over years (Fig. 1) (Al-Shami et al. 2014).

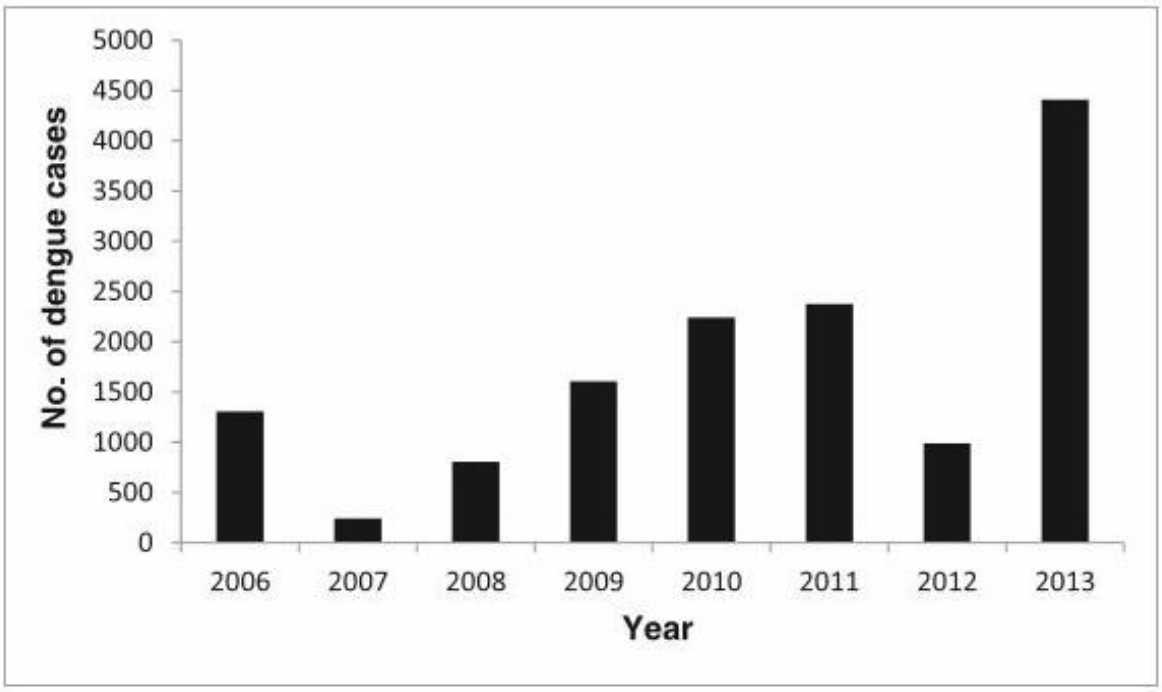

Figure 1: Number of confirmed dengue cases reported from 2006 to 2013 in western regions of Saudi Arabia (Al-Shami et al. 2014).

The symptoms of dengue fever including, body aches, joint pains, frontal headache, weakness, rash, and retro-orbital pain. The mechanisms of progression of typical dengue fever to dengue hemorrhagic fever are not well understood; however, the main important factor involving in developing DHF is exposure to another serotype after primary exposure. (Gubler 1998; WHO 2011).

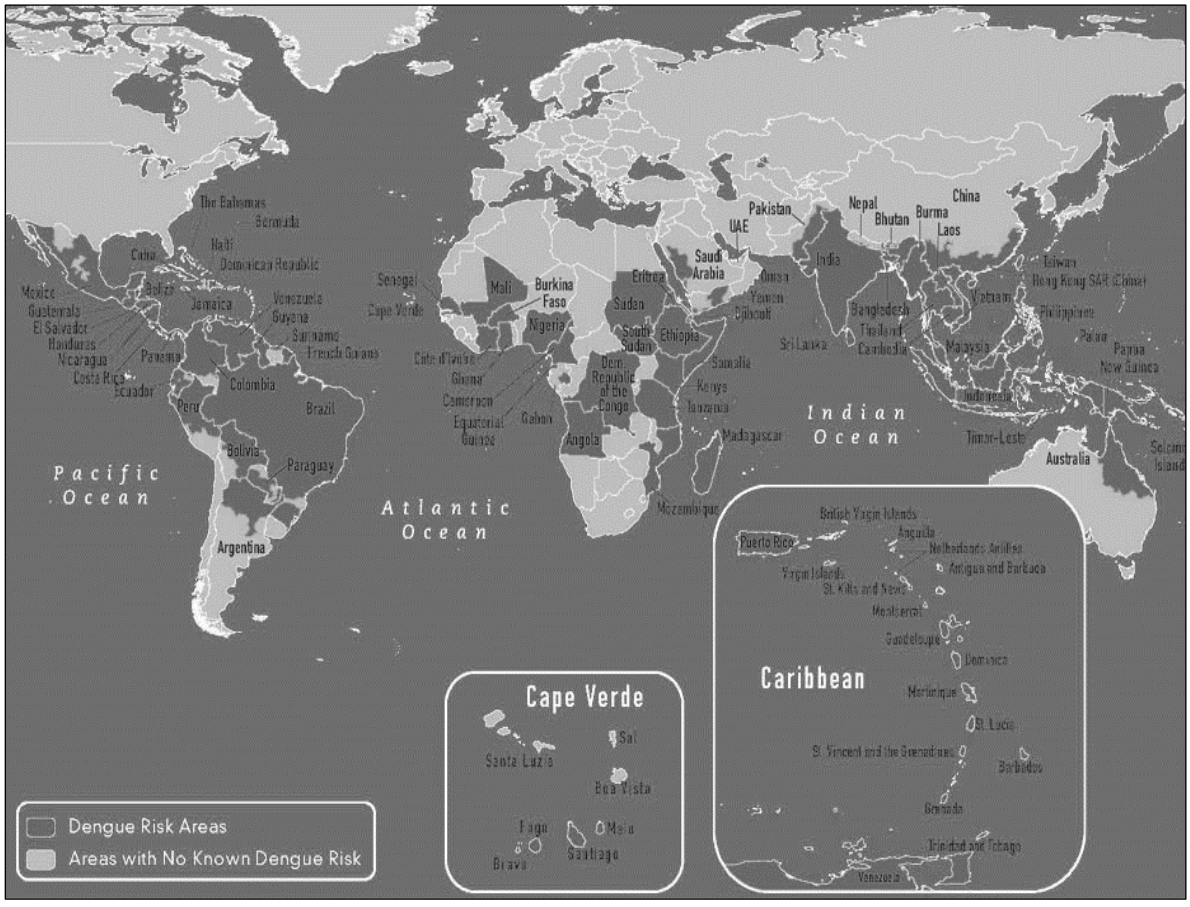

Figure 2: Distribution of dengue fever in countries or areas that are at risk as well as areas without risk of dengue fever transmission. (CDC, 2016).

Typically, geographic regions vulnerable to dengue endemicity may change from hypoendemic where one serotype is present to hyperendemic where multiple serotypes are present or to no serotype present which called non-endemic region (Gulber 1998). There are several reasons for the emergence of dengue fever in some regions, such as lack of dengue vector control, substandard living conditions, virus evolution, international travel, and climatic change. As a result, dengue fever is classified as major international public health problem because of the capability of distribution of both the mosquito vectors and the virus geographically to new areas.

DENGUE TRANSMISSION FACTORS

Transmission of dengue fever is depending on two main factors. First factor is biotic which including vector, host, and virus, whereas the second factor is abiotic, including humidity, rainfall and temperature. Dengue fever is usually transmitted during the tropical and subtropical rainy seasons where the humidity and temperature are favorable to build up *Aedes* mosquito breeding habitats as well as increase the *Aedes* mosquito survival (WHO 2011). During the dry season in the arid areas where rainfall is limited, *Aedes* mosquitoes can build up the habitats in available artificial human storage containers.

Figure 3: Geographic distribution of yellow fever mosquito, *Ae. aegypti* around the world (source: Dengue 2007, the lancet. com).

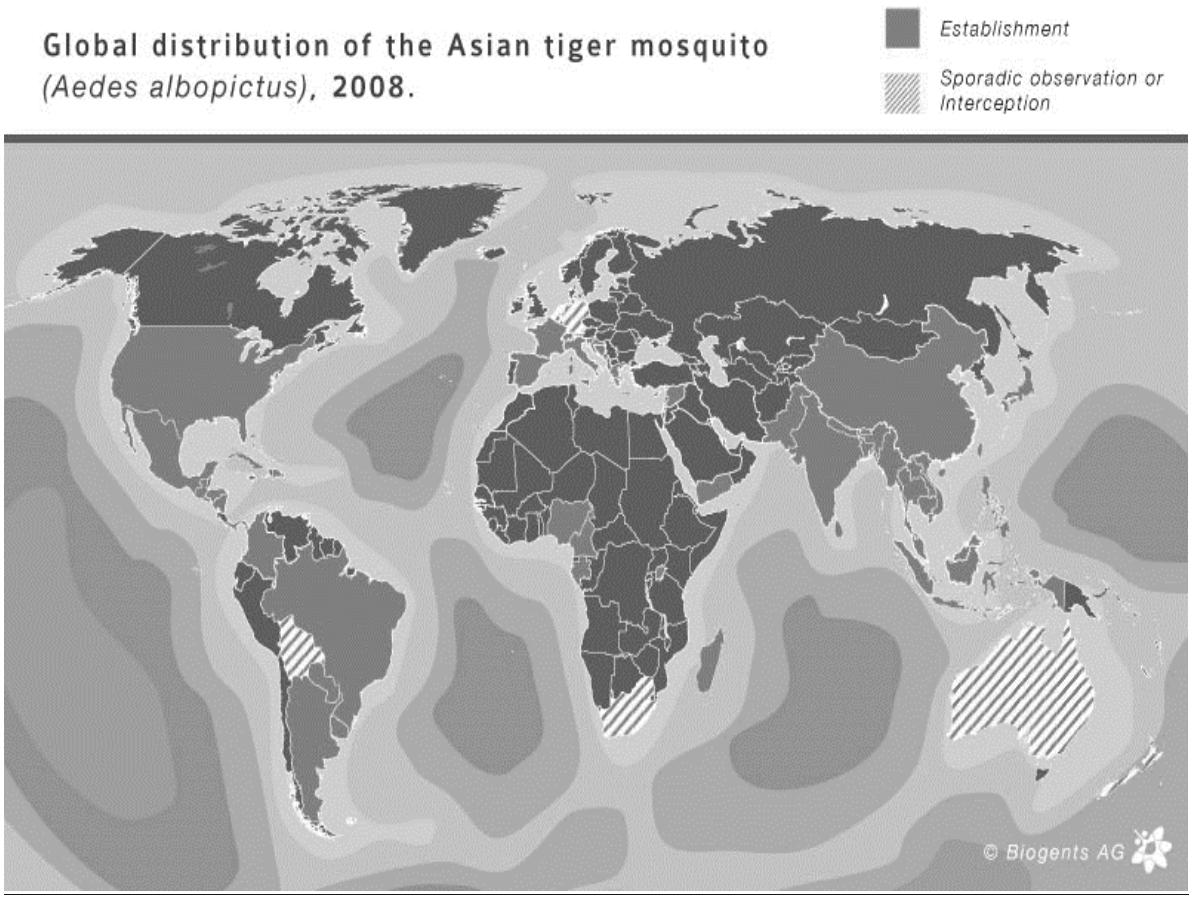

Figure 4: Geographic distribution of Asian tiger mosquito, *Ae. albopictus* around the world in 2008 (source: Asian tiger mosquito, glogster.com).

THE VIRUS

Dengue virus is genomically a small virus contains a single-stranded, positive-sense RNA virus and taxonomically classified in the genus *Flavivirus*, family *Flaviviridae* **(**Westaway and Blok 1997). Dengue virus has four virus serotypes, designated as DENV-1, DENV-2, DENV-3 and DENV-4. Each serotype elicits different antibodies, resulting in the possibility of multiple infections without cross protection. In general, all of the four serotypes are similar; however, there is some variations genetically; human response to infection ranges from no symptoms to fatal hemorrhage depending on the infected person's age and immunity. The classic course of infection is high fever, rash, and severe retroorbital and joint pain lasting for 7-10 days, followed by a period of 2-3 weeks of weakness during convalescence.

The severity of the dengue fever is also depending on the patient's age and genetic background (Bravo 1987), infecting virus strain and serotype, and the degree of the viremia. When, a person becomes infected with dengue virus, there will be an incubation period for the virus from approximately 3 to 14 days with an average of 4-7 days (WHO 2011). The four dengue virus serotypes have a geographically widespread distribution and all the serotypes of dengue virus circulate together in tropical and subtropical regions of the world. Infection with a particular dengue serotype confers life-long, neutralizing immunity only to that serotype, so a person could be infected as many as four times as a result (WHO 2011).

DENGUE TRANSMISSION CYCLES

Dengue virus transmission occurs in three types of cycles: sylvatic, urban, and epizootic (Fig. 5). As was noted above, most arboviruses are maintained in enzootic cycles involving non-human primates, birds, or rodents as reservoir hosts. If humans enter areas with such sylvatic or

enzootic transmission, the enzootic and bridge vectors could transmit virus to those humans, initiating human infection and transmission amongst humans. For dengue viruses, the enzootic transmission cycle is narrowly confined to certain forests of West Africa and Southeast Asia, where certain dengue virus serotypes are maintained by transmission by *Aedes* mosquitoes to some species of monkeys. The virus is apparently nonpathogenic in monkeys, and all four dengue virus serotypes can be transmitted to nonhuman primates by *Aedes* mosquitoes and the viremia duration is 2-3 days (Gubler 1998). However, it is entirely unclear if all four serotypes circulate in this manner in nature and one should not conclude that dengue is a sylvatic and enzootic system with occasional human involvement. By contrast, much or probably most of dengue virus transmission occurs in the urban and occasional epidemic forms amongst humans as the sole primate hosts and *Aedes* mosquitoes as vectors, without any sylvatic involvement at all (Gubler 1988).

Second cycle is the urban cycle. This cycle is considered as most important in terms of transmission cycle due to the high viremia of human infections, such that dengue viruses can be transmitted between humans and *Aedes* mosquitoes with no need for non-human primates as amplification hosts from the enzootic cycle (Gubler 1988). The urban endemic cycle is important cycle because dengue virus is maintained by *Ae. aegypti* as a principal vector and humans as principal reservoir host; it expands to epidemic transmission under conditions such as introduction of a new serotype into an area or when weather favors vector populations (De Silva et al 1999; Gubler 1988).

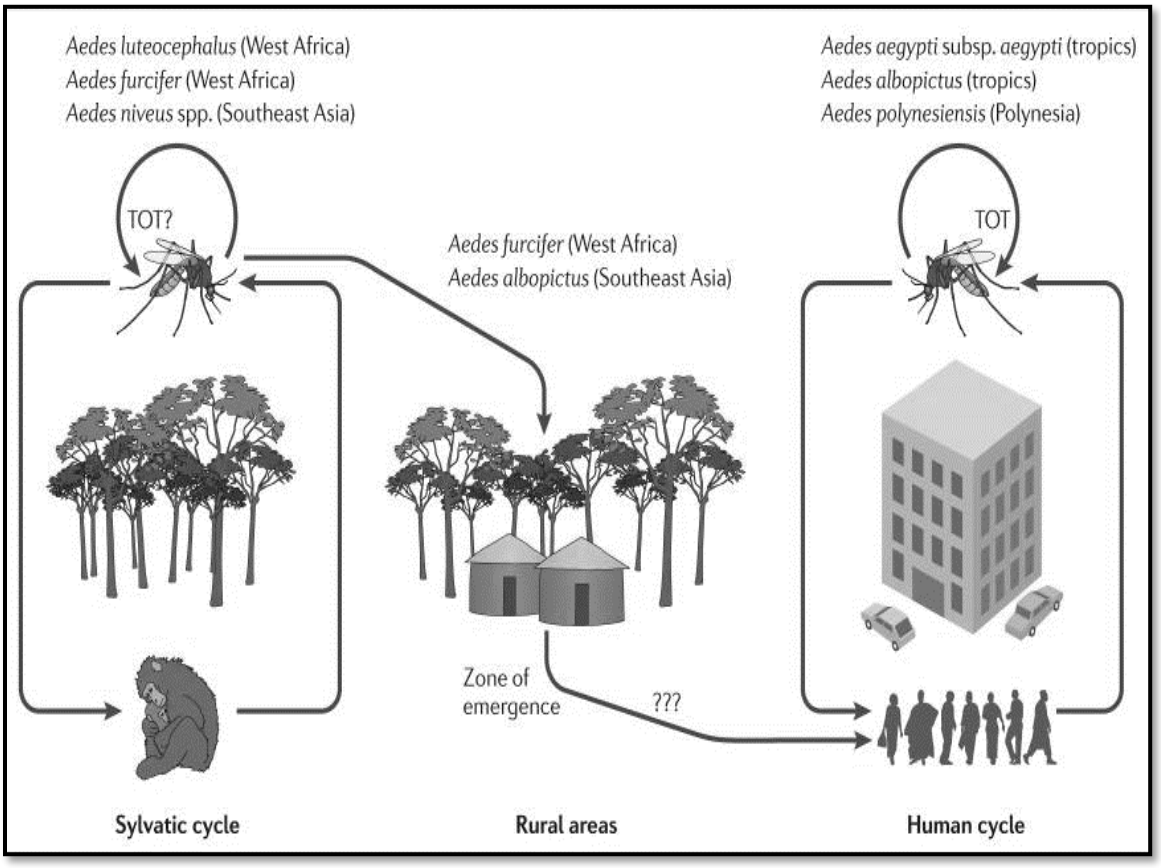

Figure 5: The dengue virus transmission cycle, showing the sylvatic cycle and the zone of emergence and human cycle. The sylvatic cycle, which origins of dengue virus, contacts with human populations in rural areas in Southeast Asia and West Africa. Dengue virus is able to persist in mosquitoes by transovarial transmission (TOT), in which virus transfer from parent to their eggs (source: Vasilakis et al. 2011).

