# EFFICACY AND RESIDUAL EFFECT OF ZEOLITE GRANULAR TEMEPHOS FORMULATION AND MONOMOLECULAR FILM OIL AGAINST LARVAE AND PUPAE OF *AEDES ALBOPICTUS* (SKUSE) (DIPTERA: CULICIDAE)

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**By**

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**KAJIAN KEBERKESANAN DAN KESAN SISA BAKI FORMULASI GRANUL TEMEPHOS ZEOLITE DAN MINYAK FILEM MONOMOLEKULER TERHADAP LARVA DAN PUPA *AEDES ALBOPICTUS* (SKUSE) (DIPTERA: CULICIDAE)**

## ABSTRAK

Ujian keberkesanan dan kesan sisa baki telah dijalankan ke atas sejenis formulasi baru granul temephos zeolite (Azai) dan minyak filem monomolekular (Agnique MMF). Pada awalnya, kajian keberkesanan telah dijalankan terhadap larva dan pupa *Ae. albopictus* di makmal dan di lapangan dengan menggunakan baldi plastik berwarna hitam (15 Liter). Dos yang digunakan untuk Azai ialah 0.50ppm, 0.75ppm, 1.00ppm, 12.5ppm and 1.5ppm a.i., manakala bagi Agnique MMF ialah 5.00ppm, 8.33ppm, 10.00ppm, 13.33Lppm and 16.67ppm a.i. Dalam lima hari, kesemua dos Azai menunjukkan peratus kematian larva (%LM) yang lebih tinggi daripada Agnique MMF. Sebaliknya, kesemua dos Agnique MMF lebih berkesan terhadap pupa berbanding Azai di makmal dan di lapangan.

Kajian kesan sisa baki telah dijalankan ke atas Azai dan Agnique MMF di makmal dengan menggunakan bekas plastik (450ml). Dos yang digunakan bagi Azai adalah sama seperti eksperimen sebelumnya tetapi Agnique MMF telah dirawat pada 40ppm a.i. (dos rendah) dan 400ppm a.i. (dos tinggi) didalam bekas plastik yang kecil. Rawatan hanya diberikan pada permulaan eksperimen dan tidak diulang. Dua jenis penambahan air telah ditentukan: tidak ditambah air

dan ditambah air. Selama 28 minggu pemantauan, semua dos-dos Azai di kedua-dua bekas yang ditambah air dan tidak ditambah air menunjukkan peratus kematian larva (%LM) dan peratus perencatan kemunculan nyamuk dewasa (% IE) yang tinggi manakala dos tinggi bagi Agnique MMF (400ppm) didalam bekas yang tidak ditambah airnya didapati mampu untuk menghapuskan lebih dari 50% larva yang didedahkan dan menghalangnya daripada menjadi dewasa sehingga empat minggu selepas diberi rawatan.

Kajian kesan sisa baki Azai dan Agnique MMF telah dijalankan di bawah keadaan lapangan di Pulau Pinang, Malaysia dengan menggunakan dos yang sama di dalam baldi (15L). Dos yang disyorkan oleh pengilang, 1 ppm a.i. memberikan kawalan yang memuaskan ke atas larva *Ae. albopictus* untuk tempoh lebih daripada sepuluh minggu selepas rawatan. Bagaimanapun, baldi yang dirawat dengan semua doa-dos Agnique MMF menjadi positif dengan kehadiran larva selepas dua minggu.

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## ABSTRACT

Efficacy and residual effect of a new formulation of temephos, a zeolite granular formulation (Azai) and monomolecular film oil (Agnique MMF) were conducted. Initially, an efficacy study was evaluated against larvae and pupae of *Ae. albopictus* in the laboratory and in the field by using black plastic pails (15 liters). The dosages for Azai used were 0.50ppm, 0.75ppm, 1.00ppm, 1.25ppm and 1.50ppm of a.i., while for Agnique MMF were 5.00ppm, 8.33ppm, 10.00ppm, 13.33ppm and 16.67ppm of a.i. In five days, all dosages of Azai showed higher percentages of larval mortality (%LM) compared to Agnique MMF. In contrast, all dosages of Agnique MMF were superior against pupae compared to Azai in the laboratory and the field.

