

A METHOD AND A SYSTEM FOR PACKAGING AND DELIVERING AN INSECTICIDE OR LARVICIDE TO TARGET AREAS

FIELD OF INVENTION

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The present invention relates to a method and a system for packaging and delivering an insecticide or larvicide to target areas for controlling growth of mosquito larvae and pupae. More particularly the present invention relates to a method and a system for packaging and delivering a graduated amount of an insecticide or larvicide such as temephos, spinosad, diflubenzuron or a biological agent such as *Bacillus thuringiensis israelensis* in a capsule or in a bag to any places that are hard to reach, inaccessible or hazardous to humans or to common mosquito breeding sites which are easily accessible to humans for controlling growth of mosquito larvae and pupae.

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15 BACKGROUND OF INVENTION

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Mosquito-borne diseases or mosquito-borne illnesses are diseases caused by viruses or parasites transmitted by mosquitoes (Family: Culicidae). The most common diseases transmitted by mosquitoes include dengue, malaria, filariasis, chikungunya, yellow fever, Japanese encephalitis and Zika fever. These mosquitoes can transmit disease without themselves being affected and thus these mosquitoes are vectors. In Malaysia, the two most common diseases transmitted by mosquitoes are malaria and dengue.

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The disease malaria is caused by a parasite, *Plasmodium falciparum*, and is transmitted by the female mosquito of the genus *Anopheles*. Worldwide, malaria is a leading cause of premature mortality, particularly in children under the age of five, with an estimated 207 million cases and more than half a million deaths in 2012 (according to the World Malaria Report, 2013 published by the World Health Organization, WHO).

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On the other hand dengue fever and dengue haemorrhagic fever are serious viral diseases borne by the mosquitoes *Aedes albopictus* and *Aedes aegypti* (Lee, 2000). Both dengue fever and dengue haemorrhagic fever are among the major problems to public health in Southeast Asian countries (Jahangir *et al.*, 2003). In Malaysia, the number of dengue

cases reported in 2015 was 120,836 cases and had killed more than 300 people (Imbas Denggi 2015, 2016).

Currently, there are no effective tetravalent vaccines or specific treatment available for the treatment of dengue fever and dengue haemorrhagic fever. Control and elimination of the vectors remain the most important option (Lee, 2000). Among the various pest control approaches, chemical control involving the use of insecticides appears to be the norm for both public health and household insect pest control.

The life cycle of a mosquito consists of the egg phase, larva phase, pupa phase and then adult phase. Larviciding is specially used to break the mosquito's life cycle by killing the larvae and pupae in their aquatic habitat before they reach maturity, so as to prevent the new mosquito generations become disease-carrying insects from developing to spread the vector-borne disease.

The chemical, temephos (*O,O,O',O'*-Tetramethyl *O,O'*-sulfanediybis(1,4-phenylene) diphosphorothioate) is an organophosphorus (OP) compound with very low mammalian toxicity. Since 1970, the World Health Organization (WHO) had recommended temephos for the control of *Aedes* mosquito larvae (WHO, 1985) and it has been used extensively for the past 35 years for the control of *Aedes aegypti* and *Aedes albopictus* larvae in Malaysia (Thevasagayam *et al.*, 1979).

Besides temephos, WHOPES (World Health Organisation Pesticide Evaluation Scheme) had also recommended a few other active ingredients for control of mosquito larvae such as *Bacillus thuringiensis israelensis*, diflubenzuron, novaluron, pyriproxyfen, fenthion, pirimiphos-methyl and spinosad. These active ingredients recommended by WHOPES are used in very small amount and the recommended dose used for container breeding sites is in the range of 0.01 to 5 mg/L of water.

In Malaysia, sand granules temephos formulation packed in 10 g sachet or 20 g sachet is commonly used by health workers and the local council workers in the vector control programme. In a study conducted to evaluate the bio-efficacy of TEMEBATE[®], containing temephos 1% w/w against mosquito larvae using the WHO bioassay

procedure, Nazni and Lee (2011, unpublished report) concluded that TEMEBATE[®] killed 99% of laboratory bred *Aedes aegypti*, *Aedes albopictus*, and *Culex quinquefasciatus* at less than 0.1 mg/L concentration. Temephos is also recommended by WHO for addition to drinking water as larvicide in controlling mosquitoes at 1 mg/L concentration (WHO, 2016). In other words, only 1 g of temephos 1% w/w is required to treat a container containing 10 L of water. Thus the study showed that only a very small amount of temephos is required to kill the mosquito larvae.