Lastly, the epizootic cycle is a condition where dengue virus transmission takes place by so-called bridge vectors from nonhuman primates to humans. This cycle was reported among macaques (Macaca sinica) in Sri Lanka (Silva et al 1999). Humans and monkeys are both amplifying hosts and the virus can be maintained by transovarial transmission. *Aedes albopictus* is considered a bridge vector between enzootic cycle and human (urban) cycle, because it will bite both nonhuman primates and humans. Also, this mosquito species can maintain the dengue virus transovarially as a reservoir more efficiently than *Ae. aegypti* (WHO 2011). fever.

VACCINE DEVELOPMENT

Dengue fever has no effective vaccine treatment; however, medical care can be an effective method to save patients' lives with the dangerous form of dengue hemorrhagic fever which required hospitalization every year. Recently, vaccine trial results have shown only partial protection, suggesting that dengue virus is going to continue impacting public health for many years (Sabchareon et al. 2012). However, the very effective and primary method to prevent and control dengue fever transmission is to reduce the main virus- carrying vector, *Aedes* mosquitoes. Moreover, understanding the disease in terms of the epidemiology, diagnosis, clinical spectrum, risk factors, and management by people and health care providers in the endemic areas together are important in preventing dengue transmission.

MOSQUITO VECTORS

Aedes aegypti

The yellow fever mosquito, *Aedes* (*Stegomyia*) *aegypti* (L) mosquito (Fig. 6) is a principal urban vector of dengue viruses as well as other viruses such as yellow fever, chikungunya, and recently Zika viruses. This species originated in Africa and was spread as a feral species, with breeding in forests away from human habitats. Later, this species become environmentally adapted to peri-domestic breeding, including storage water containers in African villages. By 1800, this species had established in many tropical coastal cities of the world. Both the transportation of water drums and tires containing *Aedes* mosquito, as well as travel of infected people have contributed to the introduction of the virus into new regions (Lounibos 2002; Gubler 1998; Harrus & Baneth 2005; Christophers 1960).

Figure 6: The yellow fever mosquito, *Ae. aegypti*, is the principal vector for dengue viruses. Here an adult female is shown feeding on a human host (From James Gathany/CDC).

Aedes aegypti is found in tropical and subtropical areas worldwide (Fig. 3) and considered as a principal and efficient vector of dengue viruses because of its high susceptibility to dengue viruses, common feeding on human blood, entering house for human host, and oviposition in water storage containers near human habitats in urban and suburban environments (Trpis and Hausermann 1975; Service 1992; WHO 1997). In addition, this species has ability to feed on multiple human hosts in short period of time for one blood meal (Scott et al. 1993). This species has increased in its distribution in Saudi Arabia where it is also considered as a main vector of dengue fever vector in that region (Al-Shami et al. 2014).

Aedes albopictus

The invasive Asian tiger mosquito *Aedes* (*Stegomyia*) *albopictus* (Skuse) (Fig. 7), known as Asian tiger mosquito, is the second most important mosquito vector of dengue viruses after *Ae. aegypti*. It is an Asian species that had distributed in Asian cities and villages. Since the early 1980s, the species has geographically spread worldwide (Fig. 4) including West Asia, Europe, Africa, and North and South America (Reiter 1998; Lambrechts et al. 2010; Kundsen 1996). This mosquito is a forest species (indeed also called the forest day mosquito) and adapted to rural, urban, suburban environments. Transportation and international shipments that containing eggs of *Ae. albopictus* are responsible for introducing the species into new areas (WHO 2011). This species has high susceptibility to dengue virus infection; however, it is not considered as efficient an epidemic vector of dengue viruses as is *Ae. aegypti* due to its relatively lower vector competence and greater breadth of blood host utilization (Gubler and Rosen 1976; Lambrechts et al. 2010). On the other hand, *Ae. albopictus* has ability to cause a serious of arboviral diseases outbreaks because of its competence to transmit at least 22 types of arboviruses, including

Chikungunya and Zika (Gratz 2004; Lambrechts et al. 2010; Shroyer 1987, Turell et al. 1988, Mitchell 1995, Gratz 2004).

Figure 7: The Asian tiger mosquito, *Ae. albopictus* shown here feeding on a human host (from Texas A&M AgriLife Extension Service photo by Dr. Mike Merchant).

These two *Aedes* species have different habitats: *Ae. aegypti* has been found in urban region with some vegetation and trees, whereas *Ae. albopictus* has been found mainly in suburban and rural, forested habitats with dense vegetation and trees. Also, *Ae. albopictus* has spread out of its native Asian range in the world with ability to exploit of man-made environments.

AEDES MOSQUITO BIOLOGY

All mosquitoes have four different stages in their life cycle (Fig. 8) including egg, larva, pupa, and adult (Goma 1966; Bates 1970). The first three stage of mosquitoes are aquatic,

whereas adult stage is flying insect with ability of feeding on plant nectars and feeding on blood meals for female only (WHO 2011).

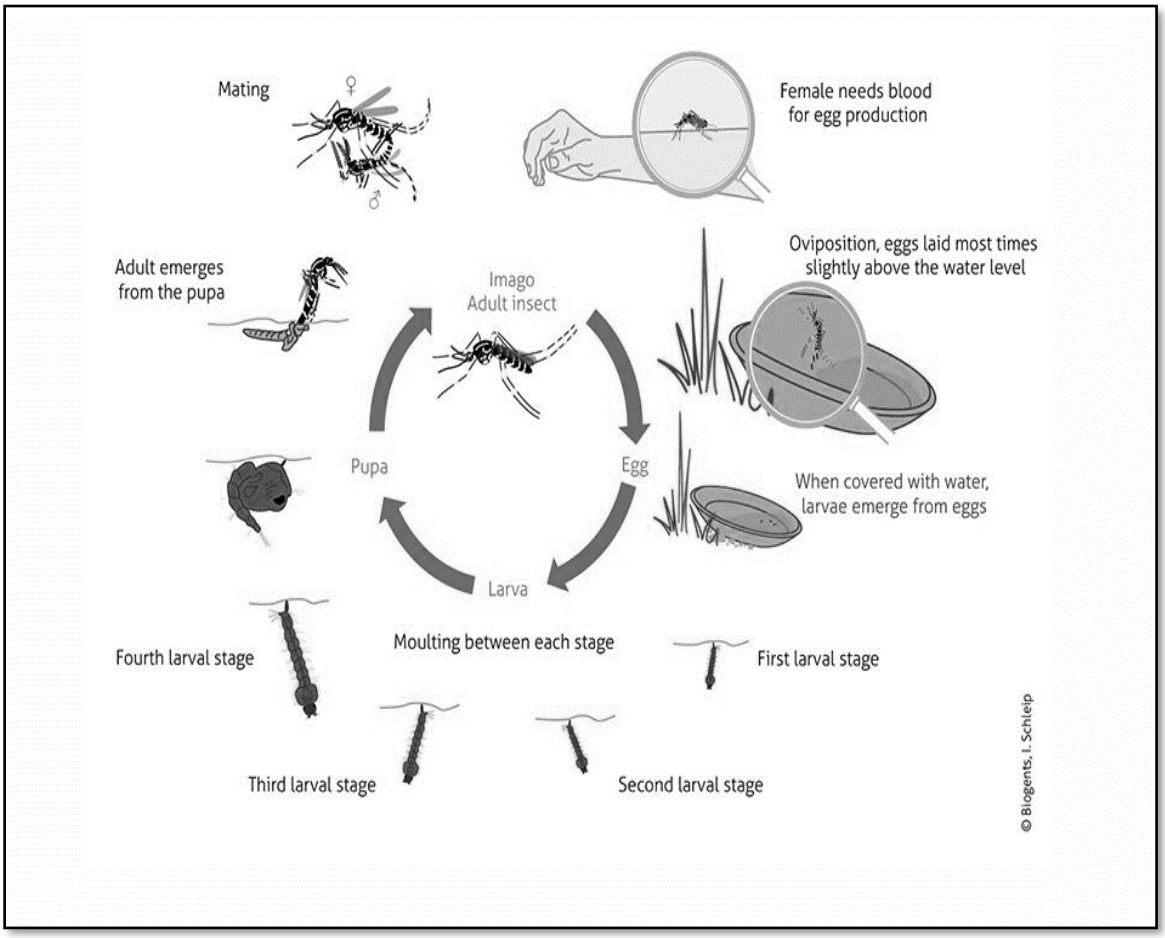

Figure 8: The life cycle of *Aedes* mosquitoes. Female *Aedes* mosquitoes commonly lay their eggs on the inner walls of natural and artificial containers, above the water line. Whenever the artificial containers fill with water, larvae will hatch from eggs. After the development of the four larval stages, the larvae metamorphose into pupae, where the pupal stage is aquatic. The adult mosquito emerges via a process of ecdysis through the pupal skin. Adult mosquitoes harden the exoskeleton, then are able to fly, mate, obtain sugar, and in the case of females blood feeding for eggs development (source: Biogents.com).

Eggs

Aedes mosquito eggs have no floats and are elongate and oval in a shape. The eggs of both *Ae. aegypti* and *Ae. albopictus* as other *Aedes* mosquitoes are similar and black (Linley 1989). Newly laid *Aedes* eggs tented to be soft white in color, and become hard black over time (Christopher, 1960; Schlaeger and Fuchs, 1974). Both *Ae. aegypti* and *Ae. albopictus* eggs are normally laid in a single batch or individually above the water line of breeding places. *Aedes* mosquito eggs are laid singly above the waterline. Usually, the development of embryonic takes about 48 hours to be completed. Eggs hatch when inundated by water. On average, female could produce 100 to 200 eggs, whereas the number of eggs laid is dependent on the bloodmeal size. Most of the eggs may not be laid once, where it can be spread during hours of even days which is depending on the availability of the oviposition sides (Clements 1999).

Larvae

The next stage is the larvae, which have four developmental instars. The development of the larvae depends on several factors including temperature, larval density and food availability (Christopher, 1960). In general, *Aedes* mosquito larvae remain at the surface of water and also swim to the bottom of the container when feeding or if disturbed (Nelson 1986). In dry and hot areas, the primary larval habitats are ground water storage and overhead tanks, while natural larval habitats which are including leaf axils, coconut shells and tree holes are mostly rare. In optimized conditions in the laboratory, larvae may take 5-10 days to become pupae; however, the development period may extend when temperature is low. Males have fast development compared with females, so that males can pupate earlier.

Pupae

The stage after the larval stage is the pupa. Mosquito pupae do not feed, actively swim and passively float. The duration of this stage is short where usually takes about one to two days to emerge (Goma 1966; Bates 1970). Newly emerged pupa appears white in color and later becomes dark (Christopher, 1960). *Aedes* mosquito pupae are rather comma-shaped (Abdel-Malek 1949).

Adult

After adult emerges, female *Aedes* mosquito mate with adult male. Mating occurs soon after emergence between adult male and female, where inseminated female can get a blood meal within 24 to 36 hours (Delatte et al 2009). Since the blood meal is an important source of protein for eggs maturation, female of *Ae. aegypti* tends to take more than one blood meal in order to complete a gonotrophic cycle. Rearing adult female *Ae. aegypti* under less than optimal conditions result in smaller size females, thus requiring at least two blood meals in order to mature an eggs batch (Chadee and Beier 1997), whereas *Ae. albopictus* as an aggressive feeder, can take a full blood meal in one time with ability to feed on nonhuman host.

Resting Behavior

Adult mosquitoes have a wide range of places. Adults are frequently found in areas where the humidity is relatively high and air is comparatively static (Goma 1966). In general, most species of adult mosquitoes prefer dark places to rest (Goma, 1966). Most of adult *Ae. aegypti* rests indoor and often on some surfaces inside building or houses, such as closets, bathrooms, hanging clothing, undersides of furniture, bedrooms, kitchens so that indoor residual spraying (IRS) is not recommended to use for dengue control as with malaria vectors (Reiter and

Gubler 1997; WHO 2011; WHO, 1995). In contrast, adult of *Ae. albopictus* prefers to rest outdoor over any place in a forest (Estrada-Franco and Craig, 1995).

Feeding Behavior

Aedes aegypti mosquito is strongly anthropophilic with close association with humans, but it can also feed on other available animals. This species has the ability to feed on more than one host for one blood meal and take multiple blood meals during a gonotrophic cycle (Scott et al. 1993; Macdonald 1956; Sheppard et al. 1969; Yasuno & Tonn 1970; McClelland & Conway 1971; Trpis & Hausermann 1986; Gould et al. 1970; Pant & Yasuno 1973), where this cycle is depending on humidity, temperature, females' size, quantity and quality of blood meal. (Clements 1999; Christopher 1960; Klowden and Lea 1978; Judson 1967). This behavior of *Ae. aegypti* can increase mosquito-human contact, thereby increasing efficiency of epidemic transmission as a result. So, it is possible to find more than one member of the same household become sick within 24 hours, suggesting that they were infected by a single infective mosquito (Gubler 1998). The peaks of biting time occur from 2 to 3 hours in the early morning after daybreak and several hours in afternoon before dark (Gubler 1998). Season and location may change the peaks of biting activity. These behaviors have important ecological and epidemiological and consequences in term of increasing the reproductive rate and fitness of mosquito as well as the rate of diseases transmission in endemic areas (Sott and Takken 2012). Generally, *Ae. aegypti* tends not to bite at night except in lighted rooms, whereas female of *Ae. aegypti* can bite even in the night if host is present (Christophers 1960; Lumsden 1957).

The Asian tiger mosquito, *Ae. albopictus*, generally maintains a more feral behavior than *Ae. aegypti*, and invades peripheral urban areas of the cities, where they can feed on both animals and humans (Hawley 1988) with preference of mammals for blood meal (Savage et al. 1993),

where it prefers to feed during the day (Lambrechts et al. 2010). The Peak feeding times are two hours before dark an at daybreak. Females of *Ae. albopictus* are able to feed on one host to complete its blood meal for a gonotrophic cycle. Therefore, *Ae. albopictus* mosquitoes are considered to have low vectorial capacity in urban cycle compared with *Ae. aegypti* as a result (Lambrechts et al. 2010). *Ae. albopictus* is more zoophagic than *Ae. aegypti* in terms of feeding habits.

Dispersal

Dengue fever spreads very fast in epidemic areas, suggesting the importance of the vector dispersal range in disease transmission dynamics (Liew and Curtis 2004). Adult female *Aedes* mosquitoes disperse in order to find blood meals, sugar, shelter, mate, or oviposition sites. In fact, the maximum flight rang of mosquito is essential in understanding the species distribution, population genetic, dynamics and patterns of mosquito- borne diseases, and the spread of pathogens to new regions (Trpis & Hausermann 1986). Epidemiologically, female dispersal to seek blood meals is an important mechanism to understand the ecology of vector, where female mosquitoes become able to acquire and disseminate diseases as a result.

Moreover, oviposition dispersal is also playing a role in propagation of diseases (McClelland & Conway 1971; Scott et al. 1993; Trpis & Hausermann 1986). For example, female *Ae. aegypti* frequently distribute their eggs in multiple oviposition sides (Christophers 1960, Reiter et al. 1995). This behavior, therefore, could increase the dispersal of *Ae. aegypti* progeny (Honorio et al 2003). According to dispersal studies, *Ae. aegypti* has a fly range of 50-100 meters (McDonald 1977; Trpis & Hausermann 1986; Muir & Kay 1998; Honorio et al 2003). However, according to mark and release- recapture study, (McDonald 1977) reported that

Ae. aegypti could fight up to 400 meters. Dispersal for a mate and carbohydrates for *Ae. aegypti* was also reported (Honorio et al 2003).

The dispersal of *Ae. albopictus* was investigated comparing with *Ae. aegypti* where *Ae. albopictus* showed to have longer disperses and flights than what *Ae. aegypti* does. According to marked and recapture studies, female *Ae. albopictus* (Skuse) showed to have dispersal within 400-600 meters (Niebylski & Craig 1994; Rosen et al. 1976). In Japan and Hawaii, dispersal of *Ae. albopictus* could be less than 200 meters (Mori 1979; Bonnet & Worcester 1946). These flight ranges of *Ae. aegypti* and *Ae. albopictus* are considered to be short comparing with other species of *Aedes*, such female *Ae. taeniorhynchus (*Wiedemann), where this species can travel up to 10 km (Honorio et al 2003).

Since the dispersal of adult mosquitoes is influenced by many factors such as, availability of oviposition sites, blood sources, housing characteristics (e.g., in urban areas), vegetation, climate (e.g., temperature, humidity, wind, rainfall), and mosquito species traits (Honorio et al. 2003), there will be probably variation of the results of dispersal of adult mosquitoes.

To sum up, *Ae. aegypti* is considered to have a short dispersal distance with preference of anthropohilic, endophilic, endophagic, and urbanized dark environments (Service 1993, Kawada et al. 2005, Christophers 1960, Gubler and Kuno 1997), whereas *Ae. albopictus* has a longer dispersal distance than former species with preference of zoophilic, exophilic, exophagic, and prefers of vegetated environments. These characteristics of the *Ae. albopictus* imply that it has a wider range of activity with ability to easily adapt to outdoor environments than *Ae. aegypti* (Higa et al 2010).

Survival

Arthropod vector survivorship is one of the most important components of the disease transmission (Garrett-Jones and Shidrawi 1969; Reisen et al. 1980; Macdonald 1956; Niebylski and Craig 1994). Increasing the survivorship of the vectors allows them to survive past the incubation period of the pathogen in the mosquito or allows them to have more offspring, become infected, and eventually infect humans or animal. The survival of female *Aedes* mosquitoes is critical and plays an important role for transmitting viruses, such as dengue, Zika, and Chikungunya, where temperature is considered as a principal for determining the *Aedes* mosquito survival (Brady et al. 2013).