The residual effect of Azai and Agnique MMF were evaluated in the laboratory by using plastic containers (450ml) against *Ae. albopictus*. The dosages of Azai used were similar to the previous experiment but Agnique MMF was applied at 40ppm (low dose) and 400ppm (high dose) of a.i. in small size plastic containers. The treatments were applied once at the beginning of the experiment. Two types of water refilling were established: non-replenishment of

water and replenishment of water. During the 28 weeks of assessment, all dosages of Azai in both replenished and non-replenished containers showed high percentages of larval mortality (%LM) and inhibition of emergence (% IE) while high dosage of Agnique MMF (40ppm a.i.) in the non-replenished containers was found to be able to eliminate more than 50% of exposed larvae and prevented them from emerging into adults until four weeks post-treatment.

The residual effect of Azai and Agnique MMF were evaluated under field conditions in Penang, Malaysia using similar dosages in the pails (15L). The recommended dosage of Azai by the manufacturer, 1 ppm a.i. provided satisfactory control of *Ae. albopictus* larvae for up to ten weeks post-treatment. However, the pails which were treated with Agnique MMF became positive with larvae after 2 week in all dosages.

# CHAPTER 1

# GENERAL INTRODUCTION

## 1.1 Introduction

Mosquitoes are among the best known groups of insects because of their importance to man as pests and vectors of some of the most worrying human diseases. They are small, two-winged insects belonging to the family Culicidae of the order Diptera (two-winged flies). This family is divided into three subfamilies: *Toxorhynchitinae*, *Anophelinae* and *Culicinae*. The last being subdivided into two tribes, *Sabethini* and *Culicini* (Goma, 1966).

These small creatures are found throughout the world except in places that are permanently frozen. Three quarters of all mosquito species live in the humid tropics and subtropics, where the warm moist climate is favourable for rapid development and adult survival and the diversity of habitats permitted the evolution of many species (Clements, 1992). There are some 34 genera and about 3100 species of mosquitoes known at present throughout the world. In Malaysia, there are 431 species of mosquitoes representing 20 genera (Abu Hassan and Che Salmah, 1990).

Mosquitoes are hosts to a variety of pathogens and parasites, including viruses, bacteria, fungi, protoctistans and nematods. The blood-sucking habit renders adult mosquitoes prone to acquire pathogens and parasites from one vertebrate host and to pass them on to another (Clements, 1992). *Aedes*, *Culex*, *Anopheles*, *Mansonia, Haemagogus, Sabethes and Psorophora* are some genera of mosquito that are medically important because of diseases such as malaria, filariasis and virus diseases (yellow fever, dengue and the mosquito-borne encephalitis) that they transmit while sucking human blood.

Nowadays, Dengue fever (DF) and Dengue haemorrhagic fever (DHF) are increasing in importance as millions of people are infected by these diseases annually (Jacobs, 2000). Dengue fever (DF) and Dengue haemorrhagic fever (DHF) are caused by the dengue virus. There are four virus serotypes (DEN-1, DEN-2, DEN-3 and DEN-4) belonging to the genus *Flavivirus*, family Flaviviridae (Gubler and Clark, 1996).

The vectors carrying these diseases are four man-biting species of *Aedes*, specifically Ae. *aegypti* (Linnaeus), Ae. *albopictus* (Skuse), *Ae. scutellaris* and Ae. *polynesiensis* (Marks) which breed efficiently in urban environments (Clements, 1992). *Aedes aegypti* and *Ae. albopictus* are major vectors in many urban areas of South-east Asia while Ae. *polynesiensis* (Marks) and *Ae. scutellaris* were incriminated in the transmission of these diseases in the Pacific islands (Smith, 1956; Rudnick, 1967; WHO, 1995).

The most important vector of DHF in the Western Pacific Region is *Ae. aegypti* while *Ae. albopictus* is an endemic species and has been associated with the transmission of DF in the Western Pacific Region since the early 19$^{th}$ century (WHO, 1995). These two species are widely distributed throughout Malaysia and have been known as vectors of dengue for years (Lo and Narimah, 1984; Yap 1984; Chan and Counsilman, 1985; Lee and Cheong, 1987; Rebecca, 1987; Lam, 1993; Lee and Inder, 1993).