Similarly, according to the study conducted by Lee and Zairi (2006), 6000 ITU/L of *Bacillus thuringiensis israelensis* (*Bti*) equivalent to 4 mg of *Bti* in 2 L of water or 80 mg of *Bti* in 40 L of water was effective against both *Aedes aegypti* and *Aedes albopictus* for a minimum of 25 days. *Bti* is also recommended by WHOPES in controlling mosquito larvae at 1 – 5 mg/L. Based on this study, it was concluded that only a very small amount of *Bti* is required to control mosquito larvae.

In Malaysia the most productive *Aedes* breeding habitats at home were identified as (i) domestic/household containers (ii) flower pots/plates/trays (iii) ornamental containers (iv) plants and plant axils, and (v) toilet bowls/cisterns. These five habitats are similar to the findings conducted by the National Environment Agency of Singapore (NEA, 2016). The top five habitats as listed above usually hold a water volume of less than 5 L. To kill the *Aedes* mosquito larvae in these breeding habitats, larvicides such as temephos and *Bacillus thuringiensis israelensis* are widely used by local council and local health authority.

The common packaging for mosquito larvicide such as temephos and *Bacillus thuringiensis israelensis* are currently packed in the bottle or sachet form which is ideal to treat a large area and is not practical enough to treat a small area which contained less than 5 L of water. Furthermore, packing the insecticide or larvicide in bottles or sachets has the following disadvantages:

a) the insecticide or larvicide can only be placed in common mosquito breeding areas which are easy to reach by humans, such as for example, flower pots in the garden, drains, etc.;

b) the amount of the insecticide or larvicide used vary from one end-user to another as the end-user put different amount of the insecticide or larvicide in the water;

c) health and local council workers cannot place the insecticide or larvicide in hard to reach areas in a building, or in places which are hazardous in nature or in terrain that is inaccessible to them; and

d) the possibility of the insecticide or larvicide in the form of a dust or sand granule coming into contact with the end-users or the health and local council workers, thus posing a threat on the safe use of the insecticide or larvicide.

In view of these and other shortcomings, it is advantageous to provide a novel way to pack and to deliver directly to target areas graduated amount of insecticide or larvicide to any places that are hazardous, hard to reach or inaccessible to humans as well as other common mosquito breeding sites which are easily accessible to humans for controlling growth of mosquito larvae and pupae in an effective and simplistic manner.

SUMMARY OF THE INVENTION

The present invention provides for a method and a system for controlling growth of mosquito larvae and pupae in any place with water, any place that may accumulate or hold water or any place suspected as mosquito breeding site.

In a first aspect of the present invention, there is provided a method for controlling a mosquito population said method comprises:

- a) a packaging means for containing the active ingredient;
- b) said active ingredient is an insecticide or larvicide;
- c) inserting a graduated amount of the insecticide or larvicide into the packaging means;
- d) delivering the packaging means containing the insecticide or larvicide directly to target area via manual application; and
- e) releasing the insecticide or larvicide within the target area when the packaging means comes into contact with water in any places with water.

The packaging means of the present invention can be a capsule or a bag which is soluble in water. In the present invention, the capsule can be made of gelatin, hydroxypropyl methylcellulose (HPMC) or biodegradable plastic material. In the present invention, the bag can be made from dimethylformamide, dimethylacetamide, ethylene glycol, propylene glycol, polypropylene, glycerol and water soluble polyglycols. Accordingly, the second aspect of the present invention is that there is provided a method for packing the insecticide or larvicide in a capsule made from gelatin, hydroxypropyl methylcellulose (HPMC) or biodegradable plastic material or in a bag made from dimethylformamide, dimethylacetamide, ethylene glycol, propylene glycol, polypropylene, glycerol and water soluble polyglycols.

The present invention also provide for a method of packaging graduated amount of insecticide or larvicide in a capsule through an “encapsulation” formulation of the insecticide or larvicide. In the present invention, the insecticide or larvicide is encapsulated in a two-part capsule and said capsule is soluble in water. Accordingly, the third aspect of the present invention is that there is provided a method of encapsulating the insecticide or larvicide in a two-part capsule, said capsule is soluble in water when the capsule comes into contact with water in any places with water.

The present invention also provide for a method of packaging the insecticide or larvicide in a bag. The bag of the present invention is water soluble, non-toxic and will degrade into water and carbon dioxide at room temperature upon contact with water in any places with water. Accordingly, the fourth aspect of the present invention is that there is provided a method of packaging insecticide or larvicide in a bag, said bag is soluble in water when it comes into contact with water in any places containing water. In a preferred embodiment of the present invention, the bag is made from non-toxic material and will degrade into water and carbon dioxide at room temperature when it comes into contact with water in any places with water.