In tropical areas, adult mosquitoes can survive as long as a few days to weeks, whereas in temperate areas the survive of adult mosquitoes become longer. In laboratory experiments the survival of *Aedes* mosquito become longer than in natural environments. For example, the longevity of *Ae. albopictus* is expected to be longer under laboratory condition than under natural environments, which is not completely known (Ho et al., 1972). Brady et al. (2013) reported that under laboratory and field experiments, *Ae. albopictus* survived longer compared with *Ae. aegypti* whereas the latter species could resist a varied range of temperatures (Delatte et al 2008). In contrast, Bhattacharya and Dey (1969) reported that adult *Ae. aegypti* (L.) survived much longer than *Ae. albopictus* under laboratory conditions, whereas in the field, *Ae. aegypti* survival becomes longer in rainy seasons, resulting in increasing dengue fever virus transmission.

In terms of mosquito sex, females are generally considered to live longer than males. For instance, female *Ae. albopictus* can live longer comparing with males, where females are usually able to live up to eight weeks (WHO 2011). High daily rates of survival can consequently

increase the chance for females to take blood meal in a viremic person and then females become infective, where they eventually become able to transmit the virus. In fact, females are able to live longer than the extrinsic dengue virus incubation period in the urban areas (Maciel de Freitas et al. 2007). As a result, the survival and dispersal capacity of adult female *Ae. aegypti* as well as *Ae. albopictus* promote spread of pathogens and affect public health (Niebylski and Craig 1994; Brady et al. 2013).

Oviposition

In general, female adults Aedes mosquito are able to lay about 100-200 eggs, where females of both *Aedes* species are able to oviposit eggs that have ability to resistant to desiccation for long time. The eggs are mainly attached to solid substrate near the edge of water. Eggs hatching will occur whenever the eggs are submerged (Reiter 2007; Service 2000; Goma 1966; Harwood and James, 1979). Clean water with a high organic content is an important feature of containers to be selected by female of *Ae. aegypti* and *Ae. albopictus* for oviposition (Clements 1992, Delatte et al. 2008). The majority of eggs laid by the *Aedes* species occurs at two hours before sunset and two hours after sunrise (Delatte et al 2009; Chadee and Corbet 1990).

Yellow fever mosquito *Ae. aegypti* commonly laid its eggs artificial water-filled containers such as, flower pots, metal cisterns, wooden barrels, discarded tires, plastic cups, bottles, tin cans, rainwater containers, buckets, flower vases, and drums as larval habitats (Focks and Chadee 1997; Service 1992; Gubler 1998; WHO 2011). Majority of female *Ae. aegypti* laid their eggs in more than one oviposition sites which is known as “skip oviposition” which may, therefore, reduce the sibling competition as well as the risk of eggs mortality over several sites (Reiter 2016). In contrast, *Ae. albopictus* has a wide range of artificial and natural containers

such as bamboo stumps, rock holes, leaf axils, catch basins, pots, discarded tires and flower plates (Delatte et al. 2008; Sota et al. 1992; Hawley 1988). Moreover, *Ae. albopictus* has been found in tree-hole habitat to share with the inhabitant of *Ae. triseriatus* in Florida (Lounibos et al. 2001).

The total lifetime fecundity of *Ae. aegypti* and *Ae. albopictus* was compared and reported that *Ae. aegypti* to fecund more than *Ae. albopictus* (Sucharit and Tumrasvin 1981; Black et al. 1989). Also, female of *Ae. aegypti* found to lay more eggs per batch than *Ae. albopictus* using java strains (Soekiman et al. 1984). In contrast, *Ae. albopictus* has been reported to be more fecund according to (Galliard 1962). Also, Hein (1976) reported that female *Ae. albopictus* could lay more eggs than *Ae. aegypti* per milligram of blood ingested, whereas there was a correlation between rainfall and *Ae. albopictus* oviposition rate (Ho et al. 1971), abundance (Khan 1980), and biting rate (Gould et al. 1970). At high temperatures, *Ae. albopictus* egg production was significantly reduced, whereas *Ae. aegypti* had a slight reduce (Sames 1999).

AEDES MOSQUITO CLASSIFICATION

In general, the family of Culicidae has three main subfamilies (Table 1): Anophelinae, Toxorhynchitinae, and Culicinae (Dahl 1997; WHO 2011). First Subfamily is Anopelinae, this subfamlily has three genera where *Anopheles* is the most medical importance genera (Service, 2000) and around 40 species of *Anopheles* mosquitoes are considered to be vectors of human malaria. Also, some species of *Anopheles* can transmit other disease, such as filariasis, dog heartworm, and o'nyong'nyong virus (ONNV). Toxorhynchitinae is the second subfamily of Culicidae which has a single genus, *Toxorhynchites* that consisting the largest species of mosquito in size with a proboscis covered backwards. This genus is known as not medically important because of adult females of *Toxorhynchites* do not feed on blood. The last subfamily is

Culicinae. which has the major arboviruses vectors. The most medically important genera in this subfamily are *Aedes, Culex,* and *Mansonia* mosquitoes (WHO 1997; Service 2000; WHO 2011), where this subfamily includes the main dengue vectors *Ae. aegypti* and *Ae. albopictus*.

Subfamily	Tribe	Representative genera
Anophelinae	Anophelini	***Anopheles***, *Bironella, Chagasia*
Culicinae	Aedini*	***Aedes***, *Haemagogus, Ochlerotatus, Psorophora, Armigeres, Stegomyia, Belkinius, Kompia, Bothaella, Collessius , Finlaya , Udaya , Scutomyia, Opifex, etc.*
	Culicini	***Culex***, *Deinocerites, Galindomyia*
	Aedeomyiini	*Aedeomyia*
	Culisetini	*Culiseta*
	Ficalbiini	*Ficalbia, Mimomyia*
	Hodgesiini	*Hodgesia*
	Mansoniini	*Coquillettidia,* ***Mansonia***
	Orthopodomyniini	*Orthopodomyia*
	Sabethini	*Sabethes, Trichoprosopon, Wyeomyia, Isostomyia, Topomyia, Shannoniana, Johnbelkinia, Kimia, Limatus, Malaya, Maorigoeldia, Onirion, Runchomyia, Tripteroides,*
	Uranotaeniini	*Uranotaenia*
Toxorhynchitinae		*Toxorhynchites*

Table 1: List of mosquito subfamilies with their representative genera, where the most medically important genera are in bold font. Also, there are about 82 genera of Aedini, whereas just 14 genera are listed (Foster and Walker 2009).

Morphologically, *Ae. aegypti* and *Ae. albopictus* have a main difference in their adult stages which can be distinguished by looking to the side of their thorax. In instance, Ae. *aegypti* mosquito has two straight lines surrounded via curved lyre-shaped lines on the side of the thorax, whereas *Ae. albopictus* mosquito has a single broad white line scales located in the middle of the thorax (Fig. 9). The identification between male and female of *Aedes* mosquito can be observed by the differences between the length and shape of the palps in the head. In female, the palps are much shorter than proboscis, whereas male's palps are longer than proboscis with tapered tips.

In the larval stage, *Aedes* species have different features. For example, the shape of the comb scales of *Ae. aegypti* larva has developed lateral denticles, whereas *Ae. albopictus* larva has no lateral denticles (Fig. 10). Also, the shape of the pectin teeth on the siphon of *Ae. aegypti* larva has less defined denticles and for *Ae. albopictus*, the larva has three well defined pointed denticles (Lee and Cheong, 1986; WHO 1995; Christophers 1960).

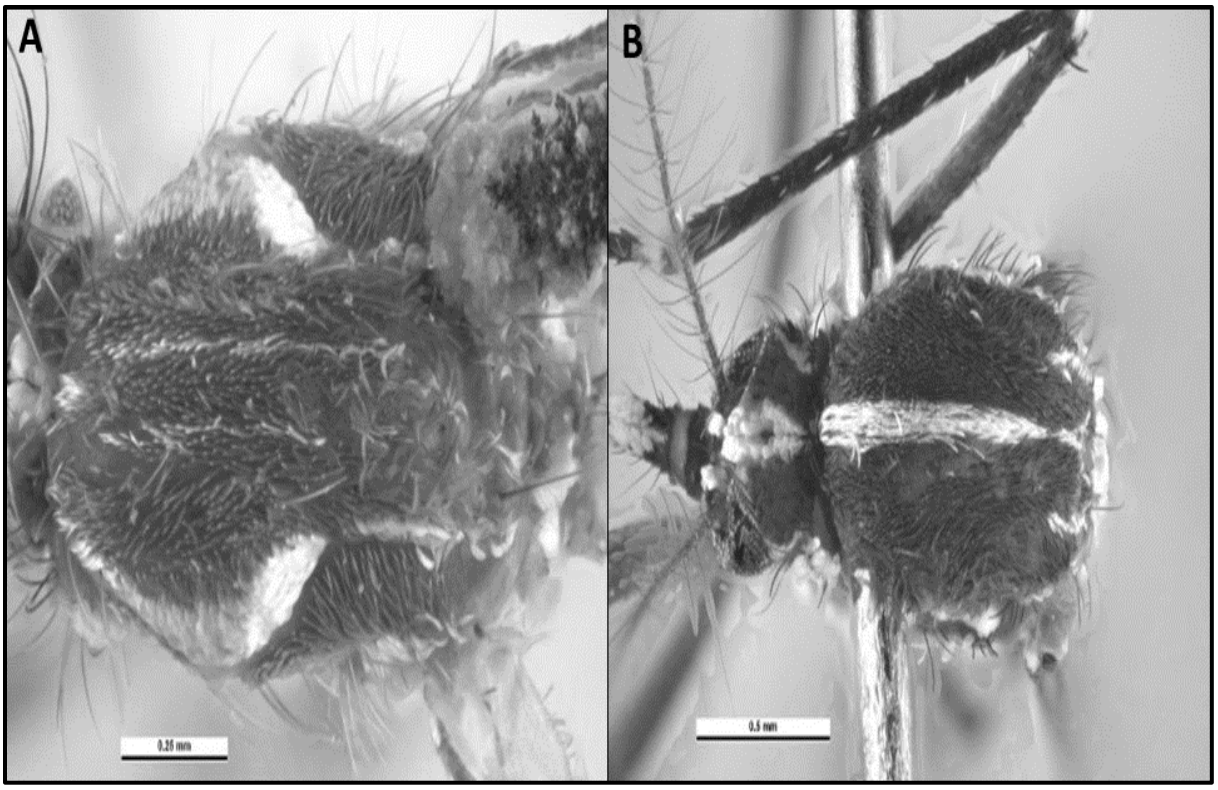

Figure 9: (A) Close picture of the “lyre” on the thorax of the adult female of yellow fever mosquito, *Ae. aegypti* and (B) Close picture of the single broad white line on the thorax of the adult female of Asian tiger mosquito, *Ae. albopictus*. Photograph by Simon Hinkley and Ken Walker, Pest and Diseases Image Library, Bugwood.org.

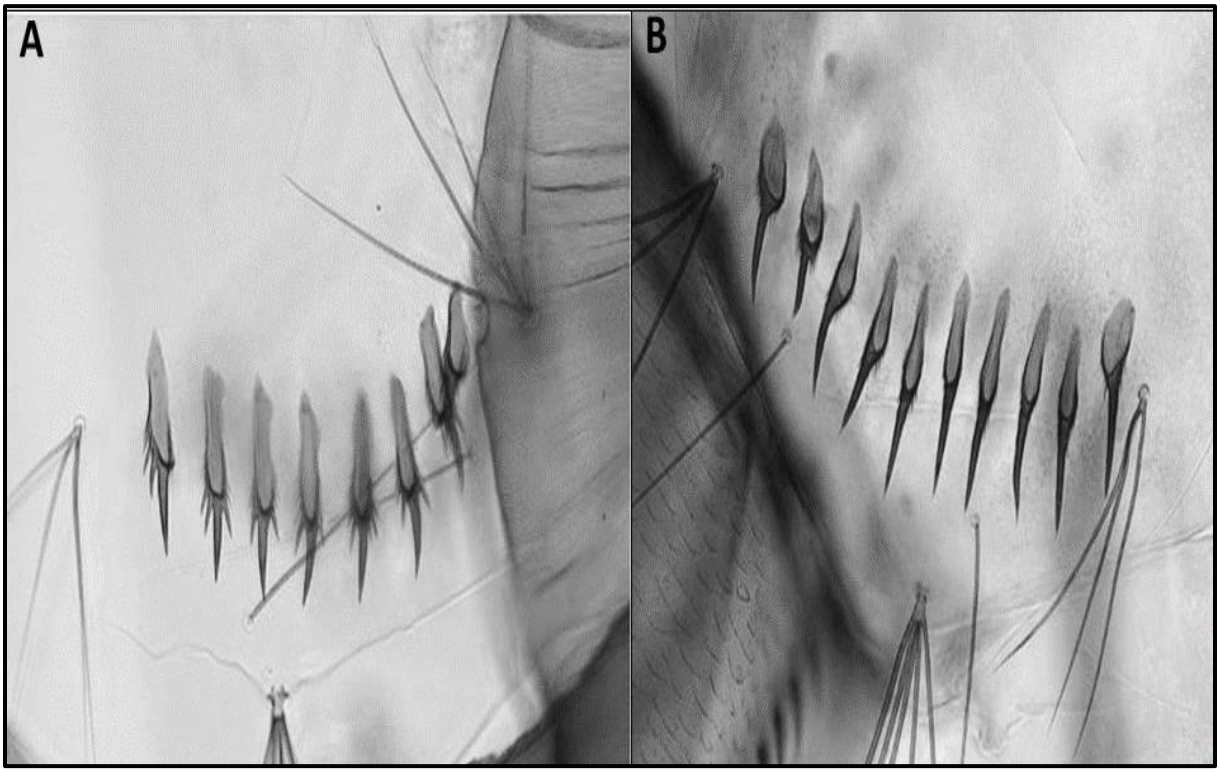

Figure 10: (A) Close-up picture of the larval comb scales of the yellow fever mosquito, *Ae. aegypti* and (B) Close-up picture of the larval comb scales" no lateral denticles" of the Asian tiger mosquito, *Ae. albopictus*. Photograph by Simon Hinkley and Ken Walker, Pest and Diseases Image Library, Bugwood.org.

MOSQUITO MANAGEMENT AND CONTROL

Environmental Management

Environmental management is one of the mosquito control measurements, where it is involving monitoring, manipulation, and modification of environmental factors. For example, solid waste management, improved house design, source reduction. The major environmental management purposes are to minimize mosquito breeding sites and reduce human-mosquito contact (WHO 2011).

Personal Protection

Personal protection is another way to protect humans from being bitten by mosquitoes. For instance, some cloth material is very thick so when it is covered person's legs and arms the chance of being bitten by female mosquito will be reduced. The use of the repellents is considered as a very common way of personal protection against mosquito bites. Chemical repellents can provide human protection for several hours against female of *Ae. aegypti* and *Ae. albopictus* bites. Also, Insecticide-treated mosquito nets (ITNs) are effectively utilized to prevent sleepers from mosquito bite especially for malaria mosquito control. For dengue control, however, ITNs are not considered to be efficient since the dengue vector known to bite during the day, but it could be effective for persons who sleep during the day under ITNs.

Biological Control

Biological control is an effective approach for pest control where it is based on using some organisms in order to reduce the target species populations (WHO 2009; WHO 2011). Using biological control for mosquito control is mainly targeting the larval stages in their

habitats and breeding sides. While this method of control could avoid contamination of chemical of the environment, there are, however, some limitations such as large-scale rearing organisms and limiting utility in aquatic breeding sites, where the pH, organic pollution, and temperature may affect negatively on the organism used.

Another important factor that can restrict the role of biological control is desiccation where *Aedes* eggs can survive in dry conditions for long time (Rezende et al. 2008; WHO 2011), whereas many of biological control organisms do not resistant to the desiccation. However, people in communities can play an important role in introducing of organisms into, for example, wells, and large storage containers of water as well as monitoring and destroying the temporary containers (WHO 2011).

Bacteria

The use of *Bacillus thuringiensis* (Bt. Serotype H-14*)* and *Bacillus sphaericus* (Bs) as endotoxin producing bacteria, a bacteria that produces proteins which are toxic to insects, are very effective agents for mosquito larvae control. For instance, *Bacillus thuringiensis* (Bt.H-14) *is* considered to be effective against mosquitoes especially *Ae. aegypti*, dengue vector (Ansari and Razdan 1999). Bt.H-14 has been used widely for mosquito larvae control in containers due to its low level of toxicity to mammals. *Bacillus sphaericus* (Bs) is also found to be effective to control larvae of *Cx. quinquefasciatus, An. gambiae, An. stephensi* (Hougard et al 1993; Kumar et al 1994; Karch et al. 1992). This species has high levels of efficacy, low toxicity to environment, and persistence to environment (Regis et al. 2001).

Fish

In various parts of the world, different fish species have been used as a biocontrol against the larval stage of mosquitoes mainly in natural mosquito breeding sites (Fletcher et al 1993; Nelson and Keenan 1992; Lee 2000; Martinez-Ibarra et al. 2002, Hurst et al. 2004; Bay 1985). *Poecilia reticulate* (guppy) and *Gambusia affinis* (mosquito fish*)* are the common species of larvivorous fish that feed on larval stages of mosquitoes. These species were introduced to control of mosquito larvae population in laboratory as well as in large water containers, large waterbodies, or domestic storage water containers in many regions (Seng et al. 2008; Manna et al. 2008; Ghosh et al. 2011; Saleeza et al. 2014) whereas, the World Health Organization (1982) has discouraged the introduction of exotic species due to the ecological potential of negative consequences.

In general, there are some characteristics of larvivorous fish that should be met to be more effective such as easy to rear, prefer mosquito larvae over other type of foods in water, no food value for other predators, and small size to adopt in shallow water (Chandra et al. 2008) Basically, type of containers used could determine the applicability and efficiency of this method of control (WHO 2011).

Chemical Control

Since 50 years ago, the control methods of *Aedes* mosquito is mostly based on the use of insecticides, which have been used widespread to control adult and larvae of mosquito, where adult mosquito control is in most cases applied as response of disease outbreaks (WHO 1997). When DDT insecticide had been discovered in the 1940s, this insecticide became a very common tool in eradicating, for example, *Ae. aegypti* mosquito in north and South America.