The earliest report of dengue fever (DF) in Malaysia was from Penang in 1902. However, the first report of Dengue haemorrhagic fever (DHF) was made only in 1962 in Penang Island and since then, the disease has become endemic throughout the country (Singh, 2000). The first major national DF and DHF outbreak occurred in 1973. There were 969 cases and a case fatality ratio of 5.6/100 as reported by Kementerian Kesihatan Malaysia (2009).

In 2008, a total of 49,335 cases of dengue fever were reported in Malaysia. Out of these reported 49,335 cases, 94% or 46,518 cases were of dengue fever (DF) while Dengue haemorrhagic fever (DHF) accounted for 6% or 2,817 cases. The number of related deaths from these diseases increased from 98 deaths in 2007 to 112 deaths in 2008 (Kementerian Kesihatan Malaysia, 2009).

*Aedes aegypti* and *Aedes albopictus* were also responsible for the transmission of the Chikungunya virus. Chikungunya virus is a member of the genus *Alphavirus*, in the family *Togaviridae*. Chikungunya mainly occurs in areas of Africa and Asia. Countries that have experienced recent outbreaks of chikungunya fever include India, Indonesia, Malaysia, Singapore, and Sri Lanka. In Malaysia, a total of 3,452 cases of Chikungunya fever were reported until November 2008 (Kementerian Kesihatan Malaysia, 2008). However, no fatal cases were reported.

Currently, neither chemotherapeutic agents nor effective vaccines are available for Dengue and Chikungunya and the only method of controlling or preventing these diseases is combating the vector mosquitoes. The available vector control methods are mainly based on environmental management, biological control and the use of chemicals. With rare exceptions, environmental management and biological control have limited applicability on their own and chemical control is still considered as the most important element in the integrated control of these vector-borne diseases (WHO, 1996).

In Malaysia, vector control is still heavily dependent on the use of chemical insecticides. Larviciding is the first step in chemical mosquito control, since the mosquitoes are killed at the breeding site, prior to dispersing and infesting a community. Since early 1970, WHO has recommended temephos sand granules (Abate) for the control of *Aedes* mosquitoes (WHO, 1985) and it has been used extensively in the past 30 years for the control of *Ae. aegypti* and *Ae. albopictus* in Malaysia.

To develop and avail more options for control, Azai and Agnique MMF were evaluated. Azai is a new formulation of temephos larvicide which contains temephos 1% (w/w) and the inert mineral clonoptilolite (zeolite) 99% (w/w), whereas Agnique MMF is an ethoxylated alcohol non-ionic surfactant made from plant oils which can control immature mosquitoes through physical mode of action. The active ingredient in Agnique MMF is poly (oxy-1, 2-ethanediyl), α-Isooctadecyl-ω-hydroxyl.

## 1.2 Objective

The objective of the study is to evaluate the efficacy and residual effect of a new formulation of temephos larvicide (Azai) and a monomolecular surface film product (Agnique MMF) against *Ae. albopictus* larvae and pupae in laboratory and field conditions.

# CHAPTER 2

# LITERATURE REVIEW

## 2.1 Biology of *Aedes albopictus*

The Asian tiger mosquito (*Aedes albopictus*) is characterized by its black and white striped legs and small black and white body. This mosquito has become a significant pest in many communities because of its aggressive daytime human-biting behavior and ability to vector many pathogens and viruses that cause diseases such as West Nile, LaCrosse**,** Yellow fever, Encephalitis, Dengue fever and Chikungunya fever. This mosquito undergoes complete metamorphosis, passing through four distinct stages during their life cycle which consists of egg, larvae, pupae and adult (Miyagi and Toma, 2000).