The insecticide or larvicide used in the present invention can be an organophosphate, carbamate, pyrethroid, neonicotinoid, avermectin, pridazinone, phenylpyrazole, insect growth regulator, biological agent or a combination thereof. Accordingly, the fifth aspect of the present invention is that there is provided a method for controlling a mosquito population wherein the insecticide or larvicide used is selected from the group

comprising an organophosphate, carbamate, pyrethroid, neonicotinoid, avermectin, pridazinone, phenylpyrazole, insect growth regulator, biological agent or a combination thereof. In a preferred embodiment of the present invention, the insecticide or larvicide used is an organophosphate, preferably a temephos.

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In the present invention, the amount of insecticide or larvicide used in the packaging can be graduated according to a desired quantity. In the present invention, the quantity of insecticide or larvicide inserted in the capsule ranges from 0.01 g to 5 g, preferably 1 g; whereas the quantity of insecticide or larvicide inserted in the bag ranges from 1 g to 500 g, preferably 10 g. Accordingly, the sixth aspect of the present invention is that there is provided a method for controlling a mosquito population wherein the quantity of insecticide or larvicide inserted into the capsule or the bag is graduated according to a desired quantity, preferably 0.01 g to 5 g for the capsule and 1 g to 500 g for the bag.

15 The water soluble capsule or the water soluble bag containing the insecticide or larvicide is delivered to target areas by manual application, preferably by throwing the capsule or the bag in an upward or downward direction using the hand or a mechanical device. In the present invention, the targeted areas can be hazardous, inaccessible or hard to reach areas and comprise the roof gutters, water tanks, high rise apartments or dwellings, tree
20 holes, lakes, drains, gully traps, and ponds. The targeted areas can also be common mosquito breeding sites such as the cistern, flower pots, ornamental containers, containers, etc. Accordingly, the seven aspect of the present invention is that there is provided a method of delivering the water soluble capsule or the water soluble bag containing the insecticide or larvicide to target areas via manual application, preferably
25 by throwing the capsule or the bag in an upward or downward direction using the hand or a mechanical device.

The present invention also provide for a system for controlling growth of mosquito larvae and pupae in any place with water, any place that may accumulate or hold water or any place suspected as mosquito breeding site said system comprises:

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- a). a capsule or a bag containing graduated quantity of an insecticide or larvicide;
- b). directing the capsule or bag to target areas via manual application;

- c). releasing the insecticide or larvicide within the target areas when the capsule or bag comes into contact with water in any places with water; and
- d). killing the larvae or pupae present in the water.

5 The present invention consists of several novel features and a combination of parts which will hereinafter fully described and illustrated in the accompanying description and drawings; it being understood that various changes in the details may be made without departing from the scope of the invention or sacrificing any of the advantages of the present invention.

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BRIEF DESCRIPTION OF THE DRAWINGS

The present invention will be fully understood from the detailed description given herein below and the accompanying drawings which are given by way of illustration only, and
15 thus are not limitative of the present invention, wherein:

Fig. 1 shows ten III-IV instar mosquito larvae that were released into each container containing 5L of water.

Fig. 2 (a) is the physical appearance of water soluble gelatin capsule at post - 1 minute of
20 exposure in the water. The temephos granule was encapsulated in the gelatin capsule.

Fig. 2 (b) is a close up view showing the appearance of water soluble gelatin capsule.

Fig. 3 (a) is the physical appearance of water soluble gelatin capsule at post - 15 minute of exposure in the water. The gelatin capsule was partially disintegrated in the water and the temephos granule was partially released from the capsule.

25 Fig. 3 (b) is a close up view showing the appearance of water soluble gelatin capsule.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

The present invention relates to a method and a system for controlling growth of
30 mosquito population, and more particularly to a method and a system for controlling growth of mosquito larvae and pupae in hazardous, hard to reach, or inaccessible areas as well as common mosquito breeding sites. In particular, the present invention provides for a method and a system for controlling growth of a mosquito population comprising:

- a) a packaging means for containing the active ingredient;
- b) said active ingredient is an insecticide or larvicide;
- c) inserting a graduated amount of the insecticide or larvicide into the packaging means;
- d) delivering the packaging means containing the insecticide or larvicide directly to target area via manual application; and
- e) releasing the insecticide or larvicide contained therein within the target area when the packaging means comes into contact with water in any places with water.