After decades of excessively used of this compound, the resistance of DDT was emerged. As alternative, organophosphate insecticides were used for control of adult *Ae. aegypti* with malathion, fenitrothion, and fenthion, whereas temephos was used for *Ae. aegypti* mosquitoes in their larval stages. Generally, larval mosquito control is based on reduction of larval sources or treatment of the storage water container using one of the larvicides either methoprene, insect growth regulator (IGR), temephos, an organophosphate, or Bti, *Bacillus thuringiensis* var. *israelensis*. Currently, space spraying and application of larvicides are the common approaches of applying insecticides for mosquito control (WHO 2011).

Larvicides

Chemicals have been widely used to control dengue vector *Ae. aegypti* either in their adult stages or larval stages. Use of larvicides for dengue vectors control is effective where they are usually applied to domestic containers that cannot be eliminated, destroyed or managed. In some cases, applying larvicides either in natural site including tree hole, deep wells, and leaf axils, which are common *Ae. albopictus* habitats, or indoor *Ae. aegypti* larval habitats such as plant vases and storage water containers are hard to reach and also difficult to apply for long-term control. Due to these difficulties, therefore, larvicides can be best used in certain periods of time and localities, where outbreaks may potentially occur. Larvicides should have low toxicity to non-target species with no change in the odour, colour, or taste of the water (WHO 2016 web). In addition, households can play an important role in the process of mosquito larvae control, such as source reduction, emptying containers, and recycling of discarded tires and containers in order to minimize mosquito breeding sites. Also, it is possible for households to use and apply some of larvicides, such as IGR, temephos, and Bacillus thuringiensis (Bt.H-14).

Adulticides

Adulticides are another method of control which target the adult stages of mosquito vectors. The main effects of adulticides is to have immediate impact on mosquitoes' densities and other transmission parameters. the application of adulticide can have quick result in reducing the mosquito population in particular areas as well as reducing the number of infected mosquito in order to decrease disease transmissions (CDC 2016 web). Adult mosquitoes can be controlled with the use of insecticides by either thermal fogging where spraying a small size of smoke particles or ultra-low volume sprays which has been recommended to use only when the dengue outbreaks occurred. However, these methods of control can fail in terms of targeting adult indoor resting mosquitoes which makes the intervention inefficient as a result (Castle et al 1999; Perich et al 2000).

Yet, adulticides remain an important approach in fighting against epidemics, especially to reduce quickly mosquito density (Ranson et al 2010). The main insecticides classes are organophosphates, carbamates, organochlorines, and pyrethroids. In dengue control programs, there is a concern of spread and evolution of resistance to the insecticides where due to this concern just a few classes of insecticides are available to use for control in public health (Ranson et al 2010).

Since there is no efficient vaccine and specific treatment for dengue fever, the most effective method to eliminate and reduce dengue fever transmission is to control dengue vectors. Insecticides play an important role in this cause. However, dengue vectors including *Ae. aegypti* in many countries (WHO 2011) has developed resistance to several insecticides such as DDT, and other compounds that have the similar modes of action and target sites, such as pyrethroids, which makes the control programs inefficient. Therefore, there is an urgent need to evaluate new

classes of insecticides that have different modes of action and target sites as well as environmental acceptability.

SPINOSYNS

Spinosyns comprise a family of bacterial secondary metabolites produced by natural fermentation; they have insecticidal properties and a toxicological profile indicating very low and negligible mammalian toxicity (Kirst 2010). A bacterial strain isolated from soil collected in a sugar/rum still in the Virgin Islands was named *Saccharopolyspora spinosa* as a new species of soil actinomycete (Mertz and Yao 1990). The chemical class name spinosyns was given in order to connect them with their producing bacteria *Saccharopolyspora spinosa* (Thompson et al. 1995). Initial testing of spinosyns showed a broad-spectrum activity against a variety of important insect pests (Kirst 2010).

This spectrum includes several species of Diptera and Lepidoptera along with some other members of insect orders and arthropods, for example, cockroaches, leafhoppers, planthoppers, spider, and mites (Boeck et al. 1994; Thompson et al. 1995; Sparks et al. 1996). Insecticidal activity of spinosyns was investigated and shown to have more than one method of delivery including oral toxin assays and contact (Kirst 2010). Persistence of spinosyns varies with environmental conditions (Salgado and Sparks 2005; Thompson and Sparks 2002).

Spinosad

Fermentation of *Saccharopolyspora spinosa* produces a mixture of (Fig.11 A) spinosyn A and (Fig.11 B) spinosyn D, which in combination for purposes of insecticide formulation is known as Spinosad (Thompson et al. 1995). Spinosad is effective against insects of many orders, including Orthoptera, Diptera, Coleoptera, Hymenoptera, Isoptera, Homoptera, Thysanoptera, and Lepidoptera. In addition to the wide range of effectiveness against important insect pests, they have also such a low mammalian and environmental toxicological profile (Crouse and Sparks 1998; Sparks et al. 1998, 1999). Moreover, Spinosad has been found not to be carcinogenic, teratogenic or mutagenic to mammals (Thompson et al. 2000). Comparing with many other of insecticdes, spinosad is showing to have a safety profile to mammals and other animals (Salgado and Sparks 2005; Thompson and Sparks 2002).

Spinosad was evaluated and accepted in United States of America for listing by World Health Organization Pesticide Evaluation Scheme in terms of effectiveness and safety (WHO 2007). Spinosad is showing a promising activity for mosquito control (Bond et al. 2004). Currently, it has been evaluated for mosquito control programs in USA and in some other countries (Liu et al. 2004, Darriet and Corbel 2006, Perez et al. 2007, Legocki et al. 2010). Spinosad insecticide was registered in EPA as a Reduced- Risk Pesticide Initiative in 1997 where it showed efficacy, low toxicity to human with favorable and safety environmental profiles which enabled spinosad to receive the Presidential Green Chemistry Award in 1999 (Dripps et al. 2008).

In 2008, spinosad was registered for use against mosquitoes under trade name of Natular^{R} (Ravichandran 2011). Dow AgroSciences, Indianapolis, IN, USA, has produced some products contain spinosad as their active ingredient in series of their naturally control of insect

agents (Dow AgroSciences). Some examples of these products are SpinTor and success which is for controlling insects on many field of crops, Conserve is for controlling insects on ornamental plants and turf, Tracer is for a major worm pests control, and Entrust is an organic formulation for controlling insects such as fire ant in baits traps and fruit fly (Dow AgroSciences, Racke 2007). Using these products in integrated pest management is useful as well as in insecticide resistance management strategies.

Recently, in the United States controlling chewing and sucking lice on cattle and horn flies by Elector, spinosad as active ingredient, was approved as well as Elector PSP for control beetles and flies (Elanco.com, White et al 2007, White et al 2007a). Ticks are another important blood-feeding pest which need to be controlled, Spinosad shows to have activity against to some tick species in laboratory and to *Boophilus* ticks on cattle (Cetin et al. 2009, Davey and George 2001, Davey et al 2005). Some studies were performed to determine the potential of spinosad against some insect parasites including tsetse flies and screwworm (Coronado and Kowalski 2009, De Deken et al. 2004). Blood-feeding insect causes a huge health problem for livestock and animals. Several studies have indicated that spinosad was effective in control fleas on dogs (Snyder et al. 2007; Robertson-Plouch et al. 2008, Franc and Bouhsira 2009). Human lice is a parasitic health problem for humans, spinosad was showing activity against permethrin-susceptible body louse and also permethrin-resistant head lice in laboratory bioassays (Cueto et al. 2006).

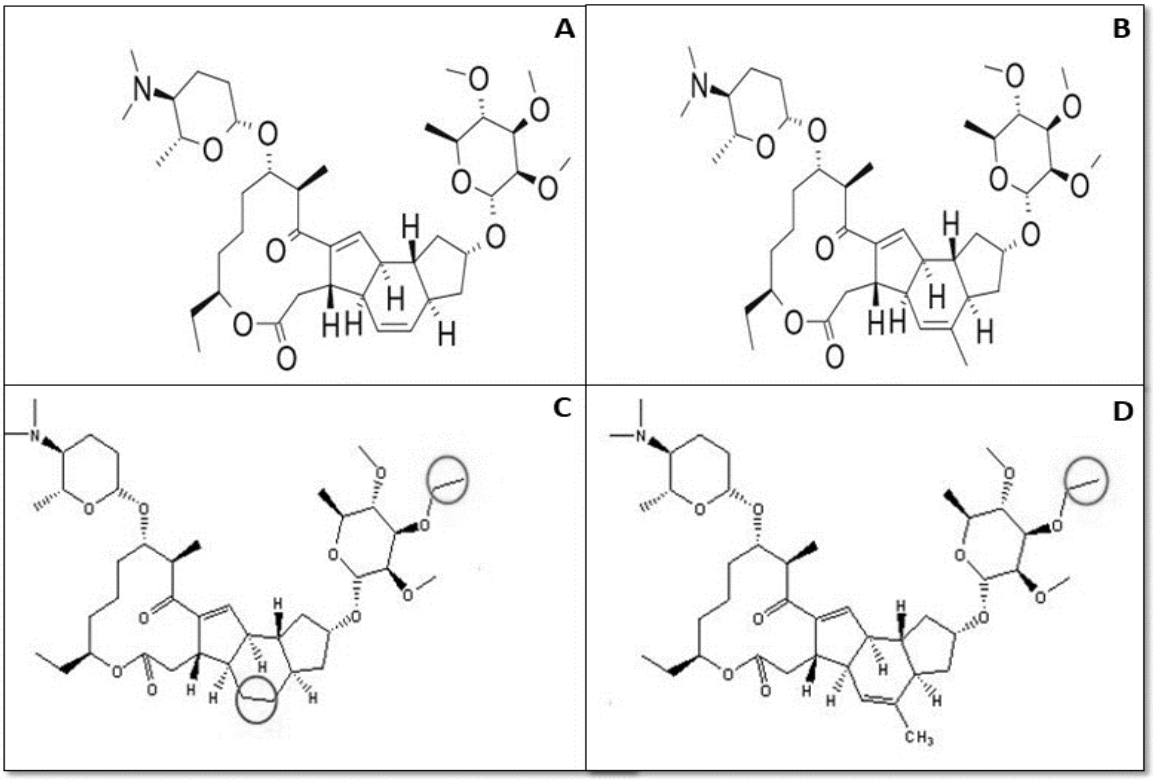

Figure 11: Structures of spinosyn used in binding studies. (A) Spinosyn A and (B) Spinosyn D (spinosad is a mixture of spinosyn A and spinosyn D), Whereas (C) Spinosyn J and (D) Spinosyn L (spinetoram is a result of synthetic modifications of spinosyns J and L).(Source: Orr et al. 2009; Dripps et al. 2008).

Spinetoram

Following spinosad discovery, there was a concern if the residual and efficacy of spinosad can be improved by specific modifications to the structure of spinosyns (Galm and Sparks 2016). Spinetoram discovery and development started with the discovery of spinosad in early 80s at Eli Lilly and Company (Galm and Sparks 2016). The major components of spinosad are spinosyn A and D, whereas spinosyns (Fig.11 C) J and (Fig.11 D) L are the major components of the new generation product, spinetoram, which was more active and residual than spinosad while maintaining the same favorable and safety toxicological and environmental profiles as spinosad (Galm and Sparks 2016). Therefore, Spinetoram received the Presidential Green Chemistry award in 2008 where Spinetoram was registered and launched in the United States in 2007 (Galm and Sparks 2016; Dripps et al.2008).

In other countries around the world, spinetoram are anticipated to be registered and developed (Dripps et al.2008). Spinetoram is exhibiting a wider spectrum activity with more insecticidal potency and efficacy (Galm and Sparks 2016). Currently, in the United States there are two products of spinetoram as Radiant™ SC insecticide and Delegate™ WG insecticide which are for insects control (Dripps et al. 2008).

Spinosyns Mode of Action

The primary action of spinosyn is affecting nervous system of insect and causing contractions on the involuntary muscle which is led insect to paralysis and eventually dead (Salgado 1998; Salgado et al. 1998). Later spinosys has been found to targeting the nicotinic acetylcholine receptors (nAChRs) subunit D α 6 (Fig. 12) which could implicate toward spinosyn mechanism of action (Watson 2010).

Since the spinosyn has a different mechanism of action compared with other insecticides, therefore, cross-resistance between spinosyn and other insecticides is initially low or none (Salgado and Sparks 2005; Scott et al. 2000). This enable spinosyn to be considered as effective insecticides for mosquito control.

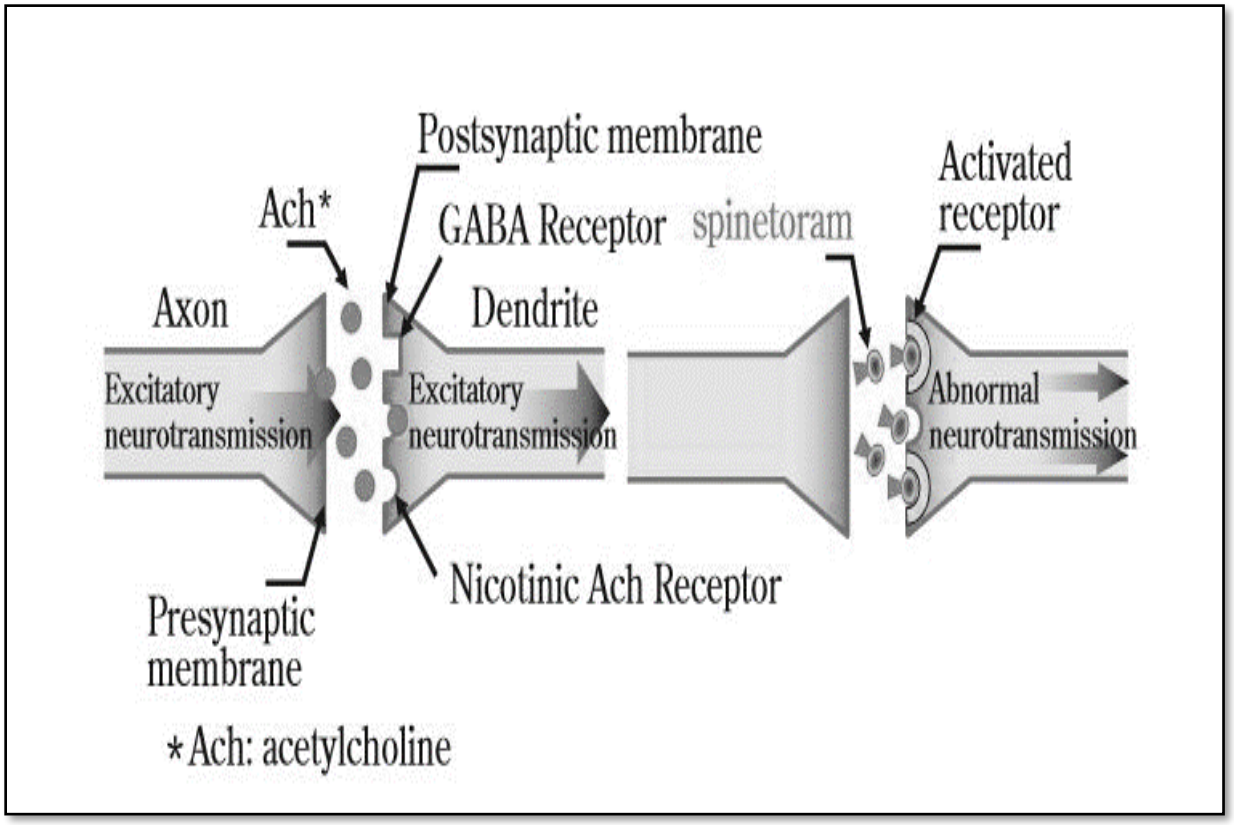

Figure 12: The mode of action of spinosyn and use spinetoram as an example which affects nicotinic acetylecholine receptors in insect nervous systems (source: Shimokawatoko et al. 2012).

CHAPTER 2: TOXICITY OF SPINOSAD AND SPINETORAM IN SUGAR SOLUTION TO THE MAIN DENGUE VECTORS *AEDES AEGYPTI* AND *AEDES ALBOPICTUS* (DIPTERA: CULICIDAE)

ABSTRACT

Aedes aegypti (Linnaeus) and *Aedes albopictus* (Skuse) mosquitoes (Diptera: Culicidae) are principal vectors of dengue fever in tropical and subtropical regions around the world. Disease management is mainly based on control of mosquito vectors by using insecticides applied to resting surfaces and through space sprays. Attractive toxic sugar baits (ATSB) is an effective method of control that targets adult mosquitoes based on their sugar feeding behavior. Spinosad and spinetoram are new naturally derived insecticides with a novel mode of action, low mammalian toxicity and low impact to environment. In the present study, acute toxicity of spinosad and spinetoram delivered in sugar solution as oral toxin to adult males and females of *Ae. aegypti* and *Ae. albopictus* were evaluated in the laboratory. Median lethal concentrations of spinosad sugar solution (LC_{50} in ppm) at 24-h exposure for males and females *Ae. aegypti* were 34.8 and 37.4 respectively; and for *Ae. albopictus*, 36.0 and 40.0 respectively, whereas spinetoram sugar solution (LC_{50} in ppm) for males and females *Ae. aegypti* were 32.0 and 36.8 respectively; and for *Ae. albopictus*, 25.0 and 28.9 respectively. The LC_{50} values in ppm of spinosad sugar solution at 48-h exposure for males and females *Ae. aegypti* were 24.4 and 26.9, respectively; and for *Ae. albopictus*, 22.4 and 24.8 respectively, whereas spinetoram sugar solution (LC_{50} in ppm) for males and females *Ae. aegypti* were 12.1 and 17.0 respectively; and for *Ae. albopictus*, 13.8 and 14.7 respectively. These results indicated that the new generation of spinosyn, spinetoram sugar solution was relatively more toxic based on acute mortality results but not statistically significant compared to spinosad. However, median lethal concentrations after 48-h of exposure showed that spinetoram sugar solution was significantly more toxic than

spinosad sugar solution to males of *Ae. aegypti*, suggesting that spinetoram is more likely to be an effective insecticide to use for ATSB technology for control of adult mosquitoes and other sugar feeding insect pests.