### 2.1.1 Eggs

*Aedes albopictus* eggs are black cigar-shaped like those of *Aedes aegypti* (Abu Hassan and Yap, 1999) (Plate 2.1). They are blunt at the anterior end and more tapering in the posterior region (Estrada-Franco and Craig, 1995). They are laid singly and can withstand dryness for a longer period of time. When dried under natural conditions the eggs can retain their viability for up to six months or longer (WHO, 1995).

Plate 2.1: The eggs of *Aedes albopictus*

The eggs must be submerged in water in order to hatch (Abu Hassan and Yap, 1999). They hatch after the containers become flooded naturally (rainfall) or artificially (human water storage). However, hatching only occurred when the eggs above dry line were soaked by rain but not flooded by rising water (Kloter et al., 1983). After embryonic development has been completed, hatching can occur within minutes of flooding and larval growth and development can proceed immediately. However, sometimes the eggs of *Aedes* take several weeks to hatch after wetting in addition to requiring relatively calm environment for breeding (Foo et al., 1985).

Flooding and submerging these dried eggs can induce partial hatching from the eggs batches. Subsequent drying and flooding can induce further hatching from the remaining unhatched eggs of the same batch (WHO, 1995).

### 2.1.2 Larvae and Pupae

*Aedes albopictus* larvae have been found to require clear, but not necessarily clean water (Lee, 1991). They have been known to breed in septic tanks and other polluted water sources where polluted water breeding mosquitoes such as *Culex quinquefasciatus* are commonly found (WHO, 1995). Examples of natural habitats for the mosquito are tree holes, bamboo holes and stumps, coconut shells, plant axils, ground pools and rock pools. Aedes *albopictus* also oviposits in artificial containers such as rubber tires, tin cans, drums, earthenware containers, bottles, flower pots, cisterns and buckets (Estrada-Franco and Craig, 1995). Generally, the immature stage of *Aedes* mosquitoes requires about seven days before adult emergence in a tropical environment. As such, any container, natural or artificial, that can accumulate water for that length of time can become a potential breeding habitat for *Aedes* (WHO, 1995).

The larvae have four developmental periods or instars (Abu Hassan and Yap, 1999). These are called 1st, 2nd, 3rd, and 4th instars with each succeeding stage larger than the last. At the end of each instar, the larvae shed its skin by a process called molting. In most of efficacy studies on larvicides, 3rd and 4th instars are usually used because the larvae are more resistance to the larvicide and their biological conditions are more stable at these stages (Busvine, 1971). The larvae are active feeders, feeding on particulate organic material in the water. The larvae have a breathing tube and must occasionally come to the surface of the water to get oxygen. Upon maturity, the 4th instar larvae molts into the pupal stage.

The pupa is the last immature stage before a mosquito emerges into an adult. It differs greatly from the larva in shape and appearance. The pupa has a comma-shaped body divisible into two distinct regions. The front region consists of the head and thorax (cephalothorax) and is greatly enlarged. It bears a pair of respiratory trumpets on the upper surface. It must periodically come to the surface to get oxygen. The second region is the abdomen which has freely-movable segments with a pair of paddle-like appendages at the tip. Feeding does not take place during the pupal stage.

The pupal stage only lasts for a few days depending on the conditions. According to Surtees (1966), under natural conditions, *Ae. albopictus* remains at the pupal stage for about two days and as in other *Aedes* species, *Ae. albopictus* males emerge before females. Hien (1975) reported that the pupal development lasted two days at 30ºC, three days at 25ºC and five days at 20ºC while Ho et al. (1972) found that *Ae. albopictus* pupae survive desiccation for up to two days at 26ºC and a relative humidity of 87%. The study also revealed that pupal mortality was about 1% under field conditions. During the pupal stage, all the larval tissues change into the adult tissues.

### 2.1.3 Adult

*Aedes albopictus* is recognized by the distinct longitudinal silver line on the scutum (Abu Hassan and Yap, 1999). Another character that can distinguish between *Ae. aegyti* and *Ae. albopictus* is the clypeus. In *Ae. albopictus* females, the clypeus is covered only with dark scales while *Ae. aegyti* shows white scales (Estrada-Franco and Craig, 1995).