Hereinafter, this specification will describe the present invention according to the preferred exemplary of the present invention. However, it is to be understood that limiting the description to the preferred exemplary of the invention is merely to facilitate discussion of the present invention and it is envisioned that those skilled in the art may devise various modifications and equivalents without departing from the scope of the appended claims.

In the present invention, the packaging means can be a capsule or a bag. The capsule or the bag is soluble in water when the capsule or the bag comes into contact with water found in any places with water. In the present invention, the capsule can be made of gelatin, hydroxypropyl methylcellulose (HPMC) or biodegradable plastic material. The biodegradable plastic material is made from cone, sugarcane, plant starch, polylactic acid (PLA) or polyglycolic acid (PGA). In the present invention, the bag can be made from dimethylformamide, dimethylacetamide, ethylene glycol, propylene glycol, polypropylene, glycerol and water soluble polyglycols.

The present invention also provide for a method of packaging graduated amount of insecticide or larvicide in a capsule through an "encapsulation" formulation of the insecticide or larvicide. The capsule used in the present invention is a two-part capsule and the insecticide or larvicide is encapsulated in the capsule using a mechanical process which is known in the art.

The present invention also provide for a method of packaging graduated amount of the insecticide or larvicide in a bag. The bag of the present invention is water soluble, non-toxic and will degrade into water and carbon dioxide at room temperature upon contact

with water in any places with water. In the present invention, the bag used for containing the insecticide or larvicide is purchased in bulk from commercial bag supplier. The packaging of graduated amount of the insecticide or larvicide into the bag is then carried out at the inventors' premises using a mechanical process which is known in the art.

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The insecticide or larvicide used in the present invention can be an organophosphate, a carbamate, a pyrethroid, neonicotinoid, an avermectin, a pyridazinone, a phenylpyrazole, a thiourea, an insect growth regulator, a biological agent or combinations thereof. The organophosphate used in the present invention comprised dimethoate, methamidophos, chlorpyrifos, temephos, pirimiphos-methyl, and fenthion. The carbamate used in the present invention comprised carbofuran, carbosulfan, propoxur, and bendiocarb. The pyrethroid used in the present invention comprised cypermethrin, alpha-cypermethrin, beta-cypermethrin, delta-methrin, lambda-cyhalothrin, and bifenthrin. The neonicotinoid used in the present invention is comprised imidacloprid. The avermectin used in the present invention is comprised emamectin benzoate and abamectin. The pyridazinone used in the present invention is comprised pyridaben. The phenylpyrazole used in the present invention is comprised fipronil. The thiourea used in the present invention is comprised diafenthiuron. The insect growth regulator used in the present invention is comprised buprofezin, diflubenzuron, novaluron, and pyriproxyfen. The biological agent used in the present invention is selected from the group consisting of *Bacillus sphaericus*, *Bacillus thuringiensis israelensis*, and spinosad. In a preferred embodiment of the present invention, the insecticide or larvicide used is an organophosphate, preferably a temephos; or an insect growth regulator, preferably diflubenzuron or a biological agent, preferably spinosad and *Bacillus thuringiensis israelensis*.

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The amount of insecticide or larvicide contained in the packaging can be graduated according to a desired quantity. In the present invention, the quantity of insecticide or larvicide inserted in the capsule ranges from 0.01 g to 5 g, whereas the quantity of insecticide or larvicide inserted in the bag ranges from 1 g to 500 g. In a preferred embodiment of the present invention, the graduated amount of insecticide or larvicide inserted in the capsule is 1 g whereas the graduated amount of insecticide or larvicide inserted in the bag is 10 g.

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The water soluble capsule or the water soluble bag containing the insecticide or larvicide is delivered to target areas by manual application, preferably by throwing the capsule or the bag using the hand or a mechanical device. When using the invention by hand application, the capsule or the bag is thrown in an upward or downward direction
5 directed to the targeted area.

In the present invention, the targeted areas can be any places with water, places which may accumulate or hold water, or suspected as mosquito breeding sites. The suspected mosquito breeding sites can be a hazardous, inaccessible or hard to reach areas such as
10 for example, roof gutters, water tanks, high rise apartments or dwellings, and tree holes. Other places which accumulate or hold water include but not limited to lakes, drains, ponds, abandon cars, abandon tyres, construction sites, and recycling centres, as well as common mosquito breeding sites such as flower pots, water containers, gully traps and cisterns. In the present invention, the targeted areas can also be ornamental containers
15 and household containers.