INTRODUCTION

Mosquito-borne diseases are currently considered to be a major public health concern causing a large number of human and animal diseases throughout the world. Dengue fever is the most important mosquito- borne diseases because of the increasing of its incidence in many sub-tropic and tropic areas in world (WHO 2000; Guzman et al. 2010). Around 2.5 billion people who live in dengue-endemic regions are at risk of the infection (WHO 2008). Annually, around 500,000 causes of dengue hemorrhagic fever, which the worst form of dengue fever, need a medical care, especially children under the age of five who have the large proportion comparing with adults. Also, there are probably about 2.5 % chance of death among those affected (WHO 2009). This virus is transmitted primarily by a bite of an infected female of *Aedes* mosquito species.

Yellow fever mosquito, *Ae. aegypti* (L), is a principal vector of dengue virus as well as other diseases, such as yellow fever virus and chikungunya virus (Gubler 1989, 2002; Barrett and Higgs 2007; Powers and Logue 2007). Recently the newly emerged disease, Zika virus is transmitted by *Ae. aegypti* as well (Chouin-Carneiro et al. 2016). This mosquito has adapted to breed in areas near human habitats where the biting time is usually occurred during the day with preference of human (i.e. anthropophilic) than any other hosts (WHO 2011).

Asian tiger mosquito *Ae. albopictus* is the second main vector of dengue virus and also considered as a major public health concern (Lambrechts et al. 2010; Gubler 1998). Since the early 1980s, this species has become global in distribution (Reiter 1998; Lambrechts et al. 2010). *Ae. albopictus* is introduced to new areas by regional and international shipping containing their eggs (WHO 2011). *Aedes albopictus* mosquito has ability to cause serious of arboviral diseases

outbreaks because of its competence to transmit at least 22 types of arboviruses (Gratz 2004), which is more than of what *Ae. aegypti* mosquito can transmit.

Since there is no effective vaccine or drug treatments for dengue fever yet available, disease management has depended on vector control measures, such as breeding sites reduction and use of insecticides. Adult mosquitoes are controlled mainly by aerial or ground ultra-low volume application of insecticides. However, these application techniques risk environmental pollution because of the large quantities of insecticides applied over such large areas. There are many of synthetic pesticides available for vector control programs around the world (Walker 2000; Curtis and Davies 2001), where the widespread use of these pesticides has caused concerns about their impact on environmental and human health as well as development of insecticide resistance (Schmutterer 1990; Tremblay 1982). For instance, the pyrethroid class of insecticides are widely used for vector control and accounting for about 81% of the total surface area that receiving insecticide treatments globally from 2000 to 2009 (Van den Berg et al. 2012). Also, there have been concerns raised regarding the development of resistance due to intensive application of pyrethroid insecticides (Van den Berg et al. 2012), which can potentially lead to loss of diseases control (Luz et al. 2011).

In addition to the biological challenges of disease control, vector control programs around the world also face social and economic challenges as financial resources and the public sector human decrease (WHO 2004). Thus, world health organization urged vector control programs to involve in implementing the integrated vector management to be more efficient, friendly to the environments, cost effective, and sustainable (WHO 2012). There is a need to develop an effective and safe alternative method of control for adult mosquitoes.

The need of adult mosquito for a carbohydrate source is very well known (Foster 1995), where adult male and female mosquitoes need carbohydrates for energy production, survival, and flight. These needs can be often met from natural sources, such as flowers, honeydew, plant tissues, and extrafloral nectaries (Yuval 1992; Foster 1995). When *Ae. aegypti* mosquito is in close of a sugar solution, it is able to readily ingest the solution (Stell et al. 2013). Field and laboratory studies have indicated that mosquitos need regular sugar meal for energy and nutrition (Xue et al. 2008; Braks et al. 2006; Xue et al. 2010). Obtaining a sugar meal by males of all species of mosquito is critical and occurring frequently and probably more than one time a day (Gary and Foster 2006). Female mosquitoes typically take sugar meal soon after emergence, and some females strongly prefer sugar sources over blood meal (Foster 1995; Gary and Foster 2006; Foster and Takken 2004). Therefore, this need of carbohydrate source for both males and females could be exploited to develop a novel method of control.

There is a new method with potential to exploit the mosquito's need for a carbohydrate source as well as achieving the purpose of the integrated vector management and WHO programs which is known as attractive toxic sugar bait (ATSB). This method is a highly effective and promising for vector control, which exploits mosquito's sugar feeding behavior (Müller and Schlein 2006, 2008; Schlein and Müller 2008; Beier et al. 2012; Müller et al. 2008, 2010). This novel approach is developed and tested in the Middle East, Africa, and United States, where it has been shown effective control of local populations of *Aedes*, *Culex*, and *Anopheles* mosquito species (Müller and Schlein 2006, 2008; Beier et al. 2012; Khallaayoune et al. 2013; Gu et al. 2011; Müller et al. 2008, 2010; Qualls et al. 2012; Naranjo et al. 2013; Qualls et al. 2014; Fulcher et al. 2014; Revay et al. 2014). Exploiting this mosquito's physiological requirement showed that foliar application of sugar baits that contained boric acid was successfully control

mosquito species in St. Augustine, FL, USA (Naranjo et al. 2013; Xue et al. 2006). Furthermore, adult mosquitoes were able to feed on flower nectaries that had been treated with a pesticide (Müller and Schlein 2006; Schlein and Müller 2008), and sugar-boric acid baits (Müller et al. 2010; Xue and Barnard 2003) and also spinosad-treated baits stations (Müller et al. 2008).

Attractive toxic sugar baits can be applied on either vegetation spots or as a baits station which can attract adult mosquitoes and kill them (Khallaayoune et al. 2013). For instance, when ATSBs applied in the areas, where plants are mostly absent, they were very attractive for adult mosquitoes (Müller and Schlein 2006). Use of attractive sugar feeding centers was potential for control when spraying them with a sugar baits with toxin. Sugar insecticide baits were used to screen some adulticides for toxicity to mosquito species of *Aedes*, *Culex*, and *Anopheles* (Allan 2011). These studies, as a result, indicated that adult mosquitoes would ingest sugar baits even with the presence of insecticides.

Therefore, it is important for ATSB to include a safe oral toxin that can be ingested in order to circumvent problems that are associated with use of contact insecticides (Müller and Schlein 2008; Enayati and Hemingway 2010; Xue et al. 2006). A highly efficacy of using ATSB has been shown in field experiments using different active ingredients, such as spinosad (Müller and Schlein 2008; Müller et al. 2008; Müller et al. 2010), eugenol (Revay et al. 2014; Qualls et al. 2014), boric acid (Müller et al. 2010; Naranjo et al. 2013; Beier et al. 2012; Xue et al. 2006), pyriproxyfen (Fulcher et al. 2014), and dinotefuran (Khallaayoune et al. 2013). The use of different low-risk ingestible active ingredients could enable ATSB to become a potentially valuable tool to fight against develop of resistance and traditional contact insecticides problem (Allan 2011).

Spinosyns, a new class of insecticide, are effective against numerous of insect orders, such as Coleoptera, Diptera, Lepidoptera, Thysanoptera, Orthoptera, Homoptera, Hymenoptera, and Isoptera, while maintaining a safe toxicological profile to mammals and environment (Crouse and Sparks 1998; Sparks et al. 1998, 1999). Spinosad is a naturally derived insecticide which is mixture of two active components, spinosyn A and D, where produced by the bacteria *Saccharopolyspora spinosa* fermentation, and it has been shown a promising activity against mosquitoes (Bond et al. 2004). According to USEPA (1997) Spinosad insecticide was registered as a reduced- risk pesticide Initiative in 1997, where it showed efficacy, low toxicity to human with favourable and safety environmental profiles, which enabled spinosad to receive the Presidential Green Chemistry Award in 1999 (Dripps et al. 2008).

A new generation spinosyn product, spinetoram, is generally more active and has longer residual than spinosad, while maintaining the same favourable safety toxicological and environmental profiles (Galm and Sparks 2016). Spinetoram received the presidential green chemistry award in 2008, where it was registered and launched in the United States in 2007 (Dripps et al. 2008; Galm and Sparks 2016). Spinetoram is exhibiting a wider spectrum activity with more insecticidal potency and efficacy (Galm and Sparks 2016).

Since several of insecticides were evaluated and tested for their toxicity to adult mosquitoes in sugar solution. Studies evaluating different natural derived insecticides in sugar solution as an oral toxin are limited. At present, no data available in evaluating the toxicity of the new generation, spinetoram to adult *Aedes* mosquito species in sugar solution. Therefore, the aim of this study was to determine the acute toxicity of spinosad and spinetoram in sugar solution in laboratory bioassays against the main dengue vectors, *Ae. aegypti* and *Ae. albopictus* to estimate the LC_{50} and LC_{90} at 24-h and 48-h of continuous exposure for both males and females.

MATERIALS AND METHODS

Mosquitoes

Aedes aegypti (Linnaeus) and *Aedes albopictus* (Skuse) mosquitoes were obtained as eggs from Dr. Barry Alto at University of Florida, Florida Medical Entomology Laboratory, Vero Beach. These two strains were sampled from wild populations at White City, Florida. *Aedes* mosquitoes used in this study were established in colony at Michigan State University. These colonies were reared and maintained in a Percival Scientific incubator (Perry, IA) at the following conditions: temperature was 28°C ± 1°C (mean ± standard deviation); relative humidity was 50% ± 10%; and a photoperiod of 12:12 h (light/ dark).

Adult mosquitoes were held in front opening, collapsible insect cages (BugDorm, BioQuip Products, Rancho Dominquez, CA). Adults were provided as a carbohydrate source a 10% sucrose solution (hereafter, "sugar water") *ad libitum*, via reservoirs fitted with dental rolls as wicks (Coltene, Cuyahoga Falls, HO). Adult females were blood fed twice weekly for a minimum of 30 minutes by using fresh defibrinated bovine blood (Hemostat Laboratories, Dixon, CA) via artificial membrane feeder heated to around 36.5°C. Two to three days after females had bloodfed, oviposition cups were placed inside the cages. They consisted of small dark jars containing water and lined with brown paper towels as an oviposition substrate. The eggs laid on the paper were collected one to two times from oviposition containers and then stored in self-sealing plastic bags in order to retain a high relative humidity, enhancing egg survival. All bags were placed inside a sealed plastic storage container and kept at optimal temperature of 20 ± 5°C for a minimum of one week prior to use, ensuring embryonation of the eggs. This method of storage can keep eggs alive for several months (Clemons et al. 2010). After that, eggs were placed in trays containing tap water and allowed to hatch over several days.

After hatching, larvae were transferred to plastic containers containing fresh tap water warmed to room temperature. Larvae were fed *ad lib*. a mixture of liver powder (Sigma-Aldrich, life science, MO) and ground Tetramin fish flakes (Tetramin, Blacksburg, VA), added daily. In order to eliminate food particles, water was changed and added as needed. Larvae were raised at low densities until reaching the fourth instar. Newly emergent pupae were then transferred via pipette to small plastic cups and placed in cages for adult emergence.

Bio-insecticides

Spinosad (Entrust® SC, a Naturalyte® commercial formulation, Dow AgroSciences LLC, Indianapolis, IN) was a liquid containing 22.5% active ingredient (AI). Spinetoram (Delegate® WG Insecticide, Dow AgroSciences LLC, Indianapolis, IN) contains 25% (AI) in a water dispersible granule.

Assessing the Purity of Spinosad and Spinetoram

To determine the purity of the commercial formulations used in the following experiments, samples of them were analyzed by high-performance liquid chromatography [HPLC] system at the Pesticide Analytical Laboratory, Michigan State University. The results of the test confirmed the purity of 22.5 % for spinosad and 25 % for spinetoram.

Spinosad and Spinetoram Sugar Solution Residue Profile Analysis

Samples of spinosad and spinetoram sugar solution were submitted to the Michigan State University Pesticide Analytical Laboratory in order to determine the amount of residue in the sugar solution solutions. A 0.6 g of spinosad and spinetoram sugar solution solutions were placed in 20 ml of HPLC grade dichloromethane (Burdick & Jackson, Muskegon, MI), 4 g $MgSO_4$ and

1 g NaCl. Then, the extracts were vacuum filtered, and the filtrate was passed through 5 g of anhydrous sodium sulfate. After that, the samples were dried using rotary evaporation and brought up to 2 ml in acetonitrile. Any remaining of particulates were then removed via passing the sample through a 0.45-µm PTFE syringe filter.

Samples were analyzed for spinosad and spinetoram residue (parent compound) with a Waters 2690 Separator Module HPLC equipped with a Waters Acquity mass spectrometer (Waters) monitored for 746.5 m/z for spinosad and 748.5 m/z for spinetoram and a C18 reversed phase column (4.6-mm bore, 5-mm particle size) was used. The mobile phase was water/acetonitrile (80:20) at 25°C. The HPLC level of quantification was 0.05 ppm for both analytes. The samples were quantitated against a standard curve. A 10-µl injection was used in HPLC analysis

Laboratory Bioassays

Toxicity bioassays were carried out in 473 ml clear plastic cups (Deli serve, WNA, Chattanooga, TN) covered with fabric nets fastened with rubber bands. Toxicity bioassays consisted of different concentrations of spinosad and spinetoram in 10% sugar solution to determine the LC_{50} and LC_{90} for males and females of *Ae. aegypti* and *Ae. albopictus*.

Primary stock solutions of 10,000 ppm of spinosad and spinetoram were prepared in 10% sugar water (w/w) and stored at room temperature until use. This stock solution was diluted further to achieve at least ten concentrations ranging from 0.01 to 1000 ppm. For each concentration, three replicates were performed in oral toxicity exposures. Controls were 10% sugar water without insecticides. For each replicate, groups of ten unfed females and males were transferred to the bioassay cups with a mouth aspirator (John W. Hock Company, Gainesville,

FL). Each bioassay cup contained a reservoir of sugar water with a particular concentration of insecticide, made available to the mosquitoes with a cotton wick of dental roll. The wick was inserted through the lid of the reservoir, extending to the bottom, and the liquid in the reservoir naturally saturated. Bioassay cups were held in an incubator (Percival Scientific incubator, Perry, IA) at 28°C ± 1°C, relative humidity of 50% ± 10% and a photoperiod of 12:12 (light: dark) during the test.

Assessing the Results of Bioassays

Due to the slow acting nature of the insecticides tested, mortality was determined after 24 and 48 hours from initial exposure. Adult mosquitoes were considered as dead if they were either not able to move their wings, fly, or stand. At each observation, dead mosquitoes counted and sexed. After the final mortality recording at 48 hours, all bioassay cups were held at cold room for at least 24 hours to kill surviving male and female mosquitoes.

Statistical Analysis

Concentration - response results for males and females *Ae. aegypti* and *Ae. albopictus* were corrected for control mortality according to Abbott's formula (Abbott 1925) and analyzed by Probit Analyzes (Finney 1952) using SAS 9.4 statistical software (SAS Institute 2004) to estimate LC_{50} and LC_{90} with their 95% confidence intervals (C.I.s). In this study, statistical differences between LC_{50} and LC_{90} values were determined based on overlap of 95% confidence intervals.

RESULTS

Concentration – response results were observed for both males and females of *Ae. aegypti* and *Ae. albopictus* in the sugar- spinosad and spinetoram feeding bioassay. The mortality rates were variable among the two insecticides in sugar solution. Results reported in (Table 2) indicate the LC_{50} values of spinosad sugar solution after 24-h of exposure was estimated for males and females of *Ae. aegypti* at 34.8 and 37.4 ppm respectively, whereas the LC_{50} values for males and females of *Ae. albopictus* was estimated at 36.0 and 40.0 ppm respectively. While the concentration of spinosad sugar solution that required to kill 90% tended to be lower for males than for females of *Ae. aegypti* and *Ae. albopictus*, the differences were not statistically significant based on their overlapping 95% confidence intervals.

After 24 hours of exposure to spinetoram sugar solution, the LC_{50} value was estimated at 32.0 ppm for males and 36.8 ppm for females of *Ae. aegypti*, whereas males and females *Ae. albopictus* LC_{50} were estimated at 25.0 ppm and 28.9 ppm respectively (Table 3). Spinosad sugar solution appeared to be less toxic to males and females of *Ae. aegypti* and *Ae. albopictus* compared with spinetoram sugar solution toxicity after 24 hours of exposure (Table 2 and 3). Also, males of *Ae. aegypti* and *Ae. albopictus* were most susceptible to spinosad and spinetoram sugar solution than to females of *Ae. aegypti* and *Ae. albopictus* at 24 hours but statistically not significant (Table 2 and 3). Also, males of *Ae. aegypti* and *Ae. albopictus* were more susceptible, statistically not significant, to spinosad and spinetoram sugar solution than to females of *Ae. aegypti* and *Ae. albopictus* at 24 and 48 hours of exposure (Table 2 and 3). In general, the LC_{50} and LC_{90} values of spinosad and spinetoram sugar solution decreased for both males and females *Ae. aegypti* and *Ae. albopictus* following the 48 hours of exposure comparing with 24 hours (Table 2 and 3).

Exposure time		24-h						48-h					
Species and Sex	N	LC_{50}	95% C.I.	LC_{90}	95% C.I.	Slope	χ^2	LC_{50}	95% C.I.	LC_{90}	95% C.I.	Slope	χ^2
Aedes aegypti													
Male	210	34.8	30.35-40.29	77.2	62.61-106.99	3.70± 0.47	2.0	24.4	20.71-28.42	61.1	49.17-85.44	3.21± 0.42	1.7
Female	210	37.4	32.28-44.23	90.7	70.73-135.79	3.33± 0.45	0.1	26.9	22.91-31.49	69.0	54.76-99.22	3.13± 0.41	1.0
Aedes albopictus													
Male	210	36.0	28.77-47.80	157.0	96.66-425.85	2.00± 0.37	0.3	22.4	18.58-26.54	63.5	49.51-95.27	2.83± 0.40	3.1
Female	210	40.0	31.87-55.06	177.3	105.68-523.66	1.98± 0.37	0.9	24.8	18.87-31.67	119.7	75.92-311.99	1.87± 0.36	3.2

Table 2: Acute toxicity of spinosad in sugar solution to adult male and female of *Ae. aegypti* and *Ae. albopictus*. The median lethal concentration, LC_{50} and LC_{90} with their 95% confidence intervals were calculated after 24 and 48 hours of continuous exposure. Concentrations are in (ppm). Slope results are presented as slope ± SE.