Both female and male adult mosquitoes feed on nectar which they use for energy. Only the females feed on blood in order to develop her eggs. The adult female *Aedes* mates and takes its first blood meal about 48hours after emergence and take multiple blood meals between different gonotropic/ generational cycles. It is an indiscriminate blood feeder and more zoophagic than *Aedes. aegypti* (WHO,1999). Studies of *Ae. albopictus* revealed a preference for humans among wide range of blood meal hosts including mammals, birds, reptiles and amphibians (Hawley, 1988).

Once a female has completely engorged, she flies to a shaded environment until her eggs are completely developed. *Ae. albopictus* usually prefer to rest outdoor in shrubs and trees. It utilizes the undersides of leaves as resting places (Gubler, 1971). However, it can also be found in forest canopy (Rudnick, 1965).

Once the eggs are developed, a gravid female begins to search for a desirable place to lay her eggs. The act of laying eggs by mosquitoes is known as oviposition. Engorgement to oviposition usually takes two to five days (WHO, 1995). Generally, a single female lays about 60-100 eggs in initial oviposition. Laboratory and field studies indicate that both *Ae. albopictus* and *Ae. aegypti* appear to prefer darker colour backgrounds for oviposition, with special preference for red and black over lighter colours (WHO,1999).

If the female survives her oviposition activity, she will very soon start searching for another blood meal after which she will lay another batch of eggs. Laboratory studies on adults survival potential for both *Ae. albopictus* and *Ae. aegypti* indicate that the male and female mosquitoes survive an average of 20 and 30 days, respectively. Thus, each *Aedes* female can theoretically deposit up to four batches of eggs with subsequent blood meals (WHO, 1995).

*Aedes albopictus* is an aggressive biter which prefers to feed during the daytime hours (Lambrecht, 1971). By using the human-bait baiting method, Del Rosario (1963) found *Ae. albopictus* to be most active during the early hours of the morning. In field observations, Ho et al. (1973) found two defined peaks of biting activity by *Ae. albopictus* in Singapore, with the morning peak occurring just after sunrise (0730 hours) and the evening peak occurring before or immediately after sunset (1730-1830 hours).

After feeding on a person whose blood contains dengue virus, the female mosquitoes normally require an incubation period of 8 to 10 days, when the virus multiplies in the mosquito salivary gland. Thereafter, the mosquito becomes infected and will transmit the dengue virus to the next human host when feeding occurs again. In man, the incubation period is from five to seven days. The female *Aedes* mosquito can also transmit the virus immediately from an infected person to another individual by a change of host when its blood meal is interrupted. This is termed “mechanical transmission”.

The flight range of *Aedes* is rather short in comparison with other genera of vector mosquitoes such as *Anopheles*, *Culex* and *Mansonia*. Generally, adult can be found around 50 meters from the breeding sources with maximum flight distance of around 200 meters (WHO, 1995). However, its flight range may be up to 500 metres (WHO, 1999).

## 2.2 Distribution of *Aedes albopictus*

*Aedes albopictus* originally indigenous to South-east Asia, islands of the Western Pacific and Indian Ocean. It has spread during recent decades to Africa, the mid-east, Europe and the Americas (north and south) after extending its range eastwards across Pacific islands during the early 20th century. The majority of introductions are apparently due to transportation of dormant eggs in tyres (Gratz, 2004).

*Aedes albopictus* is primarily a forest species that has become adapted to rural, suburban and urban human environments. It oviposits and develops in tree holes, bamboo stumps and leaf axils in forest habitats and in these plus artificial containers in urban settings. Unlike *Ae. aegypti*, some strains are cold adapted in Northern Asia and America, with eggs that spend the winter in diapause (WHO, 1999).

## 2.3 *Aedes albopictus* as vector of diseases

Over the last 50 years, *Aedes albopictus* has spread to all continents in the old and new world. This anthropophilous species is able to adapt to most climates. Although long considered as a secondary disease vector, it has been shown to be competent for arbovirus transmission under laboratory conditions. In several locations that it has invaded, this mosquito has played a major role in arbovirus transmission, both dengue fever and chikungunya (Pages et al., 2009).

