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4. Nanobiotechnology's Efficient Role for Control of Mosquito-Borne Diseases Via Aqua Nano Emulsions

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4.1 Introduction:

Mosquitoes are well known vectors for transmission of many pathogen-borne diseases like dengue, chikungunya, Malaria, Filariasis etc. These diseases have resulted into global morbidity and mortality. Considering the human health, it is essential to control mosquito population so that people can be protected from mosquito borne diseases (WHO 2015). The larval forms of mosquitoes are controlled by a number of aquatic predators, including odonate young instars, tadpoles, fishes, copepods and water bugs (Kalimuthu *et al* 2014). Larval stage is most susceptible stage to attack mosquitoes because these are immotile and concentrated in smaller areas. So, one of the effective methods for mosquito control is to interrupt their life cycle at larval stage (Srivastava *et al* 2008). Generally, the chemical control is carried out by the indoor residual spraying of insecticides such as hexachlorocyclo hexane, benzene hexa chloride, melathion and synthetic pyrethroid (Soni and Prakash 2013). However, synthetic chemicals have strong negative effects on human health and environment and induce resistance in number of mosquito species (Wattanachai and Tintanon 1999). Varying amount of resistance to commonly used insecticides like temephos, fenthion, melathion and dichlorodiphenyltrichloroethane (DDT) has been reported (Tikar *et al* 2008). Moreover, chemical insecticides are associated with many concerns like harmful effect on non-target species (Sarwar *et al* 2009), long persistence in environment and entry in food chain (Linde 1994). In view of these facts eco-friendly insect control agents which are safe and effective are urgently needed.

Recently, extensive research has been carried out to investigate the efficacy of botanical products against mosquito vectors (Pavela 2015). Essential oils are particular plant products made up of volatile substances found in variety of species (Weinzieri 2000). The main characteristics of essential oils are that they are easily extractable, ecofriendly and biodegradable. They possess low toxicity against mammals and are effective against wide spectra of insect pests (Elango *et al* 2009). Literature revealed that essential oils with better lethal activity against mosquitoes come from only five botanical families: Lamiaceae, Cupressaceae, Rutaceae, Apiaceae and Myrtaceae. In particular, the oil extract of eucalyptus leaves possesses a wide spectrum of biological activity including anti-microbial, fungicidal, insecticidal/insect repellent, larvicidal, herbicidal, acaricidal and nematicidal. The leaf essential oil from *Eucalyptus* spp. had shown excellent inhibitory effects against both *Ae. aegypti* and *Ae. albopictus* larvae (Cheng *et al* 2009). Thus, essential oils from effective constituents of leaves of eucalyptus may be explored as a potential environmental-benign larvicide. The main drawback of using essential oils as larvicidal agent in the natural habitat of larvae which are water bodies is immiscibility of water and oil. Oil forms a separate layer over the surface of water body which leads to various problems like light penetration, breathing problems and disturbing the other non-target species.

This issue can be overcome by downsizing the oils using nanotechnology via making Nano emulsions and they could act as larvicidal agent (Anjali *et al* 2012). Nanotechnology has developed immensely in the last decade and was able to create many innovative materials with wide range of applications of nanoparticles in medical, pharmaceutical, industrial, biotechnological and scientific fields. In recent years, it is emphasized on the application of nanotechnology in insect pest management (Prabhakar *et al* 2017).

At present, application of nanotechnology has been extended in field of mosquito control by synthesis of nanoparticles from environmentally acceptable plant extracts (Priya and Santhi 2014). Nanotechnology includes nanoparticles having one or more dimensions in the order of 100 nm or less (Auffan *et al* 2009). "Nano" a Greek word means "dwarf or small". In scientific and mathematical terms its basic metrical unit is the nanometer which is equal to one billionth part of a meter (1nm $=10^{-9}$ m). Nanotechnology is one of the most vigorous promising research areas in revolutionary science. It is an ancient science which was applied in the preparation of Ayurvedic Bhasmas and Homeopathic medicines and are still used even today (Rai and Ingle 2012).