An advantage of the present invention is that due to the small size of the capsule, it can be easily delivered to any hazardous, inaccessible or hard to reach areas by manually throwing the capsule in an upward or downward direction directly towards these targets.
20 Where the target areas involved high rise buildings or dwellings, hard to reach areas such as roof gutters and tree holes, the capsule can be delivered directly to these targets by hand throwing or by using a mechanical device, for example a catapult. The small sized capsule which contained graduated amount of the insecticide or larvicide is also suitable for application to common mosquito breeding sites such as the cistern, flower pots,
25 ornamental containers, or household containers. The capsule upon reaching the target area will dissolve in the water and release the insecticide or larvicide directly within the target area, thus killing the mosquito larvae or pupae in the water.

An advantage of using the bag containing graduated amount of insecticide or larvicide is
30 that it is suitable for application to places having a large volume of water such as lakes, ponds or drains. The water soluble bag of the present invention is delivered directly to these targets by hand throwing or by using a mechanical device, such as a catapult. The bag upon reaching the target area will dissolve in the water and release the insecticide or

larvicide directly within the target area, thus killing the mosquito larvae or pupae in the water.

EXAMPLE 1

- 5 The effectiveness of the encapsulated temephos against mosquitoes, in particular *Aedes* mosquito was conducted and the result was presented below and discussed using the accompanying Fig. 1 to 3.

Study Site

- 10 The trial was conducted in the laboratory of the Institute for Medical Research (IMR) at Jalan Pahang, Kuala Lumpur.

Mosquitoes

- 15 Wild type mosquitoes were collected from various hotspot areas in Selangor. The eggs were bred under laboratory conditions until they reached III-IV instar, which were used to assess the larviciding effect of the insecticide. Strain collected from Ridzuan Condominium, Jalan PJS 10/11, Bandar Sunway, 46150 Petaling Jaya, Selangor, Malaysia was used in this study.

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Treatment Schedule

No.	Insecticide	Active Ingredient	Application rate (g / 5 L water)	Concentration of pure temephos ^d
1.	TEMEBATE [®] ^a	Temephos 1% w/w	0.5 g ^b	1 mg/L
2.	TEMEBATE [®] ^a	Temephos 1% w/w	1.0 g ^c	2 mg/L
3.	CONTROL	-	-	-

^a Commercial product supplied by Imaspro Resources Sdn. Bhd. Imaspro Resources Sdn Bhd is the owner of the trademark "TEMEBATE"

^b 0.5 g of TEMEBATE[®] was packed in a water soluble gelatin capsule

25 ^c 1.0 g of TEMEBATE[®] was packed in a water soluble gelatin capsule

^d Concentration of pure temephos was calculated as follow:

0.5 g of temephos 1% w/w in 5 L of water

= $\frac{500 \text{ mg} \times 1}{100}$

30 5 L

= 1 mg/L a.i. of temephos

1.0 g of temephos 1% w/w in 5L of water

$$= \frac{1000 \text{ mg} \times 1/100}{5 \text{ L}}$$

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= 2 mg/L a.i. of temephos

Evaluation of testing

- 10 A batch of 10 III-IV instar mosquito larvae was released into each container or replicate containing 5L of water as shown in Fig. 1. The container size used during the whole trial was 19 cm (W) x 32 cm (L) x 10.5 cm (H). Fish pellet as larval food was added into each container. A container with 10 mosquito larvae and without exposure to any other chemicals acted as untreated control. The larvae in the control were fed in the same
15 manner as those in the treated containers. The trial was conducted at room temperature of $27^{\circ}\text{C} \pm 2^{\circ}\text{C}$.

- A water soluble gelatin capsule containing 0.5 g of TEMEBATE[®] was introduced into the container holding 5 L of water to achieve 1 mg/L a.i. of temephos. The condition of
20 the water soluble gelatin capsule was observed and recorded until the capsule was completely dissolved. The number of moribund larvae at 30 minutes, 1, 2, 3, 4, and 6 hours were recorded. The number of dead larvae was recorded after a 24 and 48 hours holding period. Three replicates were conducted for this study.

- 25 The trial was repeated for a higher dosage at 2 mg/L a.i. of temephos. In order to achieve 2 mg/L a.i. of temephos, a water soluble gelatin capsule containing 1.0 g of TEMEBATE[®] was introduced into the container holding 5L of water. Observation on the condition of capsule and larvae were recorded as mentioned above.

- 30 Dead larvae are those that cannot be induced to move when they are probed with a needle in the siphon or the cervical region. Moribund larvae are those incapable of rising to the surface or not showing the characteristic diving reaction when the water is disturbed.