Among public health authorities in the newly infested countries and those threatened with the introduction, there has been much concern that *Ae. albopictus* would lead to serious outbreaks of arbovirus diseases. *Aedes albopictus* is a competent vector for at least 22 arboviruses. Results of many laboratory studies have shown that many arboviruses are readily transmitted by *Ae. albopictus* to laboratory animals and birds, and have frequently been isolated from wild-caught mosquitoes of this species, particularly in the Americas (Gratz, 2004).

In some areas of Asia, *Ae. albopictus* has been occasionally incriminated as the vector of epidemic Dengue Fever/Dengue Haemorrhagic Fever, though it is much less important than *Ae. aegypti*. In the laboratory, both species can transmit the dengue virus vertically from a female through the eggs to her progeny, although *Ae. albopictus* does so more readily (Gratz and Knudsen, 1996). Eventhough both species was also responsible as vector of Chikungunya virus, the recent study by Delatte et al. (2008 a & b) found that *Ae. albopictus* was considered as the main vector of Chikungunya virus.

## 2.4 Control of *Aedes albopictus* as a dengue vector

Among many diseases carried by *Ae. albopictus*, Dengue is the most concerning disease because it affects millions in the tropical and subtropical regions of the world. In the absence of an effective vaccine and the specific drugs treatment, vector control is the only known option to control it. The prevention and control measures against *Ae. aegypti* and *Ae. albopictus* have been described by World Organization Health in the Regional guidelines on dengue/DHF prevention and control (Regional Publication 29/1999) (WHO, 1999).

### 2.4.1 Environmental Management

According to WHO (1995), the most effective means of vector control for *Ae. aegypti* and *Ae. albopictus* is environmental management. The approach involves any change that prevents or minimizes vector breeding and hence reduces human-vector contact.

In 1980, The WHO Expert Committee on Vector Biology and Control defined three types of environmental management:

a) Environmental modification - long lasting physical transformations of vector habitats

b) Environmental manipulation - temporary changes to vector habitat as a result of planned activity to produce conditions unfavorable to vector breeding.

c) Changes to human habitation or behavior-efforts to reduce human-vector-pathogen contact.

Environmental management methods to control *Ae. aegypti* and *Ae. albopictus* and to reduce human-vector contact are source reduction, solid waste management, modification of man-made larval habitats and improvement of house design. Environmental management should focus on destruction, alteration, disposal or recycling of containers and natural habitats that produce the greatest number of adult *Aedes* mosquitoes in each community. The programmes should be conducted concurrently with health education programmes and communications that encourage community participation in the planning, execution and evaluation of container-management programmes (e.g. regular household sanitation or clean-up campaigns) (WHO, 1999).

### 2.4.2 Biological control

Interventions based on the introduction of organisms that prey upon, parasitize, compete with or otherwise reduce the numbers of *Ae. aegypti* or *Ae. albopictus* remain largely experimental and information on their efficacy is based on the results of small-scale field operations.

Larvivorous fish and the biocide *Bacillus thuringiensis* H-14 (BTI) are two organisms most frequently employed (WHO, 1995). Larvivorus fish (*Gambusia affinis* and *Poecilia reticulata*) have been extensively used for the control of *An. stephensi* and/or *Ae. aegypti* in large water bodies or large water containers in many countries in South-East Asia. The applicability and efficiency of the control measure depend on the type of containers.

On the other hand**,** the endotoxin-producing bacteria, *Bacillus thuringiensis* serotype H-14 *(Bt.H-14)* has been found to be most effective against *An. stephensi* and *Ae. aegypti*. There is a whole range of formulated *Bti* products produced by several major companies for control of vector mosquitoes. Such products include wettable powders and various slow-release formulations including briquettes, tablets and pellets. Further developments are expected in slow-release formulations. *Bt.H-14* has an extremely low level mammalian toxicity and has been accepted for the control of mosquitoes in containers storing water for household use (WHO, 1999).