There was toxicity attenuation of spinosad sugar solution after 48 hours of exposure, where the slope of the fitted regression line was decreased for males and females of *Ae. aegypti* and for females of *Ae. albopictus* with opposite effects for males of *Ae. albopictus* (Table 2). For spinetoram sugar solution, the slope of the fitted regression line was reduced for both males and females of *Ae. albopictus*, whereas the slope for males and females of *Ae. aegypti* was increased after 48 hours of exposure (Table 3). Spinetoram sugar solution tended to be more toxic even though statically not significant to males and females of *Ae. albopictus* than males and females of *Ae. aegypti* after 24 hours of exposure (Table 3).

In addition of remaining toxic with low concentrations after 48 hours of exposure for both *Ae. aegypti* and *Ae. albopictus* comparing with spinosad sugar solution (Table 2 and 3). In general, spinosad and spinetoram sugar solution, the 95% confidence intervals for the LC_{50} and LC_{90} values of males and females of *Ae. aegypti* and *Ae. albopictus* did overlap which indicated that there were no statistically significant differences, except for the median lethal concentration of spinetoram sugar solution after 48-h of exposure which showed that spinetoram to be significantly more toxic to males of *Ae. aegypti* comparing to spinosad sugar solution (Table 2 and 3).

Exposure time		24-h						48-h					
Species and Sex	N	LC_{50}	95% C.I.	LC_{90}	95% C.I.	Slope	χ^2	LC_{50}	95% C.I.	LC_{90}	95% C.I.	Slope	χ^2
Aedes aegypti													
Male	208	32.0	6.58-53.86	163.4	81.13-343	1.8± 0.5	7.7	12.1	4.92-17.78	44.7	34.56-68.34	2.2± 0.5	6.0
Female	204	36.8	27.95-46.17	157.7	105.91-351.93	2.0± 0.3	5.9	17.0	9.51-22.92	64.5	49.30-106.94	2.2± 0.4	1.9
Aedes albopictus													
Male	200	25.0	18.60-30.61	73.2	58.04-107.48	2.7± 0.4	4.2	13.8	6.66-19.31	44.7	35.04-66.63	2.5± 0.5	5.7
Female	210	28.9	20.98-36.19	116.1	83.42-217.74	2.1± 0.3	6.4	14.7	7.02-20.74	60.1	45.74-100.53	2.0± 0.4	6.4

Table 3: Acute toxicity of spinetoram sugar solution to adult male and female of *Ae. aegypti* and *Ae. albopictus.* The median lethal concentration, LC_{50} and LC_{90} with their 95% confidence intervals were calculated after 24 and 48 hours of continuous exposure. Concentrations are in (ppm). Slope results are presented as Slope ± SE.

DISCUSSION

Naturally derived insecticides, such as spinosad and spinetoram, have potential to use in sugar baits for control of adult *Aedes* mosquito species and other sugar feeding insect pests. Reduced risk insecticides and pest control methods are continually being developed and evaluated to limit environmental impacts, the effect on non-target species, and development of insecticide resistance. Developing a successful toxic sugar baits system with use of some new naturally derived insecticides will achieve the purpose of integrated vector control while reducing the concerns regarding the environmental impacts. Both spinosad and spinetoram have been classified as reduced risk insecticides by Environmental Protection Agency (EPA) based on factors such as photostability and hazards to environment, humans, and animals (Allan 2011).

There has been an evidence of the sugar as an important phagostimulant for ingestion of solutions containing insecticide (Jiang and Mulla 2006). Stell et al. (2013) indicate that when females of *Ae. aegypti* are near a sugar solution, they will readily ingest it. Therefore, using sugar could enhance uptake of solutions that containing insecticides and could also cause mortalities among targeted species through the ingestion.

This is the first evaluation toxicity study of newly developed spinosyn, spinetoram in sugar solution against adult of *Ae. aegypti* and *Ae. albopictus*. Spinetoram, which was developed to improve its efficacy as well as expanded the activity spectrum, in sugar solution showed to be significantly more toxic to *Ae. aegypti* males after 48-h of exposure comparing to spinosad in sugar solution. In addition of being more toxic with no statistically significant to other adult males and females of both species of *Aedes* mosquito species as an oral toxin when comparing to spinosad in laboratory bioassays. There are limited data available for use of spinetoram against mosquitoes in literatures. According to Shah et al. (2016) who reported that spinetoram was

significantly more toxic than spinosad against adult females of *Cx. quinquefaciatus* Say. In another study, spinetoram was highly toxic to larval of *Cx. pipiens* and *An. multicolor* compared with Methomyl compound (Kady et al. 2008). In addition, (Besard et al. 2011) provided an evidence that spinetoram has a higher safety than spinosad by either oral exposure or direct contact to bumblebees.

Spinosad efficacy has been reported for several dipteran pests. Oral toxicity of spinosad in sugar solution has been studied where it achieved about 78 % mortality in adult laboratory susceptible strains of *Ae. aegypti* at 50 ppm, whereas *Cx. pipiens* and *An. stephensi* achieved about 30% and 66% mortality, respectively, at 50 ppm after 24 hours of exposure (Romi et al. 2006). In addition, spinosad with sugar has been commercially used for control of several Tephritid fruit flies **(**Prokopy et al. 2003; Yee and Chapman 2009). Spinosad was effective and environmentally safe in a baits formulation when spraying over large areas in Central America to control Mediterranean fruit fly**,** *Ceratitis capitata* Wiedemann (Vargas et al. 2001).

Differences in susceptibility to insecticides between the *Ae. aegypti* and *Ae. albopictus* were observed here, particularly when acute responses were measured after 48 hours of exposure, a finding which suggests variation between these mosquito species in response to toxicants in general. The efficacy of spinosad (Hertlein et al. 2010) and spinetoram (Vassilakos and Athanassiou 2013) have been shown to vary between target species. Allan (2011) reported that different mosquito species had different susceptibility to some insecticides. For example, the neonicotinoid insecticide dinotefuran was less toxic to *Cx. quinquefasciatus* than to *Ae. aegypti* and *An. gambiae* (Corbel et al. 2004).

Differences between sexes in susceptibility to spinosad and spinetoram in sugar solution could be due to variation in size, although it was not measured here. Female mosquitoes are typically larger in size than males. Allan (2011) reported that females of *Cx. quinquefasciatus* were less susceptible than males.

Spinosyns act on the nicotinic acetylcholine receptor of insect nervous system and have been shown to possess a unique mode of action which is not shared by any other chemical classes (Salgado and Sparks 2005; Salgado 1998; Orr et al. 2009; Dripps et al. 2011; Hertlein et al. 2011; Sparks et al. 2012). For instance, larval bioassays comparing the LC values of strains of different species of mosquitoes showing resistance to various insecticides (pyrethroids, organophosphates, or carbamates) revealed no cross-resistance to spinosad for *Cx. quinquefasciatus, Ae. aegypti, Ae. albopictus*, or *An. gambiae* (Liu et al. 2004b; Darriet et al. 2005; Liu et al. 2004a). Spinosad paradoxically had relatively low toxicity to a susceptible strain of *Cx. quinquefasciatus*, however, when tested against strains resistant to permethrin and other insecticides, spinosad was the most toxic insecticide (Lui et al. 2004).

Because toxins in sugar solution must be ingested, beneficial insects that generally do not feed on plant nectar, sugary exudates, and overripe fruits will have a lower chance of being affected. Further, the spinosyn family of insecticides have low impact on non-target organisms. For example, spinosad has low toxicity effects on mammals, predatory beneficial insects, and birds (Liu et al. 1999; Williams et al. 2003; Galvan et al. 2006). Moreover, Besard et al. (2011) concluded that spinosyns, spinosad and spinetoram had no negative effects on the foraging behavior of bumblebees. These findings reduce the concerns that spinosad and spinetoram, when mixed with sugar and presented to adult mosquitoes in nature in devices like baits stations, will increase risk of toxicity to beneficial and non-target insects.

In conclusion, the efficacy of spinosad and spinetoram sugar solution is promising against adult males and females of *Ae. aegypti* and *Ae. albopictus* based on the laboratory bioassays. There are differences in susceptibility among the sex of adult mosquitoes as well as in species level even though statistically not significant. For example, males of *Ae. aegypti* and *Ae. albopictus* were more susceptible than females to both insecticides. By species, spinosad sugar solution were more toxic, for example, to female *Ae. aegypti* (LC_{50} of 37.4ppm) than *Ae. albopictus* (LC_{50} of 40.0ppm), whereas spinetoram sugar solution were more toxic to females *Ae. albopictus* (LC_{50} of 28.9ppm) than *Ae. aegypti* (LC_{50} of 36.8ppm) at 24 hours of exposure. Considering both species together, spinetoram sugar solution appeared to be more effective and toxic to *Aedes* mosquitoes when compared with spinosad. For instance, median lethal concentration of spinetoram sugar solution after 48-h of exposure indicated that spinetoram to be significantly more toxic to males of *Ae. aegypti* comparing to spinosad. This result suggests that this new naturally derived insecticide, spinetoram, could be more effective and safe to use for adult mosquitoes control. Attractive toxic sugar baits are a promising new vector control method, and should be further investigated by field trials of spinetoram. Incorporating low risk insecticides with sugar baits will enhance of the versatility of the attractive toxic sugar baits approach and its role in integrated mosquito management for controlling adult mosquitoes and other sugar feeding flies.

CHAPTER 3: THE EFFECTS OF SUACUTE EXPOSURE OF SPINOSAD AND SPINETORAM IN SUGAR SOLUTION TO THE MAIN DENGUE VECTORS *AEDES AEGYPTI* AND *AEDES ALBOPICTUS* (DIPTERA: CULICIDAE)

ABSTRACT

Most studies related to the insecticides efficacy on mosquito are mainly accessed by estimating the lethal (acute) effects using mortality data. Besides mortality, sub-lethal concentrations of insecticides could have some effects on mosquito' physiology, biology, or behavior. In present study, the effects of an oral 24-h exposure to an LC_{50} concentration of spinosad and spinetoram sugar solution were carried out to evaluate the effects on adult *Ae. aegypti* and *Ae. albopictus* survival, fecundity, and fertility under laboratory conditions. The results indicated that the survivorship of males and females of *Ae. aegypti* were significantly reduced following the exposure to spinosad and spinetoram sugar solution compared to unexposed females, whereas the fecundity of *Ae. aegypti* was not significantly affected following the exposure. Furthermore, males and females of *Ae. albopictus* survivorship were also significantly reduced following the exposure, whereas the fecundity of *Ae. albopictus* was significantly increased compared to unexposed females. On the other hand, fertility as hatch rate of eggs was significantly reduced in the exposed females of *Ae. aegypti* and *Ae. albopictus* to spinosad and spinetoram sugar solution compared with that of unexposed females. The assessment of these sub-lethal effects is important in order to acquire a more accurate estimation of the compatibility of these naturally derived insecticides in sugar baits in the integrated mosquito management programs.

INTRODUCTION

Dengue fever (DF) is very important arboviral infection disease in terms of the mortality and morbidity and considered as major emerging diseases around the world. This is internationally caused a major public health problem in more than 100 sub-tropical and tropical regions. *Aedes aegypti* (Linnaeus) and *Ae. albopictus* (Skuse) are the principle vectors of the dengue fever as well as chikungunya, yellow fever, and recently Zika viruses. According to (WHO 2011), *Ae. aegypti* mosquito is found in many of the tropics and sub-tropics areas worldwide, whereas *Ae. albopictus* has mainly distributed in Asian cities and villages.

Male and female mosquitoes require a source of carbohydrate in order to obtain energy (Anderson et al. 2016). Adult mosquitoes are mostly able to obtain their needs of sugar meal from nectar that on stems, leaves, from floral nectar or honeydew droplets excreted by a group Homoptera (Foster 1995; Yuval 1992). Thus, honeydew and plant-derived sugars are very important nutritional components for mosquitoes to obtain energy for flight, survival, fecundity, and maintaining nutritional reserves (Nayar and Sauerman 1971; Nayar and Sauerman 1975; 1975a; Foster 1995; Breigel 2003).

Attractive toxic sugar bait (ATSB) is one of the new methods for control adult mosquitoes by exploiting mosquito sugar feeding behavior (Scott et al. 2016). Attractive toxic sugar baits method was developed and tested in the fields in Middle East, Africa, and the United States, where it has clearly showed its ability in reducing the local population of culicine and anopheline mosquito species (Müller and Schlein 2006, 2008; Müller et al. 2008, 2010; Gu et al. 2011; Beier et al. 2012). The concept is to formulate a toxin into a sugary material and present it to the mosquitoes in nature in such a way that they encounter it, ingest the baits with toxin, and die. The first commercial formulation of a toxic sugar baits registered by the US EPA for

mosquitoes is ATSB™ by Westham Inc. The concept is similar to other baited toxins formulated to attract and kill insects by ingestion of the baited materials. The baits presentation can be broadcast by spray, or in a station.

Spinosyns, in combinations and formulations marketed as spinosad, are a class of insecticides effectively against many orders of insects including Orthopteran, Diptera, Coleoptera, Hymenoptera, Isoptera, Homoptera, Thysanoptera, and Lepidoptera. Spinosad is secondary fermentation metabolites of the actinomycete bacteria, *Saccharopolyspora spinosa*. In addition to the wide range of effectiveness against important insect pests, spinosad has also satisfied mammalian and environmental toxicological profiles to permit classification as a class IV toxin, a noncarcinogen, and an organic substance (Crouse and Sparks 1998; Sparks et al. 1998, 1999).

Spinetoram, a similar insecticide blend of spinosyn-like compounds derived from fermentation of the same bacterial species, exhibits a wider spectrum activity with more insecticidal potency and efficacy but with similar toxicological profiles as spinosad (Galm and Sparks 2016). In both cases, the relative toxicity to adult mosquitoes is not well known yet owing to the particular toxicological modes of action, these compounds are attractive, particularly when resistance to other insecticides, such as pyrethroids is present in populations. Subacute exposures of insecticides are important considerations in insect control, because exposures may commonly lead to non-lethal yet negative effects. Exposures that do not lead to death (i.e., sub-lethal exposures) could occur for a variety of reasons in nature, and may lead to several consequences other than acute toxicity, such as reduced survivorship, fecundity, or lifetime fertility.

Use of the ATSB method for targeting adult mosquito species by either spraying insecticide sugar bait solutions as toxin baits directly onto plant surfaces, where mosquitoes rest and forage for sugar, or by a bait station could be affected by either rainfall, density of vegetation cover, humidity, or other environmental factors. consequently, adult mosquitoes might ingest a non-lethal concentration. Ail et al. (2006) reported that oral ingestion of a sub-lethal concentration of boric acid (0.1%) sugar baits affected survival, fecundity, and fertility of adult *Stegomyia albopicta* (i.e., *Aedes albopictus*) mosquito. In contrast, Anderson et al. (2016) indicated that (0.1%) boric acid sugar baits mixture with pyripoxfen had no significant effects on survival and fecundity of female of *Ae. aegypti*. Also, egg laying of *An. quadrimaculatus* was reduced following the exposure to DDT, chlordane, BHC, aldrin and rotenone (DeCoursey et al. 1953). Sub-lethal doses of three pyrethroid insecticides reduced female of *Ae. aegypti* blood engorgement as well as the number of eggs laid (Liu et al. 1986).

Our previous chapter has showed that spinosad and spinetoram in sugar solution can be promising new naturally derived insecticides for control of adult *Aedes* mosquito species. However, when the lethal concentrations were not achieved, a number of adult mosquitoes may be able to survive and fecund in the presence of the sub-lethal concentration of spinosad or spinetoram sugar solution. In fact, this is an important issue of concern because it is more likely that new mosquito progeny will inherit traits that allow them to survive in the presence of spinosad or spinetoram residues. In addition of possibility to develop resistance in targeted population against those products. Therefore, the objective of this research was to evaluate the effects of subacute exposure to an LC_{50} concentration of spinosad and spinetoram sugar solution on the survival, fecundity and fertility of *Ae. aegypti* and *Ae. albopictus*.

MATERIALS AND METHODS

Mosquito Maintenance

Two species of *Aedes* mosquito used in this research were: *Aedes aegypti* (Linnaeus) and *Aedes albopictus* (Skuse). The eggs of these species were obtained from Dr. Barry Alto at the University of Florida, Florida Medical Entomology Laboratory, Vero Beach. The strains originated from wild populations sampled at White City, Florida, USA. These strains had successfully established as viable colonies in insect microbiology laboratory at Michigan State University. The colonies were maintained in a Percival Scientific incubator under relative humidity of 50% ± 10% and temperature of 28°C ± 1°C (mean ± standard deviation) and a photoperiod of 12:12(light/ dark) hours.

Adult mosquitoes were held in front opening, collapsible insect cages (BugDorm; BioQuip Products, Rancho Dominquez, CA), where they had constant access to 10% sucrose solution (hereafter, "sugar water") provided *ad libitum* with small cotton wicks (Coltene, Cuyahoga Falls, HO) fitted to plastic reservoirs. Adult female mosquitoes were bloodfed with fresh, defibrinated bovine blood (Hemostat Laboratories, Dixon, CA) via artificial membrane feeder heated to around 36.5°C for at least 30 minutes at two times a week. After females had been bloodfed, a small dark jar lined with brown paper towel, provisioned to half-full of water, and serving as an oviposition container was placed inside the cages.