Conventional synthesis of nanoparticles can involve expensive chemical and physical processes that often use toxic materials with potential hazards such as environmental toxicity, cytotoxicity and carcinogenicity (Biazar *et al* 2011). The toxicity problems arise from hazardous substances, such as organic solvents, reducing agents and stabilizers that are used to prevent unwanted agglomeration of the colloids. In addition, some have also been found to be toxic due to factors such as composition, size, shape and surface chemistry. Thus, there is currently widespread interest in developing clean, reliable, biologically compatible, benign, and environment friendly green processes to synthesize nanoparticles (Kulkarni and Muddapur 2014). Now a days Au, Ag, Cu, Si etc. nanoparticles are being used alone or mixed with fungal (Soni and Prakash 2012) and plant extracts to control the population of insects like *Drosophila* (Vecchio *et al* 2012), *Anopheles* (Priyadarshini *et al* 2012), *Aedes* (Patil *et al* 2012, Sundaravadivelan *et al* 2013) and *Culex* species (Subarani *et al* 2013). Biological systems such as bacteria, plants, fungi are recognized as suitable candidates for nanoparticle synthesis because of their features like rapid growth rate, increased protein expression and bioaccumulation of the metal nanoparticles by bioreduction (Khan *et al* 2017).

Biomolecules present in plant extracts (leaves, flower, stem, roots) reduce metal ions to nanoparticles in a single step green synthesis process. Nanoparticles have a great potential as 'magic bullets' loaded with herbicides, fungicides, larvicides and targeting specific tissues in desired plants or insects to release their charge to desired part of target animal to achieve desired results (Duhan *et al* 2017).

The Nano formulations have ability to act as larvicide (Ghosh *et al* 2013), adulticide (Veerakumar and Govindarajan 2014) and also repellent agent (Nuchuchua *et al* 2009). It is a new pathway which can be effectively utilized for the efficient killing of mosquitoes without harming the environment and non-target species.

Attention to insecticides of natural origin, particularly plant derived products, has been recently revived. Some phytochemicals act as general toxicants to all life stages of mosquitoes, whereas others interfere with growth and reproduction or act on the olfactory receptors, eliciting responses of attractancy or repellency (Sukumar *et al* 1991). Workers reported that these oils are competitive inhibitors of acetyl cholinesterase and another possible target for essential oil activity is the octopaminergic system of insects (Kostyukovsky*et al* 2002). Preiestley *et al* (2003) showed interference with GABA- gated chloride channels. Eucalyptus is one among most significant plant belonging to family Myrtaceae possessing more than 700 species (Menut *et al* 1995) and is most widely planted genera. Eucalyptus leaves are well known for their aromatic oils with steam volatile components of molecular weight less than 250 amu (Brophy and Southwell 2002). These oils can be recovered by steam distillation and thus they are referred to as essential oils (Denny 2002). The facility with which essential oils are obtained from aromatic plants and their diverse chemical compositions makes them potential sources of natural pesticides (Isman 2000). Among essential oils, eucalyptus oil, in particular, is more useful as it is easily extractable commercially (industrial value) and possesses a wide range of desirable properties worth exploiting for pest management (Barton 2000). The main constituent in leaf extracts is 1, 8-cineole, γ-Terpinene, α- Pinene and Globulol. Among these various components of eucalyptus oil, 1, 8-cineole is the most important one and, infact, a characteristic compound of genus Eucalyptus, and is largely responsible for a variety of its pesticidal as well as larvicidal properties (Sandhu and Vashishat 2022).

Many studies demonstrated that essential oil from *E. globulus* is toxic to *Ae. aegypti* larvae and showed LC_{50} of 32.4 ppm (Lucia *et al* 2007). Seyoum *et al* (2003) reported that burning of leaves of *E. citriodora* provides a cost- effective method of household protection against mosquitoes in Africa. Ethanolic extracts derived from three species of the Piperaceae family, *Piper longum, P. rbesoides* and *P. sarmentosum* were evaluated for efficacy against early 4^{th} instar larvae of *Ae. ageyti* mosquitoes using larvicidal bioassays. The highest larvicidal efficacy was established from *P. longum* with LC_{50} value of 2.23 ppm (Chaithong *et al* 2006). Senthil *et al* (2005) considered pure limonoids of neem seed, testing for biological, larvicidal, pupicidal, adulticidal and antiovipositional activity against *Anopheles stephensi*. Same test was done by using *Melia azedarach* leaf and seed extracts (Senthil 2006).

