Brief review on materials used as carrier agents for larvicide formulations

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Abstract. Mosquito-borne diseases are great concerns of people in the worldwide, especially dengue. There are 390 million people infected with dengue in a year. According to Dengue Situation Update Report for Western Pacific Region, there is an increment in the number of the infected areas as compared to the same period of previous year. World of Health Organization (WHO) and Ministry of Health (MOH) from every country pay the highest attention towards this issue. Other than inventing new formulation of adulticides, application of larvicide is newly emerging as an effective method to control mosquitoes from spreading virus. In this brief review, several recent studies about ways of the virus transmitted through mosquito biting behaviour, larvicide formulation and types of carrier agents being used to carry larvicide will be discussed. In order to sustain the aquatic ecosystem, materials for carrier agents should be environment-friendly. This review paper will give insight into the recent development in carrier system for mosquitoes larvicide formulations.

1. Introduction
Weather in Southeast Asia region which located in the Equator is hot and humid throughout the whole year. This makes the countries in this region desired habitats and breeding sites of female mosquitoes and it leads to a proliferation of mosquito-borne diseases in these countries [1]. Dengue and malaria transmitted by Aedes aegypti bring high mortality rate around the world. There are approximately 390 million infection cases around the world in a year. Mosquito-borne diseases take away lives more than a war, terrorism, gun violence or other human activities [2]. Table 1 shows the statistics of dengue cases in Southeast Asia countries from 1 January to 20 June 2019.

The proliferation of mosquito-borne diseases after war rises alarm of society towards the reduction of diseases transmittance in the shortest time. Therefore, insecticides and mosquito repellent start to emerge in markets at the late few decades of the 20th century. The spread of the diseases was well controlled after the precaution mentioned were taken. However, rapid urbanisation, dense population growth and modernisation of transportation system prior to economic growth after World War II have driven the diseases to another high new level of uncontrollable infection [3].

Mosquito control is the main way to prevent the transmission of mosquito-borne diseases. After years of application of pyrethrin-based products on adult mosquitoes, there are findings stated that killing larvae is a more effective method to reduce the number of mosquitoes because larvae are living in water and they are not as mobile as adults [4, 5]. The breeding sites are easier to be banished by removal of stagnant water. Mosquito control is carried out mostly by using insecticides on their breeding sites such as poor drainage system, tyre or rubbish in urban areas. However, this leads to environmental pollution and harms other non-targeted organisms [6]. In addition, extensive usage of
synthetic insecticide could lead to insecticide resistance and undesirable effects on non-target organism and human as well as the environment.

There is a variety of formulations in larvicides available on the commercial market. Essential oil (EO) from plants emerge as an important role in larvicide formulation in these few decades. Despite there are many active ingredients which also known as secondary metabolites in EO, insects react slower towards insecticides that synthesised from plants and those secondary metabolites are biodegradable. EO is much cheaper than those synthetic larvicides. EO has low water solubility, thus emulsification by oil in water (o/w) has to be done in terms of stabilising the structure [7].

In this brief review, the transmittance of diseases virus via mosquito biting behaviour, larval source management and properties of nanoparticles material for carrying larvicides has been compiled.

Table 1. Statistics of dengue cases and number of death cases in Southeast Asia countries [8].

| Country   | Number of dengue cases | Number of death cases |
|-----------|------------------------|-----------------------|
| Malaysia  | 56,819                 | 88                    |
| Cambodia  | 2,490                  | 5                     |
| China     | 142                    | (N/A)                 |
| Lao PDR   | 4,216                  | 14                    |
| Philippines | 83,570               | 354                   |
| Singapore | 4,720                  | (N/A)                 |
| Vietnam   | 70,491                 | 4                     |

a Not available.