If mortality was above 20% in the concurrent controls, the whole test was rejected. If mortality in the controls was above 5%, results from the treated sample were corrected using Abbott's formula as below:

$$\frac{\% \text{ test mortality} - \% \text{ control mortality}}{100 - \% \text{ control mortality}} \times 100$$

As there was no dead larvae recorded in the control throughout the study, the mortality was calculated as follow:

$$\% \text{ Mortality} = \frac{\text{Number of dead larvae}}{\text{Number of test larvae}} \times 100$$

Data analysis

Moribund and mortality's data collected were subjected to analysis of variance at 5% level of probability. Differences between treatments mean were evaluated using Duncan's Multiple Range (DMRT) Test. Only data collected from 1 hour, 2 hours, 4 hours, 6 hours, 24 hours and 48 hours was reported.

Results and discussion

The condition of TEMEBATE[®] capsule, containing temephos 1% w/w at post – 1 minute exposure of water and post - 15 minutes exposure in the water is shown in Fig 2 and 3. Fig 2 showed the temephos granule was “encapsulated” in the gelatin capsule, while Fig 3 showed that the water soluble gelatin capsule was disintegrating in the water and TEMEBATE[®] granules were partially released from the capsule after 15 minutes of exposure into the water. Complete disintegration of TEMEBATE[®] capsule was observed within an hour.

The moribund and mortality effects of TEMEBATE[®] capsule on *Aedes aegypti* larvae are summarized in Table 1. In containers treated with 1 mg/L a.i. of temephos, more than 60% larvae mortality rate was observed at post-24 hour treatment. At post-48 hours treatment, complete larvae mortality was observed in container treated with both 1 mg/L and 2 mg/L of temephos, suggesting that TEMEBATE[®] effectively control mosquito larvae at the treated dosage. No significant difference was found between container

treated with 1 mg/L and 2 mg/L of temephos at post-48 hours treatment ($P < 0.05$), suggesting that both temephos at 1 mg/L & 2 mg/L provide equal control to *Aedes aegypti* larvae. In comparison to treated containers, the mortality of larvae in control container (without larvicide) was 0%. Moribund effect was observed after 2 hours of exposure in container treated with 2 mg/L of temephos. Similar effect was observed after 4 hours of exposure in container treated with 1 mg/L of temephos. Statistical analysis showed that there was a significant difference between the 1 mg/L and 2 mg/L of temephos, suggesting that moribund effect was faster at 2 mg/L of temephos compared to 1 mg/L of temephos.

Table 1: Moribund and mortality effects of TEMEBATE[®] capsule on *Aedes aegypti* larvae.

Treatment	% Moribund			% Mortality	
	2 h	4 h	6 h	post-24 h	Post-48 h
TEMEBATE [®] @ 1 mg/L of temephos	0 ^b	26.7 ^b	30.0 ^b	66.7 ^b	100.0 ^a
TEMEBATE [®] @ 2 mg/L of temephos	36.7 ^a	76.7 ^a	90.0 ^a	100.0 ^a	100.0 ^a
Control	0 ^b	0 ^c	0 ^c	0 ^c	0 ^b

Means within a same column followed by same letter are not significantly different ($P < 0.05$; DMRT's test).

Conclusion

From the results shown above, it can be seen that TEMEBATE[®] capsule containing 1% w/w temephos effectively induced high larvicidal activity to control *Aedes aegypti* at the tested dosage of 1 mg/L a.i. and 2 mg/L a.i.. The water soluble gelatin capsule fully disintegrated in the water within an hour in the water and released the temephos granules to impact on mosquito larvae in the water. This study indicated the applicability of water soluble gelatin capsule as a practical and environmental friendly way to apply TEMEBATE[®] into containers holding small amount of water such as ornamental containers and plant pots/plates. The unique packaging of water soluble gelatin capsule is also able to disperse active ingredient into hard-to-reach area or hazardous areas or inaccessible areas such as roof gutter and high-rise apartment/dwellings. Based on the

experiment conducted, TEMEBATE[®] capsule may now be considered to be included in dengue vector control programme.

EXAMPLE 2

- 5 Apart from the efficacy test of temephos on *Aedes* mosquito larvae's control, another efficacy test was conducted with similar materials and method by using *Bacillus thuringiensis israelensis* (*Bti*) as the active ingredient in the capsule. Table 2 showed the results on the evaluation of encapsulated *Bti* against *Aedes* mosquitoes. Complete mortality was observed in the container treated with both 0.04 g and 0.16 g *Bti* at post-72
- 10 hours treatment.

Table 2: Moribund and mortality effects of *Bacillus thuringiensis israelensis* (*Bti*) capsule on *Aedes aegypti* larvae.