Eggs were collected on the paper towel at three or four-day intervals, where the egg-brown paper towels were kept at incubation temperature for several days to insure the embryonation. Then they were stored in plastic zip lock bags to maintain high humidity. This method of storage enables *Aedes*' eggs to remain viable for several months (Clemons et al. 2010). For hatching, strips of paper containing eggs were placed in plastic trays containing tap

water, ca. 1:1. After hatching, tap water was added to make the trays 2/3 full. To feed newly hatched larvae, a mixture of liver powder (Sigma-Aldrich, life science, MO) and ground Tetramin fish flakes (Tetramin, Blacksburg, VA) was added to the water daily in small pinches, in order to provide adequate food but without overfeeding and fouling the water.

Larvae were raised at low densities of ca. 100 per tray until they develop to the third instar. The water in plastic trays was changed to eliminate waste and excess food material. Then, newly emergent pupae were transferred to small plastic cups containing water by using a pipette and placed them in insect rearing cages for adult emergence, where 10% sugar solution was provided as described above.

Bio-insecticides

The products tested were spinosad (Entrust® SC is a Naturalyte® commercial formulation, Dow AgroSciences LLC, Indianapolis, IN) containing 22.5% active ingredient in a concentration suspension of liquid. Spinetoram (Delegate® WG Insecticide, 25% active ingredient in a water dispersible granule formulation).

Effects of Subacute Exposure on the Survivorship

To assess the effects of subacute exposure of spinosad and spinetoram sugar solution on the survivorship of adult *Aedes* mosquitoes, adult males and females of *Ae. aegypti* and *Ae. albopictus* were transferred separately from mosquito rearing cages via a mouth aspirator, mechanical separation device, (John W. Hock Company, Gainesville, FL) to 460 mL disposable plastic cups (hereafter, “small cages”) (Deli serve, WNA, Chattanooga, TN), which were covered with fabric nets with a 1 cm hole in the top to facilitate transferring adult mosquitoes,

plus one or two rubber bands to be fastened. In these small cages, adult mosquitoes were orally exposed to a 24-h LC_{50} concertation of spinosad or spinetoram sugar solution, based on their acute dose- response toxicity tests conducted in previous chapter, by allowing them to feed through a cotton wick with reservoir that contains the insecticide mixed with sugar for 24 hours.

Subsequently, surviving adult *Aedes* mosquitoes were counted and carefully transferred to new small cages, where they had continuously access to a 10% sugar water without insecticides through a cotton wick with reservoir for the duration of their lifetime. The control individuals of this study received a 10% sugar water without previous orally exposure to LC_{50} concentrations. All small cages were held in an incubator (Percival Scientific incubator, Perry, IA) at 26°C ± 1°C, relative humidity of 50% ± 10% and a photoperiod of 12:12 (light: dark) during the experiments. Two replicated were performed. The adult mosquito survival times were recorded for both sexes of *Ae. aegypti* and *Ae. albopictus*.

Effects of Subacute Exposure on the Fecundity and Fertility

To assess the effects of subacute exposure of spinosad and spinetoram sugar solution on fecundity and fertility of adult female of *Ae. aegypti* and *Ae. albopictus* mosquitoes, adult females were selected and transferred via a mouth, mechanical separation device, (John W. Hock Company, Gainesville, FL) from mosquito rearing cages to small cages to orally expose them to a 24-h LC_{50} concentration of spinosad or spinetoram sugar solution for only 24 hours. After the exposure time, surviving female mosquitoes were carefully transferred to new cages containing healthy control males in order to ensure mating occurred. In addition, females were provided with a blood meal for at least 30 minutes.

Subsequently, 20 of blood-fed females were moved to new small cages, where they had continuously access to a 10% sugar water without insecticides through a cotton wick with reservoir as well as a cup with 15 ml of water and an oviposition substrate comprising brown paper strips serving as an oviposition site. All cages of mosquitoes were kept for about 5-7 days post blood – feeding in an incubator (Percival Scientific incubator, Perry, IA) at 26°C ± 1°C, relative humidity of 50% ± 10% and a photoperiod of 12:12 (light: dark). Four replicated were performed. The number of eggs laid by females were counted and recorded. Then ovistrips were dried out at incubation temperature for least two days to insure the egg embryonation. Finally, Eggs were placed in containers containing tap water to determine the fertility (percentage of eggs hatching). Similar method for control group except that no previous orally exposure to median lethal concentrations of spinosad or spinetoram sugar solution.

Statistical Analysis

The effects of subacute exposure to spinosad and spinetoram sugar solution on survival data of *Aedes* mosquito were analyzed by the Kaplan-Meier survival analysis (Parmar and Machin, 1995) to compare between mosquito-survivorship curves using the log-rank (i.e., Mantel-Cox) test. The comparisons of the effects of subacute exposure to spinosad and spinetoram sugar solution on female fecundity data were analyzed using the Student's *t*-test. Statistical analyses were performed with SAS 9.4 statistical software (SAS Institute 2004). The comparisons of the effects of subacute exposure to spinosad and spinetoram sugar solution on fertility data were analyzed by one-way analysis of variance (ANOVA) followed by Tukey's test using VassarStats program. The differences between treatment and control groups were considered to be statistically significant at P-value < 0.05.

RESULTS

Effects of Subacute Exposure on the Survivorship

Following the subacute exposure to median lethal concentration of spinosad and spinetoram sugar solution, effects on survivorship adult of *Aedes* mosquitoes were occurred. As shown in (Fig. 13, A), exposure to spinosad sugar solution for 24 hours significantly reduced the survivorship of females *Ae. aegypti* comparing with unexposed females (χ^2 = 15.29, P < 0.001). Also, survivorship of females of *Ae. aegypti* was significantly reduced following exposure to spinetoram sugar solution (Fig. 13, C) compared with females in control group (χ^2 = 30.89, P < 0.001). According to (Fig. 13, E), the survival curves were not significantly different for females of *Ae. aegypti* when exposed to either spinosad or spinetoram sugar solution (χ^2 = 2.53, P = 0.111).

In contrast, a statistically significant difference was observed in females of *Ae. albopictus* survival curves (Fig. 13, F) when exposed to either spinosad or spinetoram sugar solution (χ^2 = 4.43, P = 0.035). Exposure to spinosad (Fig. 13, B) did significantly affect the survivorship of female *Ae. albopictus* comparing with the females in control group (χ^2 = 40.84 , P < 0.001). Additionally, the survivorship of females *Ae. albopictus* exposed to spinetoram sugar solution (Fig. 13, D) decreased significantly compared with that of unexposed females (χ^2 = 25.68, P < 0.001).

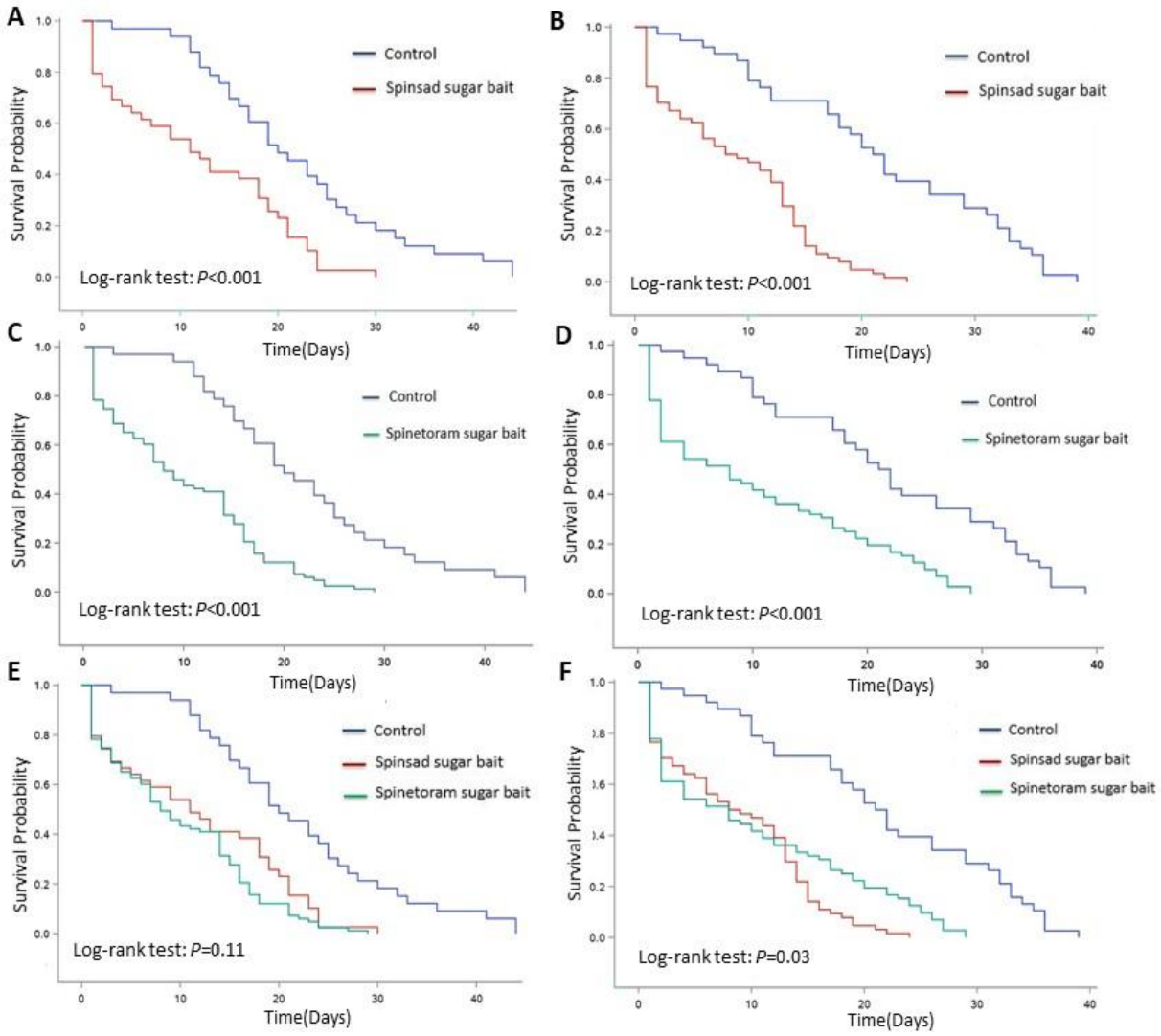

Figure 13: Kaplan-Meier analysis for the effects of subacute exposure to an LC_{50} concentration of (A) spinosad and (C) spinetoram in sugar solution and (E) the differences between spinosad and spinetoram on the survivorship of females of yellow fever mosquito *Ae. aegypti*, whereas (B) spinosad and (D) spinetoram in sugar solution and (F) the differences between spinosad and spinetoram on the survivorship of females of Asian tiger mosquito *Ae. albopictus* and unexposed females in control. Survival curves were compared using a log-rank test. *P*-value in each test is shown.

On the other hand, exposure to median lethal concentration of spinosad sugar solution (Fig. 14, A) for 24 hours significantly affected the survivorship of males *Ae. aegypti* when compared with unexposed males (χ^2 = 5.49, *P* = 0.01). Also, spinetoram sugar solution (Fig. 14, C) had significantly effect in reducing the survivorship of males *Ae. aegypti* after 24 hours of exposure (χ^2 = 5.16, *P* = 0.02), whereas there was no significant difference in survival curves of males *Ae. aegypti* following exposure to spinosad or spinetoram sugar solution (χ^2 = 0.03, *P* = 0.84) (Fig. 14, E).

The survivorship (Fig. 14, B) of males *Ae. albopictus* after 24-h of exposure to spinosad was significantly lower than those males in control group (χ^2 = 4.50, *P* = 0.03). In addition, exposed males to spinetoram sugar solution (Fig. 14, D) did significantly affect the survival of males *Ae. albopictus* compared with that of unexposed males (χ^2 = 15.23, *P* < 0.001). Spinosad and spinetoram sugar solution (Fig. 14, F) significantly differed in reducing the survivorship of exposed males *Ae. albopictus* (χ^2 = 4.23, *P* < 0.001).

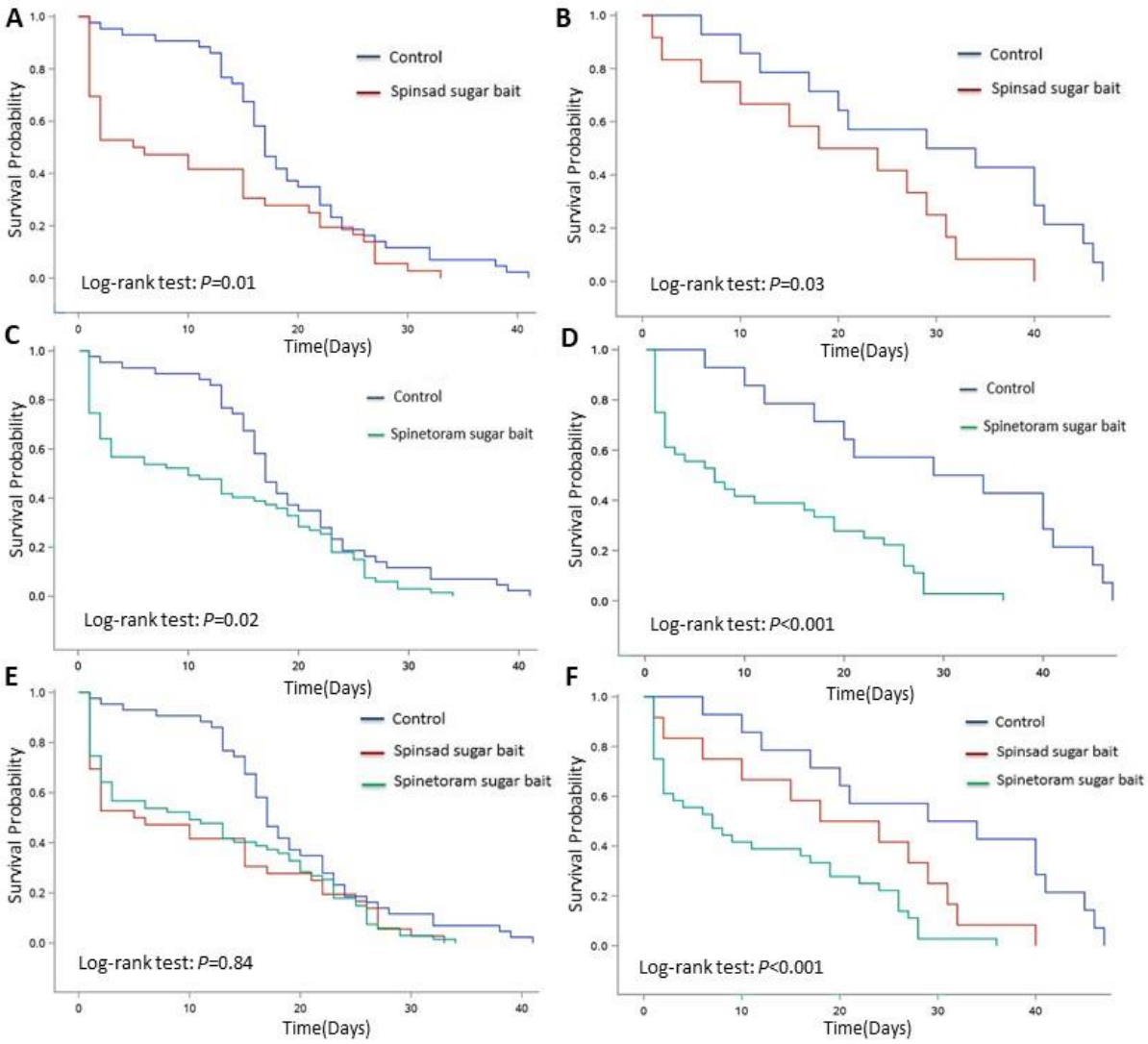

Figure 14: Kaplan-Meier analysis for the effects of subacute exposure to an LC_{50} concentration of (A) spinosad and (C) spinetoram in sugar solution and (E) the differences between spinosad and spinetoram on the survivorship of males of yellow fever mosquito *Ae. aegypti*, whereas (B) spinosad and (D) spinetoram in sugar solution and (F) the differences between spinosad and spinetoram on the survivorship of males of Asian tiger mosquito *Ae. albopictus* and unexposed males in control. Survival curves were compared using a log-rank test. *P*-value in each test is shown.

Effects of Subacute Exposure on the Fecundity and Fertility

Overall, exposed females of *Ae. aegypti* and *Ae. albopictus* to an LC_{50} concentration of spinosad and spinetoram sugar solution for 24-h affected the number of eggs laid comparing with females in control group (Table 4 and 5). On average, the fecundity of females *Ae. aegypti* was not significantly affected after the exposure to spinosad ($t = 1.41$, $P = 0.20$) (Fig. 15, A) and spinetoram ($t = 2.73$, $P = 0.06$) (Fig. 15, B) sugar solutions compared with unexposed females in control. There was no significant difference observed in the number of eggs laid by females *Ae. aegypti* when exposed to either spinosad or spinetoram sugar solution ($t = 1.44$, $P = 0.199$) (Fig. 15, C). In general, the fertility, as eggs hatch rate, was significantly decreased in the exposed females *Ae. aegypti* ($F= 23.37$, $P < 0.001$) compared to unexposed females (Fig. 17, A).

In contrast, females *Ae. albopictus* significantly produced a greater number of eggs following the exposure to spinosad ($t = 3.11$, $P = 0.02$) (Fig. 16, A) and spinetoram ($t = 4.47$, $P = 0.004$) (Fig. 16, B) sugar solutions comparing with unexposed females. As shown in Figure 16, C, when exposed females *Ae. albopictus* to spinosad or spinetoram sugar solution, there was no significantly different in fecundity ($t = 1.04$, $P = 0.339$). Exposure to spinosad and spinetoram sugar solution significantly reduced females *Ae. albopictus* fertility ($F= 26.87$, $P < 0.001$) compared to unexposed females (Fig. 17, B).