2. Virus transmitted through mosquito biting behaviour

Male mosquitoes only feed on flower nectar due to the absence of proboscis. But female mosquitoes feed on the blood of animals including human to obtain protein for oviposition purpose. They take blood from bloodstream after it pierces skin using its proboscis and this is shown in figure 1. A female mosquito could live up to a month. They are active during dawn and dusk and they feed every two or three days. Blends of volatile compounds and respective ratio and spatiotemporal of the hosts lead female mosquitoes to them to bite. They will do close-range navigation by using odorant receptor to detect odour of hosts surrounding them. After that, they will select the host that fulfill most of requirements. After selection, landing on the skin will be done. These actions might be because of odour alone or olfactory receptors of mosquitoes [9].

Besides that, the concentration of carbon dioxide during exhalation of the host could attract mosquitoes to bite because carbon dioxide could rise in plumes. Mosquitoes can sense the plumes from hundreds of metres away. Body temperature and skin emanation are preferences of mosquitoes in biting [10]. For example, Aedes species like to bite on the face as carbon dioxide emit from nose and mouth. However, it is not by carbon dioxide alone to attract mosquitoes for biting but it is a synergist in biting matter [11]. The mechanism in sweat glands of hosts will give stimuli towards antennae of mosquitoes in detecting hear emanating from warm-blooded bodies. After capillaries closest to the surface are found, proboscis will be pierce through skin and saliva will be released to numb the area and prevents the blood from clotting. Virus of the diseases will be transmitted during piercing of proboscis into bloodstream of host if the mosquito carries the virus. The host does not realise about the bite only if the itchiness caused by the minor allergic reaction is detected [12].

There is a study shows that those who have larger body size and males have higher tendency to get bitten because they have larger skin emanation area and higher carbon dioxide concentration in their exhaled air. When there are presence of additional odorants such as lactic acid, ammonia and carboxylic acid mixtures, this would increase attraction from mosquitoes [13].

Biting behaviour of mosquitoes is also affected by external factors like humidity, temperature, visual stimuli or movement. Mosquitoes could detect their hosts within range of 100 m due to the movements and colours. If the host is moving, the waves of light would be changing and this concept
is also applicable to those who wear dark-coloured clothes [10]. Some species of mosquitoes such as *Anopheles* or *Culex* which are night biters would use different light orientation to locate the host [14].

By doing these prevention steps, the frequency of getting mosquitoes bites will be less and the percentage of infected with mosquito-borne diseases will be lower.

**Figure 1.** A mosquito is taking blood from bloodstream after it pierces skin using its proboscis. Saliva carries viruses into blood streams of host once it starts to take blood [15].

### 3. Larval Source Management (LSM)

Mosquito surveillance has been a highlighted issue in order to detect abnormal changes of the mosquito population from time to time for the early warning of mosquitoes-borne diseases. Breeding rate of mosquitoes is highly influenced by meteorological factors like precipitation, temperature variation and relative humidity based on mosquitoes’ preference. These could lead to a change in life cycles of mosquitoes and susceptibility of some pathogen [16]. High occurrence of mosquito-borne diseases will be observed when the population density of mosquitoes in the area is dense. Thus, larval source management (LSM) is also one of the surveillance method chosen to monitor the change of mosquito population density [5]. *Aedes aegypti* and *Aedes albopictus* are the main vectors of dengue because they prefer to lay eggs in household and artificial containers which are available vastly in urbanised area [17]. As mentioned above, mosquito larvae live underneath the water. Mobility of larvae is only in water and this makes attacking the breeding sites of larvae much easier than habitats of adults. Adults could fly away from any encountered intervention measures like application of insecticide in the compound.

In year 2004, World Health Organization (WHO) introduced a programme named Integrated Vector Management (IVM) for controlling vector-borne diseases. More mosquitoes could be killed by targeting the larval stage before they are dispersed into the human environment. The type and scale of LSM application are defined by the ecology of the area and available financial support [18]. LSM requires high human source in surveillance and this could create job opportunities to locals [4]. Besides that, there are many hidden places to be mosquito breeding sites.