Biological synthesis of nanoparticles is safe, inexpensive and environment-friendly alternative. Microorganisms and plants have long demonstrated the ability to absorb and accumulate inorganic metallic ions from their surrounding environment. These attractive properties make many biological entities efficient biological factories capable of significantly reducing environmental pollution and reclaiming metals from industrial waste (Gowramma *et al* 2015). Plant extracts containing bioactive alkaloids, phenolic acids, polyphenols, proteins, sugars and terpenoids are believed to have an important role in first reducing metallic ions and then stabilizing them (Castro *et al* 2011).

Major nanoparticles synthesized by plant extracts are gold, silver, copper, copper oxide, palladium, platinium, titanium Dioxide, zinc oxide, indium oxide, iron oxide, lead and selenium nanoparticles (Shah *et al* 2015).

Silver nanoparticles synthesis was achieved using Kiwifruit juice, *Rumex hymenosepalus* extract, *Annona squamosa* leaf extract, *Podophyllum hexandrum* leaf extract, extracts of *Acalyphaindica Linn*., *Hibisicus cannabinus* leaf extract, *Macrotylomauniflorum* seed extract (Vidhu *et al* 2011). The larvicidal activity of silver nanoparticles synthesized by *N. nucifera* leaf extract has been evaluated against malaria and filariasis vectors (Santosh kumar *et al* 2011).

Silver nanoparticles synthesized using *P. daemia* plant latex against *Ae.aegypti*, *An.stephensi* and non-target fish *P. reticulate* has been evaluated (Patil *et al* 2012).Methanol extracts of *Allium schoenoprasum, Barbarea vulgaris, Brassica nigra, Conium maculatum, Geranium sps, Hyptis sauveoleus, Lithosperm umarvense Ocimum basilicum, Origanum majorana, Raphanussativus, Rumexcrispus and Thlaspiarvense* have shown significant reduction in adult emergence of *Ae.aegypti* (Supavarn *et al* 1974). Mwangi and Rembold (1988) evaluated the growth inhibiting and larvicidal effects of *Me. Volkensii* extracts on *Ae.aegypti* larvae. Nuchuchua *et al* (2009) studied *in vitro* characterization and mosquito (*Ae.aegypti)* repellent activity of essential-oils-loaded nanoemulsions. Paula *et al* (2009) investigated the cashew gum nanoparticles loaded with natural larvicide from *Moringa oleifera* seeds and found its efficacy against *Ae. aegypti*. Ovicidal activity of neem (*Azadirachta indica* A. Juss) seed kernels extracted with organic solvents and distilled water on *Ae. Aegypti* eggs were analysed by Umar *et al* (2007).

Bevilacqua *et al* (2011) studied the shelf-life prolongation of fruit juices through essential oils and homogenization. Pant *et al* (2012) observed that encapsulated formulations of neem and karanja oil have shelf life more than 6 months for the bioefficacy against mosquito larvae. Debnath *et al* (2011) tested the entomotoxicity of silica nanoparticles against rice weevil *Sitophilus oryzae* and compared the efficacy with bulk-sized silica. Naik *et al* (2014) tested leaf mediated Ag NPs with *Pongamia pinnata* for mosquito control and found that plant extracts showed moderate larvicidal effects. Mondal *et al* (2014) investigated the bioactive components present in root extract of *Parthenium hysterophorus* plant used for biosynthesis of Ag NPs and analysed the larvicidal effects of extract as well as Ag NPs on *Culex quinquefasciatus*.

Utilization of nanoinsecticides would be very effective measure to control insect borne diseases and reducing harmful impact of synthetic pesticides (Kumar *et al* 2013). Nanobiotechnology of natural products presents a potential larvicidal nanoemulsions prepared with essential oils. Nanoemulsion production involved a non-heating procedure, describing easy technique which may be useful for integrative control programs.

However, the use of indigenous plant-based products by individual and communities can provide a prophylactic measure for protection against various mosquito-borne diseases. Due to high larvicidal efficacy, environment safety, no toxic effects and requirement in very low amount make the water and oil nanoemulsions one among the most significant and eco-friendly control method to replace the toxic, costly and resistance causing synthetic drugs.

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