Treatment	% Moribund			% Mortality		
	50 min	1 hour	2 hour	24 hour	48 hour	72 hour
0.04 g <i>Bti</i> / capsule @ 8 mg/L	0 %	0 %	40 %	50 %	60 %	100 %
0.16 g <i>Bti</i> / capsule @ 32 mg/L	50 %	60 %	60 %	60 %	60 %	100 %
1g temephos / capsule @ 2 mg/L	0 %	20 %	60 %	100 %	100 %	100 %
Control	0 %	0 %	0 %	0 %	0 %	0 %

- From the results shown in Table 2, it can be seen that *Bti* capsule may now be considered
- 15 to be included in dengue vector control programme.

Remarks

- In Table 2 above, the amount of the concentration of each active ingredient used was calculated as follow:

20

$$\begin{aligned}
 &0.04 \text{ g of Bti in 5 L of water} \\
 &= 1 \text{ L} / 5 \text{ L} \times 0.04\text{g} \times 1000 \\
 &= \mathbf{8 \text{ mg / L water}} \\
 &= 8 \text{ mg / L} \times 3000 \text{ ITU} \\
 &= \mathbf{24,000 \text{ ITU / L water}}
 \end{aligned}$$

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$$\begin{aligned}
 &0.16 \text{ g of Bti in 5 L of water} \\
 &= 1 \text{ L} / 5 \text{ L} \times 0.16\text{g} \times 1000 \\
 &= \mathbf{32 \text{ mg / L water}}
 \end{aligned}$$

$$= 32 \text{ mg / L} \times 3000 \text{ ITU}$$

$$= \mathbf{96,000 \text{ ITU / L water}}$$

1.0 g of temephos 1% w/w in 5L of water

$$5 \quad = \frac{1000 \text{ mg} \times 1/100}{5\text{L}}$$

$$= \mathbf{2 \text{ mg/L a.i. of temephos}}$$

Conclusion

10 Based on Examples 1 and 2 above, it can be concluded that both temephos and *Bti* capsules were effective in controlling the *Aedes* mosquito larvae in very low dosage.

The present invention also provide for a system for controlling growth of mosquito larvae and pupae, said system comprises a capsule or bag containing graduated amount of the insecticide or larvicide, delivering manually the capsule or bag containing the graduated insecticide or larvicide directly to target areas or mosquito breeding sites which are hazardous, hard to reach, or inaccessible to humans or other common mosquito breeding sites, releasing the insecticide or larvicide within the target area and killing the mosquito larvae or pupae in the water.

20 The method and system of the present invention is user-friendly, financially acceptable and does not need specially trained personnel to undertake the work. The method of packaging and delivering graduated amount of the insecticide or larvicide to targeted areas as described in the present invention and the system of the present invention for controlling growth of mosquito larvae and pupae may now be included in dengue vector control programme.

It should be noted that configurations of various components, elements, systems and/or methods used in carrying out the above-mentioned embodiments are illustrative and exemplary only. One of ordinary skill in the art would recognize that those configurations, components, elements systems and/or methods used herein may be altered in a manner so as to obtain different effects or desired operating characteristics. As such, the approached as described above should not be construed as limiting, but as the best mode contemplated by the inventor for carrying out the invention and are construed to be included within the scope of following claims.

References:

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CLAIMS

1. A method for controlling mosquito population said method comprises:
 - a) a packaging means for containing an active ingredient;
 - 5 b) said active ingredient is an insecticide or larvicide;
 - c) inserting a graduated amount of the insecticide or larvicide into the packaging means;
 - d) delivering the packaging means containing the insecticide or larvicide directly to target area via manual application; and
 - 10 e) releasing the insecticide or larvicide within the target area when the packaging means comes into contact with water in any places with water.
2. A method as claimed in Claim 1 characterized in that the packaging means is a capsule or a bag.
- 15 3. A method as claimed in Claim 1 characterized in that the insecticide or larvicide is an organophosphate, a carbamate, a pyrethroid, a neonicotinoid, an avermectin, a pridazinone, a phenylpyrazole, a thiourea, an insect growth regulator, a biological agent or combinations thereof.
- 20 4. A method as claimed in Claim 3 characterized in that the organophosphate is comprised dimethoate, methamidophos, chlorpyrifos, temephos, pirimephos-methyl, or fenthion.
- 25 5. A method as claimed in Claim 3 characterized in that the carbamate is comprised carbofuran, carbosulfan, propoxur or bendiocarb.
6. A method as claimed in Claim 3 characterized in that the pyrethroid is comprised cypermethrin, alpha-cypermethrin, beta-cypermethrin, delta-methrin, lambda-cyhalothrin, or bifenthrin.
- 30 7. A method as claimed in Claim 3 characterized in that the neonicotinoid is comprised imidacloprid.