In Malaysia, the commonly used larvicides controls are application of temephos (Abate), house inspection and enforcement of Destruction of Disease-Bearing Insect Act 1975. Abate is low toxicity to organisms even human. Larval surveys are successful and this can be seen when Singapore government implemented this together with public education and enforced the law. The results show that the house index reduced from 16% to 2% [19]. In short, larval control should not be implemented alone and should implement together with others commonly used approaches such as bed nets or indoor-spraying [4].

#### 3.1 Synthetic larvicides

There are four categories of insecticides used for indoor vector control which are organochlorides, pytheroids, organophosphates and carbamates. Organophosphates is one of commonly used larvicide formulation in worldwide. Temephos and fenthion are examples of the organophosphates. Other than that, insect growth regulators are also used to control larvae. However, abuse of drugs and extensive
usage of the synthetic larvicides brings disruption of ecosystem in that area. Commonly used larvicide types have their own benefits of application and limitations and this is shown in table 2.

### Table 2. Type of larvicide, benefits of application and limitation [20].

| Type of larvicide | Benefits of application | Limitation |
|-------------------|-------------------------|------------|
| Temephos          | • Long-term efficacy    | • Highly toxic to non-targeted organisms |
|                   | • Can be applied in occasional water flow | • Incompatible with alkali |
| Bacillus thuringienesis israelensis (B.t.i) | • Highly toxic towards larvae of mosquito and black fly | • Only effective for early stages of larval |
|                   | • Not harmful to non-target organisms | • Re-treatment at regular intervals is required |
| Bacillus sphaericus (B.s) | • More effective towards Culex larvae | • Not effective towards Aedini larvae |
| Larvicidal oils   | • Convenient            | • Not effective if the oil film did not cover water surface fully |
|                   | • Applicable to water with limited surface area | • Not suitable in occasional water flow |
|                   |                         | • Reapplication is required after heavy rain |
|                   |                         | • Harm aquatic flora and fauna |
| Insect growth regulators (IGR) | • Little or no toxic towards non-targeted organisms | • Could not estimate the appropriate dosage |
|                   |                         | • Difficulty in monitoring until emergence of adults |
| Monomolecular films | • Very effective towards pupae | • Might fail kill larvae if not fully cover water surface |
|                   | • Environmental friendly |           |

#### 3.2 Essential oil based larvicides

In ancient, people could use plants in their daily life for generating smoke, decorating their habitats and applying plant extract on their bodies. In recent years, application of EO has been widely discovered in larvicide formulation. EO can be extracted from entire plant or a specific part of the plant which has abundant active ingredients needed. EO contain variety of active ingredients. Secondary metabolites of respective plants act as synergists in larvicides formulation. According to Bhatia [21], secondary metabolites of plants are extremely useful phytochemicals and they possess high chemical or structural complexity and this makes synthesis not available by using recent technology. Ingredients in EO will interfere feeding behaviour of insects by acting as an insect growth regulator or toxify their neuro system. This interference may causes protein denaturation, enzymatic inhibition or membrane disintegration and this is not adapted easily by insects. Thus, those EO that have been used as mosquito repellent also have potential to be used as larvicides (table 3) [17].
However, most EO are not soluble in water. Hence, nanoparticles acts as carrier agents to carry essential oil into water. Other than that, another limitation of EO as larvicides is application has to be focused on the most productive breeding site in an area.