The advantages of biological control measures include no chemical contamination of the environment, specificity against target organisms and the self-dispersion of some agents into sites that could not easily be treated by other means. However, the disadvantages of biological control measures include the expense of raising organisms, difficulty in their application and production and their limited utility in aquatic sites where temperature, pH and organic pollution may exceed the narrow requirements of the agents, as well as the fact that they are only effective against the immature stages of vector mosquitoes. Moreover, a reduction in larval numbers does not necessarily result in a corresponding reduction of disease transmission, since food is limited, lower larval densities may result in larger, healthier adults that are better able to survive (WHO, 1999).

### 2.4.3 Chemical control

Chemicals have been used to control *Aedes* since the turn of the century. *Aedes* larval habitats were treated with oil and houses were dusted with pyrethrins before the insecticidal properties, dichlorodiphenyltrichloroethane (DDT) was discovered. In the 1940s, DDT became the principal method for *Ae. aegypti* eradication programmes in the Americas. When resistance to DDT emerged in the early 1960s, organophosphate insecticides including fenthion, malathion, fenitrothion were used for *Ae. aegypti* adult control and temephos as a larvicide. Current methods for applying insecticides include larvicide application, perifocal treatment and space spraying (Bang and Tonn, 1993).

### 2.4.4 Larvicide

Larvicidal or “focal” control usually limited to containers maintained for domestic use that cannot be eliminated. It is difficult and expensive to apply chemical larvicides on a long-term basis. Therefore chemical larvicides are best used in situations where the diseases and vector surveillance indicate the existence of certain periods of high risk and in localities where outbreaks might occur.

Control personnel distributing the larvicide should always encourage house occupants to control larvae by environmental sanitation. Three larvicide’s can be used to treat containers that hold drinking-water: 1% temephos sand granules, the insect growth regulator methoprene in the form of briquettes and Bti (*Bacillus thuringiensis* H-14), which is considered as a biological control agent. One percent temephos sand granules are applied to containers using a calibrated plastic spoon to administer a dosage of 1 ppm. This dosage has been found to be effective for 8-12 weeks, especially in porous earthern jars, under normal water use patterns (WHO, 1999).

Insect growth regulators (IGRs) interfere with the development of the immature stages of the mosquito by interference of chitin synthesis during the molting process in larvae or disruption of pupal and adult transformation processes. Most IGRs have extremely low mammalian toxicity ($LD_{50}$ value of acute oral toxicity for methoprene (Altosid) is 34600mg/kg). In general, IGRs may provide long-term residual effects (three to six months) at relatively low dosages when used in porous earthern jars. Because IGRs do not cause immediate mortality of the immature mosquitoes, countries with legislation stipulating that the breeding of *Aedes* larvae is an offence, will require some alteration of the law, so as not to penalize home owners who use these compounds (WHO,1999).

Alternatively, *Bt. H-14*, which is commercially available under a number of trade names, is a proven, environmentally – nonintrusive mosquito larvicide. It is entirely safe for humans when the larvicide is used in drinking water in normal dosages. Slow release formulations of *Bt. H-14* are being developed. Briquette formulations that appear to have greater residual activity are commercially available and can be used with confidence in drinking water. The large parabasal body form in this agent contains a toxin that degranulates solely in the alkaline environment of the mosquito midgut. The advantage of *Bt. H-14* is that an application destroys larval mosquitoes but spares any entomophagus predators and other non-target species that may be present. *Bt. H-14* formulations tend to rapidly settle at the bottom of water containers, and frequent applications are therefore required. The toxin is also photolabile and is destroyed by sunlight (WHO, 1999).

## 2.5 Temephos and a new formulation of temephos zeolite granules (Azai)

Temephos is a non-systemic organophosphorus insecticide used to control mosquito larvae (Hii, 1979), midge (Wall and Maranian, 1973) and black fly larvae. It also may be used to control fleas on dogs and cats and to control lice on humans (WHO, 1988). Trade names for products containing the compound include OMS-786, Abate, Abathion, Biothion, Bithion, Difenphos, Ecopro, Nimitox, and Swebate. The compound may also be found in mixed formulations with other insecticides including trichlorfon. Physical properties of temephos are described as below:

| | |
|---|---|
| Common Name: | Temephos |
| Chemical name: | O,O'-(thiodi-4,1-phenylene)bis(O,O-dimethyl phosphorothioate) |
| Chemical class: | organophosphate |
| Solubility in water: | 0.03 mg/l at 25 º C |
| Solubility in solvents: | Soluble in common organic solvents. Insoluble in hexane and methylcyclohexane |
| Melting point: | 30 - 30.5 º C |