Formulations		Egg production		Total number of eggs hatched	Fertility (% egg hatch)
	N	Total	Mean		
Spinosad	20	1024	256 ± 23.90	470	45.90
Spinetoram	20	1264	316 ± 34.12	380	30.06
Control	20	883	220.75 ± 7.54	680	77.01

Table 4: The effects of subacute exposure to an LC_{50} concentration of spinosad and spinetoram sugar solution, after continuous exposure for 24-h, on fecundity and fertility of female *Ae. aegypti*, compared to the control (10% sugar water). Mean ± SE

Formulations		Egg production		Total number of eggs hatched	Fertility (% eggs hatch)
	N	Total	Mean		
Spinosad	20	977	244.25 ± 23.25	380	38.80
Spinetoram	20	1103	275.75 ± 19.55	328	29.70
Control	20	582	145.50 ± 21.62	365	62.70

Table 5: The effects of subacute exposure to an LC_{50} concentration of spinosad and spinetoram sugar solution, after continuous exposure for 24-h, on fecundity and fertility of female *Ae. albopictus*, compared to the control (10% sugar water). Mean ± SE

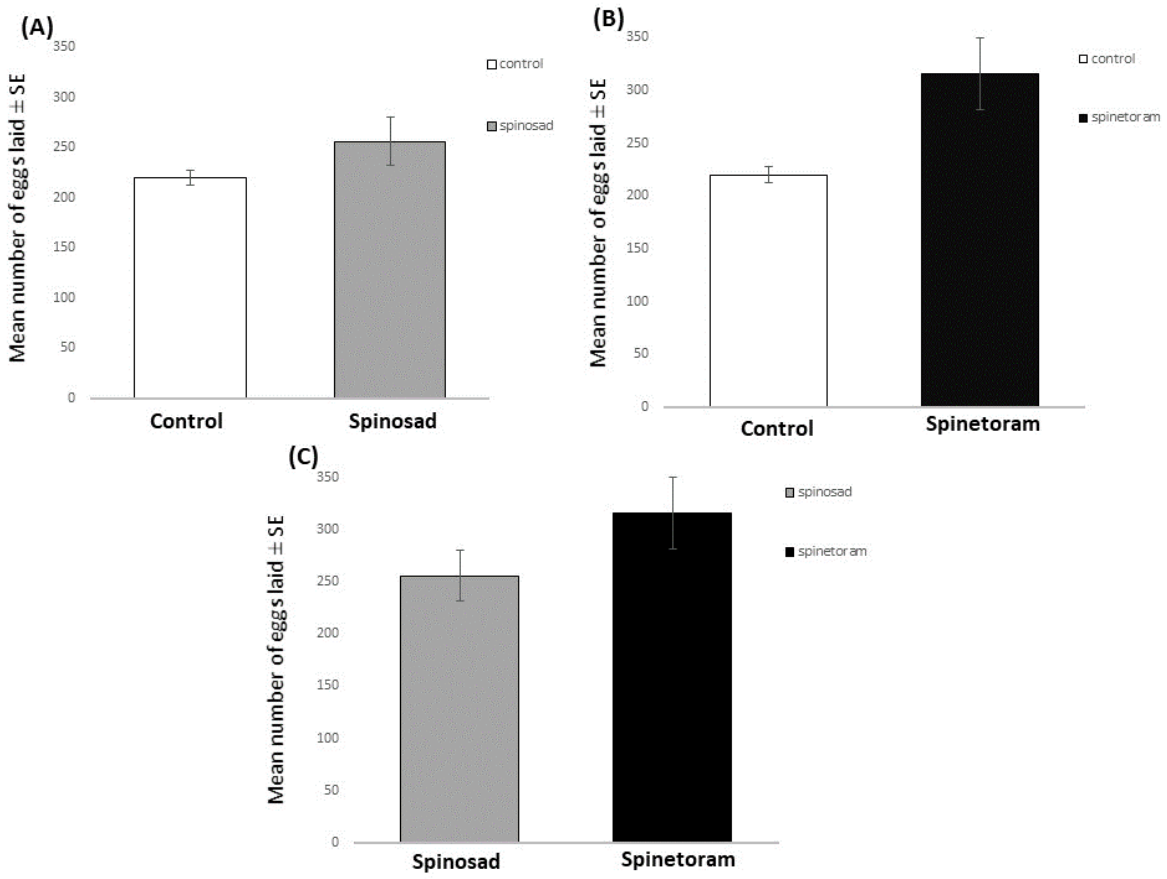

Figure 15: The effects of subacute exposure to an LC_{50} concentration of (A) spinosad and (B) spinetoram sugar solutions and (C) the differences between spinosad and spinetoram on the fecundity of females *Ae. aegypti* and unexposed females in control. The differences were compared using Student's *t*-test.

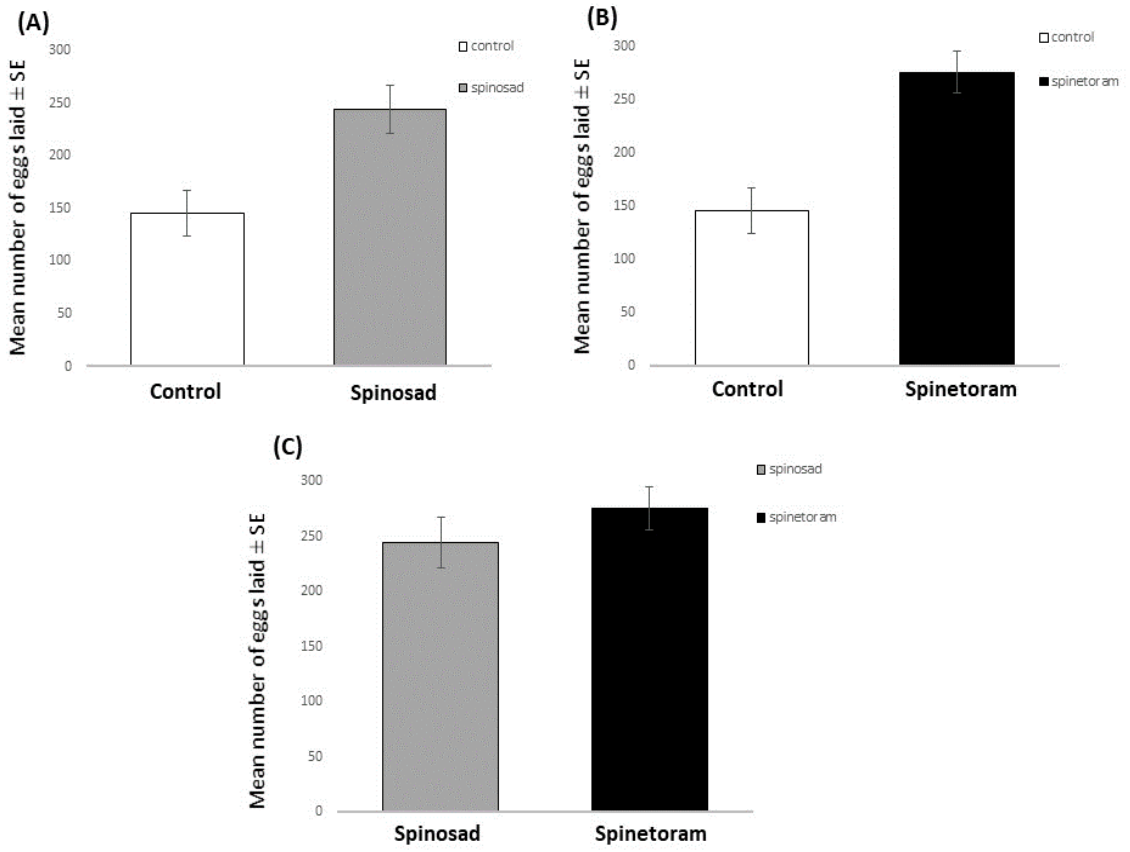

Figure 16: The effects of subacute exposure to an LC_{50} concentration of (A) spinosad and (B) spinetoram sugar solutions and (C) the differences between spinosad and spinetoram on the fecundity of females *Ae. albopictus* and unexposed females in control. The differences were compared using Student's *t*-test.

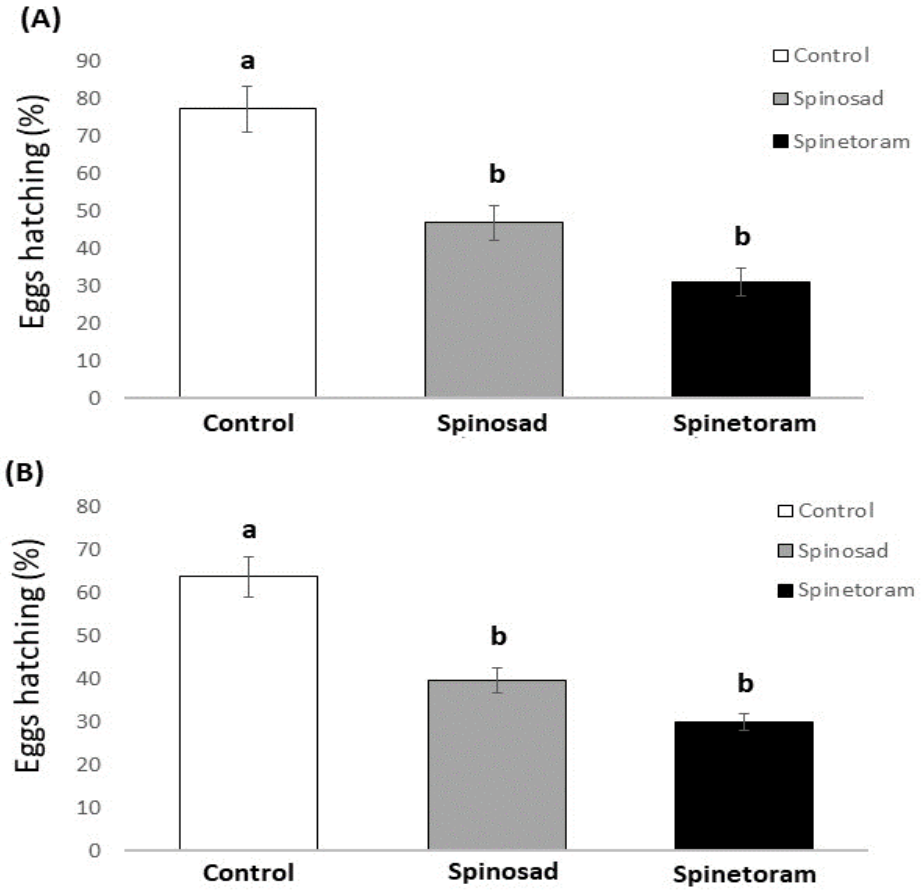

Figure 17: The effects of subacute exposure to spinosad and spinetoram sugar solutions on fertility (eggs hatch rate) of (A) females *Ae. aegypti* and (B) females *Ae. albopictus* compared to unexposed females in controls. Values are mean ± SE. Statistically significant differences are represented different letters above the bars (ANOVA and Tukey's test, $P = 0.05$).

DISCUSSION

Control of adult mosquitoes by either spraying insecticide sugar baits solution directly onto plant surface or by insecticide sugar baits stations, where adult mosquitoes rest and forage for sugar meal are more likely to be affected by either rainfall, humidity, density of vegetation cover, or other environmental factors. Consequently, targeted mosquitoes may just ingest a non-lethal concentration, which could have some impacts on their survival, fecundity, and fertility.

Our findings indicated that when a lethal concentration of spinosad and spinetoram sugar solution was not ingested, the non-lethal concentration could significantly reduce the survivorship of *Aedes* mosquito species. Also, the efficacy of spinosad and spinetoram showed to be varied between *Ae. aegypti* and *Ae. albopictus,* which could explain the difference in susceptibility among *Aedes* mosquito species. According to Allan (2011) who had reported that different mosquito species had different susceptibility to some insecticides.

The mean number of eggs laid by females *Ae. aegypti* was not significantly affected following the exposure to spinosad or spinetoram sugar solution, whereas following the exposure to spinosad or spinetoram sugar solution, the mean number of eggs laid by females *Ae. albopictus* was significantly increased compared with unexposed females. There was no significant different between spinosad and spinetoram sugar solution in terms of affecting the eggs production of females *Ae. aegypti* and *Ae. albopictus*. The percentage of fertility of eggs was significantly reduced for both *Aedes* species comparing with unexposed females in control groups. It seems that the absolute number of eggs laid were obviously greater following the exposure to spinetoram sugar solution comparing to spinosad sugar solution for both females of *Ae. aegypti* and *Ae. albopictus,* whereas females of *Ae. aegypti* was showed to be capable to produce more eggs than females *Ae. albopictus.*

There are no previous relevant data in the literature evaluating the sub-lethal effects of spinosad and spinetoram delivered in sugar solution on adult *Aedes* mosquito survival and reproduction. However, our findings are comparable with Antonio et al. (2009) who had reported that *Ae. aegypti* exposed to spinosad as larvae were not significantly affected the adult male and female longevity, whereas significantly produced more eggs and more offspring comparing with unexposed females in control.

Other insecticides can also have some effects on the survivorship and reproductive capacity of adult mosquito species. For instance, exposure larvae of *Cx. quinquefasciatus* to an LC_{50} concentration of malathion, methoprene, resmethrin or propoxur resulted in changes in adult female reproduction, whereas exposure to fenitrothion, lambda-cyhalothrin, and *Callitris glaucophyll* extracts had some changes in *Ae. aegypti* survival (Robert and Olson 1989; Shaalan et al. 2005). Survivorship of female *Ae. aegypti* mosquitoes increased when larvae were treated with temephos (Abate), which was probably due to release of surviving larvae from effects of intraspecific competition (Reyes-Villanueva et al. 1990). In another study, sub-lethal doses of tetramethrin increased egg production of female *Ae. aegypti* (Liu et al. 1986). On the other hand, exposure to sub-lethal doses of malathion reduced the number of eggs laid by *Cx. quinquefasciatus* mosquito (Robert & Olson 1989). Moreover, the number of eggs laid by female *Ae. aegypti* was reduced after dermal exposure to sub-lethal doses of dieldrin, d-phenothrin, and d- allethrin (Duncan 1963; Liu et al. 1986). Based on these results, the sub-lethal effects could be dependent on insecticides mode of action, type and length of exposure, insect species and life stage, concentrations, and other environmental factors.

In conclusion, Exposure to an LC_{50} concentration of spinosad and spinetoram sugar solution for 24-h showed significantly reducing in survivorship of both sexes of *Ae. aegypti* and *Ae. albopictus* mosquitoes. The fecundity of exposed females *Ae. aegypti* was not significantly affected, whereas significantly increased in fecundity of females *Ae. albopictus* compared with unexposed females. On the other hand, the fertility of *Ae. aegypti* and *Ae. albopictus* was significantly reduced compared to the controls. This finding indicated that ingestion of the non-lethal concentration of spinosad or spinetoram sugar solution could be to reduce the *Aedes* species population, where spinetoram showed to be relatively more effective than spinosad. Using spinosad and spinetoram in attractive toxic sugar baits technology can be effective in controlling *Aedes* mosquito species and other sugar feeding insect pests. This study was only conducted under laboratory condition, so further evaluation under field conditions is necessary, where the persistence of these chemicals in sugar baits could be also assessed.

CONCLUSIONS AND RECOMMENDATIONS

The purposes of this study were to determine the acute toxicity of spinosad and spinetoram in sugar solution against adult of *Ae. aegypti* and *Ae. albopictus* and also to evaluate the effects of subacute exposure to spinosad and spinetoram in sugar solution on survival and fecundity and fertility of *Ae. aegypti* and *Ae. albopictus* under the laboratory conditions.

The acute toxicity results showed that spinosad and spinetoram when delivered in sugar solution were toxic to males and females of *Ae. aegypti* and *Ae. albopictus.* There were no significant differences in efficacy between spinosad and spinetoram, whereas after 48-h of exposure spinetoram was significantly more toxic to males of *Ae. aegypti* compared to spinosad, suggestion that spinetoram could be relatively more toxic than spinosad in sugar solution.

Exposure to spinosad and spinetoram significantly reduced the survivorship of males and females of *Ae. aegypti* and *Ae. albopictus,* whereas exposure to spinosad and spinetoram affected fecundity (egg production) by significantly increasing it for *Ae. albopictus* but not for *Ae. aegypti*. However, the fertility as measured by hatch rate of eggs was significantly lower for *Aedes* mosquitoes that had fed on sugar solution with spinosad or spinetoram compared to controls, suggestion that spinosad and spinetoram could reduce some of the transmission parameters when the lethal concentrations were not achieved.

Overall, this research provided an accurate estimation of the compatibility of these naturally derived products in sugar solution for the integrated mosquito management programs. The new generation of spinosyns, spinetoram can be a new candidate to use in the attractive toxic sugar baits method as spinosad with recommendation of maintaining the concentrations at lethal level to overcome any problem related to the increase of the number of eggs. Spinetoram and spinosad are recommended to incorporate with sugar baits to evaluate their efficacy under field trails conditions against *Aedes* mosquito species.

APPENDIX

APPENDIX 1:

RECORD OF DEPOSITION OF VOUCHER SPECIMENS

The specimens listed below have been deposited in the named museum as samples of those species or other taxa, which were used in this research. Voucher recognition labels bearing the voucher number have been attached or included in fluid preserved specimens.

Voucher Number: 2017-11

Author and Title of thesis:

Abdullah Abdulaziz Alomar

"ACUTE AND SUBACUTE TOXICITY OF SPINOSAD AND SPINETORAM DELIVERED IN SUGAR SOLUTION TO ADULT *AEDES AEGYPTI* AND *AEDES ALBOPICTUS* (DIPTERA: CULICIDAE)"

Museum(s) where deposited:

Albert J. Cook Arthropod Research Collection, Michigan State University (MSU)

Table 6: List of voucher specimens.

Family	Genus/Species	Life Stage	Quantity	Preservation
Culicidae	*Aedes aegypti*	Adult	5 (Female)	Pinned
Culicidae	*Aedes aegypti*	Adult	5 (Male)	Pinned
Culicidae	*Aedes albopictus*	Adult	5 (Female)	Pinned
Culicidae	*Aedes albopictus*	Adult	5 (Male)	Pinned

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