**Table 3.** Essential oil that extracted from plants with respective active ingredients been used as larvicide [18].

| Essential oil   | Plant                        | Active ingredient (s)               |
|-----------------|------------------------------|------------------------------------|
| Basil           | *Ocimum spp*                 | Linalool                           |
| Catnip          | *Nepeia cataria*(L.)         | Nepetalactone                      |
| Cinnamon        | *Cinnamomum zeylanicum*      | Cinnamaldehdye                     |
| Citronella      | *Cymobogon sp*               | Citronellal                         |
| Citronella      | *Cymopogon winterianus*      | Citronellal, geraniol, citronellol |
| Clove           | *Syzygium aromaticum*        | Eugenol                             |
| Eucalyptus      | *Eucalyptus globules*        | 1.8 cineole                         |
| Garlic          | *Alium sativum*              | Ajoene                              |
| Lemon eucalyptus| *Corymbia citriodora*        | Citronellal, p-methane-3,4-diols,   |
|                 |                              | citronellol, limonene              |
| Lemon eucalyptus| *Eucalyptus citriodora*      | p-methane-3,8-diols (PMD)          |
| Lemongrass      | *Cymbopogon citratus*        | Citral                              |
| Neem            | *Azadirachta indica*         | Azadirachitin                       |
| Orange          | *Citrus sinensis*            | d-Limonene                          |
| Peppermint      | *Mentha piperita*            | Menthol                             |
| Wild tomato plant | *Lycopersicon hisatum*     | 2-undecanone                        |

4. **Nanoparticles as carrier agents for drugs**

Nanotechnology is a new branch of technology since early of the 21st century. Most of technology can be invented in nanosize to achieve similar or higher impacts towards the target. Nanotechnology is most useful in nanomedicine, nanocarrier and so forth [21]. Advantages of nanoparticles are chosen as carrier agents are high stability, small size, high surface area and high tuneable surface [22]. In addition, Hussien et al. [23] reported that nanomaterial as drug delivery is the most potential drug delivering agents due to their high loading capacity, efficient release and targeted delivery.

Drug loading in nanoparticles can be done by entrapment, encapsulation, nanoprecipitation or others. Application of nanoparticles for larvicide delivery becomes important because less amount of larvicide needed to have similar effects with the usual amount of larvicides use. Besides that, some larvicide are not compatible in water, hence water-soluble nanoparticles could carry oil-soluble larvicide into water which is the site of larvae grow.

Nanoparticles are formulated based on release mechanism. Nanoparticles will encapsulate EO and EO will be released by trigger of surroundings such as light, heat or pH. In recent years, natural polymer-based carrier agents such as chitosan, sodium alginate or gelatin become highlights in drug loading and release because of their low cost and toxicity towards non-targeted organisms and environment. Properties of nanoparticles as carrier agents will be discussed below and advantages of the carrier agents are tabulated in table 4.

4.1 **Polymer microcapsules**

Polymer microcapsules can be made from several techniques such as multiple emulsion-solvent evaporation, nanoprecipitation, dialysis or supercritical fluid technology. Encapsulation, entrapment, dissolution or adsorption of active ingredients will be done in polymer microcapsule. The most commonly used biodegradable polymer are chitosan, gelatin, polylactic acid and so forth. Besides that, polyethylene glycol and poloxamers are also used as copolymers to provide steric hindrance in order to give higher half-life of active compounds. The surface of polymer microcapsules can be manipulated by certain ligands to deliver the active ingredients to target sites more effectively or improve their circulation half-life [24].
4.2 Solid lipid micro and nanoparticles
Triglycerides, steroids, fatty acids and waxes are commonly used materials to make lipid of micro and nanoparticles and they will be in the solid state. Dispersions of solid lipid micro and nanoparticles can be stabilised by several types of emulsifier and the combinations. Examples of techniques that have been used to develop solid lipid micro and nanoparticles are high shear homogenisation, ultrasonication, solvent emulsification or evaporation, spray drying, microemulsion-based method and so forth. The extent of solubility of the drug in lipid melt, preparation method, structure and polymeric state of lipid matrix lead to loading efficiency of solid lipid micro and nanoparticles. Poor capacity to load hydrophilic molecules due to partitioning effects is usually a limit of solid lipid micro and nanoparticles but this could be solved by preparation of liquid-drug conjugate bulk via salt formation or covalent linking that later processed with an aqueous phase containing a surfactant such as Tween [24].