Typical of other organophosphate insecticides, temephos inhibits the action of the group of enzymes called cholinesterases. Specific types of these enzymes are found throughout the body including the nervous system, the brain, and the blood stream. As a nerve poison, temephos can be used not only as stomach poison but also as contact poison and fumigant (Hazardous Substance Data Base, 1993).

Temephos is moderately acutely toxic by the oral and dermal route, and has low toxicity through inhalation. Symptoms of acute exposure are also similar to other organophosphates and may include nausea, salivation, headache, loss of muscle coordination, and difficulty breathing (Hazardous Substance Data Base, 1993). In other mammals, temephos produces signs and symptoms typical of cholinesterase inhibition at moderate levels of exposure (500 mg/kg). The $LD_{50}$ of temephos ranges from 2,000 to 13,000 mg/kg for rats and is 4,700 mg/kg for mice (Smith, 1993).

Tests with various wildlife species indicate that the compound is highly toxic to some organisms and moderately toxic to other species. Most of the bird species had an $LD_{50}$ within the 35 mg/kg to 85 mg/kg range indicating a compound that is moderately to highly toxic to avian species. Freshwater aquatic invertebrates are very highly susceptible to temephos as are some marine invertebrates. The compound is nearly non-toxic to the bull frog with an $LD_{50}$ of greater than 2000 mg/kg. This pesticide is also biodegradable, cause minimum environmental pollution and slow pest resistance (Pierce et al., 1989).

There is little information available about the fate and behavior of temephos in the environment. Based on its very low solubility in water, it would probably have a high affinity for soil. Based on this, a half-life of 30 days has been estimated (Wauchope et al., 1992), indicating a low to moderate persistence.

Temephos has low persistence in water. Weekly application of temephos at twice the normal application rates on pond water resulted in the rapid disappearance of the compound from the water and from the sediments (Smith, 1993). At even higher application rates to pond water there were still only traces of the compound detected 1 week after application.

Temephos will be photolyzed in water (U.S. Public Health Service, 1995). Temephos was sprayed over an intertidal mangrove community in Florida. Between 15% and 70% of the sprayed amount reaching the leaf surface entered the water below the trees. Additional amounts were washed into the water during rainfall. Pesticide residues were detected in the water 2 hours but not 4 hours after application, indicating a very short persistence in the water. However, in simulated tide pools the compound persisted for up to 4 days. It also persisted in oysters for 2 days after application (Pierce et al., 1989).

Temephos have been used in controlling *Ae. aegypti* larvae in 18 countries and 34 islands for more than 10 years. In the United States and in the West Indies, temephos was applied in cisterns and other potable water sources in some locations for the control of mosquito larvae. Subsequent tests on the residents that had used these water sources showed no observable effects in the exposed individuals (Pierce et al., 1989).

Since early 1970, WHO has recommended temephos sand granules (Abate) for the control of *Aedes* mosquitoes in Malaysia (WHO, 1985). Treatments of temephos (Abate) are given in the area with high risk of dengue every three months by Health Department in January, April, July and October (Kementerian Kesihatan Malaysia, 2000). Similar to Malaysia, temephos sand granules have been used in Thailand since the early 1970s (Bang and Tonn, 1969 a & b; Bang et al., 1972) in operational community-based control programs at the rate of 5g/50L water in water-storage jars, barrels, water concrete tanks, metal and plastic drums and other artificial containers. The efficacy of temephos EC and temephos SG in water-storage containers and jars was studied in late 1960s (Bang and Tonn, 1969 a & b) and they found that temephos SG (1ppm a.i.) provided complete control of *Ae. aegypti* for about 2 months in one study and 13 weeks in another under simulated water use conditions. Soon after these findings, temephos sand granules were used in operational control programs in Thailand.