8. A method as claimed in Claim 3 characterized in that the avermectin is comprised emamectin benzoate, or abamectin.
- 5 9. A method as claimed in Claim 3 characterized in that the pyridazinone is comprised pyridaben.
10. A method as claimed in Claim 3 characterized in that the phenylpyrazole is comprised fipronil.
- 10 11. A method as claimed in Claim 3 characterized in that the thiourea is comprised diafenthiuron.
12. A method as claimed in Claim 3 characterized in that the insect growth regulator is comprised buprofezin, diflubenzuron, novaluron or pyriproxyfen.
- 15 13. A method as claimed in Claim 3 characterized in that the biological agent is selected from the group consisting of *Bacillus sphaericus*, *Bacillus thuringiensis israelensis*, or spinosad.
- 20 14. A method as claimed in Claim 1 or Claim 2 characterized in that the graduated amount of insecticide or larvicide is encapsulated in the capsule.
15. A method as claimed in Claim 14 characterized in that the capsule is a two-part capsule.
- 25 16. A method as claimed in Claim 1, Claim 14, or Claim 15 characterized in that the graduated amount of insecticide or larvicide encapsulated in the capsule is between 0.01 g to 5 g, and preferably 1 g.
- 30 17. A method as claimed in Claim 1 or Claim 2 characterized in that the graduated amount of insecticide or larvicide inserted in bag is between 1 g to 500 g, preferably 10 g.

18. A method as claimed in Claim 1 or Claim 2 characterized in that the capsule or bag is soluble in water when the capsule or bag comes into contact with water in any places with water.

5 19. A method as claimed in Claim 1 or Claim 18 characterized in that the capsule or the bag containing the insecticide or larvicide is delivered directly to target area via manual application.

10 20. A method as claimed in Claim 19 characterized in that the manual application is by hand or by a mechanical device.

15 21. A method as claimed in Claim 19 or Claim 20 characterized in that where the application of the invention to targeted area is by hand, the capsule or the bag is thrown in an upward or downward direction directed to the targeted area.

22. A method as claimed in Claim 1 or Claim 21 characterized in that the targeted area is a hazardous or an inaccessible or a hard to reach area by humans.

20 23. A method as claimed in Claim 1 or Claim 19 characterized in that the target area is any places with water, any places that may accumulate or hold water or any places suspected as mosquito breeding sites.

25 24. A method as claimed in Claim 1 or Claim 22 characterized in that any places containing water, any places that may accumulate or hold water or any places suspected as mosquito breeding sites comprise the roof gutters, water tanks, high rise apartments or dwellings, abandon cars, abandon tyres, construction sites, recycling centres, drains, tree holes, lakes, ponds, flower pots, ornamental containers, household containers, gully traps, and cisterns.

30 25. A method as claimed in Claim 1 or Claim 15 characterized in that the capsule is made of gelatin, hydroxypropyl methylcellulose (HPMC) or biodegradable plastic material.

26. A method as claimed in Claim 1 or Claim 17 characterized in that the bag is water soluble, non-toxic, and degrade into water and carbon dioxide at room temperature upon contact with water in any places with water or expose to moisture.

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27. A method as claimed in Claim 1 or Claim 26 characterized in that the bag is made from dimethylformamide, dimethylacetamide, ethylene glycol, propylene glycol, glycerol, water soluble polyglycols or polypropylene (PP).

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28. A system for delivering an insecticide or a larvicide to target areas for controlling growth of mosquito larvae and pupae, said system comprises a capsule or bag containing graduated amount of the insecticide or larvicide, delivering manually the capsule or the bag containing the insecticide or larvicide directly to target area which is hazardous, hard to reach, or inaccessible to humans, or other common mosquito breeding sites, releasing the insecticide or larvicide within the targeted area, and killing the mosquito larvae and pupae in the water.

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29. A system for delivering an insecticide or a larvicide to target areas for controlling growth of mosquito larvae and pupae as claimed in Claim 28, characterized in that the capsule or bag used is soluble in water when the capsule or bag comes into contact with water in any places with water.

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30. A system for delivering an insecticide or a larvicide to target areas for controlling growth of mosquito larvae and pupae as claimed in Claim 28 or Claim 29 using any method as claimed in any one of Claims 2 to 27.

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