4.3 Nanoemulsion
Nanoemulsion is a system that consists of two immiscible liquids and disperses in three ways: oil in water (o/w), water in oil (w/o) or bi-continuous. The dispersions are stabilised by an amphiphilic surfactant or co-surfactant. They are thermodynamically stable after addition of a droplet of nanoemulsion which has a size range of between 20 to 200 nm. Size and shape of particles dispersed in the continuous phase differ emulsion from nanoemulsion. The carriers are solid spheres which it is amorphous and lipophilic that carries with a negative charge. It provides better encapsulation effect for those oil-soluble active ingredients [25].

4.4 Liposomes
Liposomes is also known as phospho-lipid vesicles. They are self-assembled phospholipid bilayers formed in aqueous media. They have a hydrophilic core and hydrophobic outer layer, hence they could encapsulate both hydrophilic and lipophilic active constituents in a single platform. Performance of the loaded active ingredients will be enhanced by increasing protection, solubility and control release while undesirable interaction with other molecules is avoided. However, they do have drawbacks: poor loading efficiency of hydrophilic materials and in-vivo stability [26].

4.5 Polymeric micelles
Micelles are formed from amphiphilic polymers which have hydrophobic and hydrophilic properties in their molecular structures and unique molecules arrangement form when they are in contact with an aqueous solution. The hydrophobic portion can be encapsulated in an interior structure and a hydrophilic portion remains facing outwards the aqueous medium. Hydrophobic drugs can be encapsulated into micelles core by hydrophobic interaction or covalent bonding [24].

4.6 Nanostructured hydrogel
A nanostructured hydrogel is a three-dimensional network of hydrophilic cross-linked polymer that does not dissolve but can swell in water. They can react to the fluctuations of the environmental stimuli because they have good compatibility with bio-or other hydrophilic molecules. They are highly absorbent and can hold up to 600 times their weight in water. It will shrink when it is dried. It is highly stimuli towards the environment. Nanostructured hydrogels are swollen polymeric networks that have drugs inside it. Swelling properties are influenced by type and composition of monomers, environmental factors like temperature, pH or ionic strength and cross-linking between molecules [27].
**Table 4.** Types of nanoparticles, its advantages and example [22, 24].

| Type of nanoparticle            | Advantages                                                                 | Example                                      |
|--------------------------------|---------------------------------------------------------------------------|----------------------------------------------|
| Polymer microcapsule           | • Protect active ingredients from environment that might cause degradation. | Sodium alginate with calcium chloride        |
|                                | • Increases security of active ingredients.                                |                                              |
| Solid lipid micro and nanoparticles | • Slow release of active ingredients.                                | Lipid-core nanocapsules (LNC)                |
|                                | • Simple production method.                                                |                                              |
| Nanoemulsion/ microemulsion    | • Provide higher stability compared with classical emulsion.               | Oil in water emulsion (o/w), water in oil    |
|                                | • Thermodynamically stable                                                | emulsion (w/o)                               |
| Liposomes                      | • Biocompatible                                                            | Phospholipids                                |
|                                | • Prolonged release                                                        |                                              |
|                                | • Reduction of skin permeation and toxicity                                |                                              |
| Polymeric micelles             | • Provide long-lasting reliability                                         | N,N-diethyl-2-phenylacetamie (DEPA)          |
| Nanostructured hydrogel        | • Increase duration of reaction                                           | Poly(ethylene glycol)                        |
|                                | • Safer formulation                                                       |                                              |

5. Conclusion
Materials for carrier agents should be environmental-friendly for not harming the ecosystem in an aquatic environment. Besides that, the dosage of larvicides should be controlled so that larvicide resistance is not developed in mosquito larvae in coming few decades. Type of nanocarriers used to carry larvicide should be taken into consideration when new water-insoluble larvicide is developed. This brief review is beneficial to scientists who are working on formulation of larvicide for controlling mosquito larvae.

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