Environ Sci Pollut Res (2018) 25:10151–10163
DOI 10.1007/s11356-017-9640-y

Biological nanopesticides: a greener approach towards the mosquito vector control

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Received: 23 March 2017 / Accepted: 26 June 2017 / Published online: 18 July 2017

Abstract Mosquitoes, being a vector for some potentially dreadful diseases, pose a considerable threat to people all around the world. The control over the growth and propagation of mosquitoes comprises conventional pesticides, insect growth regulators and other microbial control agents. However, the usage of these common chemicals and conventional pesticides eventually has a negative impact on human health as well as the environment, which therefore becomes a major concern. The lacuna allows nanotechnology to come into action and exploit nanopesticides. Nanopesticides are majorly divided into two categories—synthetic and biological. Several nanoformulations serve as a promising nanopesticide viz. nanoparticles, e.g. biologically synthesised nanoparticles through plant extracts, nanoemulsions prepared using the essential oils like neem oil and citronella oil and nanoemulsion of conventional pesticides like pyrethroids. These green approaches of synthesising nanopesticides make use of non-toxic and biologically derived compounds and hence are eco-friendly with a better target specificity. Even though there are numerous evidences to show the effectiveness of these nanopesticides, very few efforts have been made to study the possible non-target effects on other organisms prevalent in the aquatic ecosystem. This study focuses on the role of these nanopesticides towards the vector control and its eco-safe property against the other non-target species.

Keywords Eco-safety · Mosquitoes · Nanopesticides · Non-target species · Vector-borne diseases

Responsible editor: Philippe Garrigues

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Introduction

Mosquitoes (Diptera: Culicidae) are a major threat to humankind worldwide, as they play a role of vectors which carry various devastating pathogens and parasites. The young instars of these mosquitos are usually targeted with the organophosphates and other insect growth regulators and controlling agents. The different strategies such as indoor residual spraying (IRS) and usage of insecticide-impregnated bed nets are also applied for vector control strategies. However, the applications of these chemical treatments have strong negative influence on the environment and human health. Mosquitoes (Diptera: Culicidae) symbolise a key danger for the global population since they act as vectors for dreadful disease-causing pathogens which include dengue filariasis and malaria (Mehlhorn et al. 2012; Murugan et al. 2015d; Benelli 2015a).

Malaria is the dreadful disease which is caused by a parasite named *Plasmodium*. This parasite is vectored to human population and other animals through bites of infected female *Anopheles* mosquitoes (Breman 2001; Jensen and Mehlhorn 2009). As per latest estimates, there are approximately 198 million cases of malaria leading to 584,000 deaths worldwide. Most of the human mortality due to malaria occurs among the children living in the African continent, where a child dies due to this disease every minute (WHO 2014a). Dengue is another viral disease spread by the mosquito. The two primary vector which is responsible for the proliferation of this disease is *Aedes aegypti* and *Aedes albopictus*. Among both of the vectors, the *Aedes aegypti* has the higher rate of transmitting the disease than the *Aedes albopictus*. As per the recent statistics, the transmission of this disease has immensely increased in urban and semi-urban tropical areas throughout the world, emerging as a significant threat in public health sector. Over 2.5 billion people are at a risk of getting infected by this dreadful disease. As per World Health Organization estimation, there may be 50–100 million human deaths occurring due to the dengue infection each year worldwide. Currently, there is no particular medication for this dreadful disease, even the development of the vaccines to control this disease is in progress (Murrell et al. 2011). The prevention and the control of this disease entirely depend on the effective strategies and measures towards the supervision of these dreadful vectors (Suresh et al. 2015; WHO 2015).

Lymphatic filariasis, also termed as elephantiasis, is one of the examples of a neglected tropical disease. It is approximated that this disease is spread over the 74 countries infecting about 1.4 million people worldwide. An estimate of 25 million men gets infected with this genital disease, and over 15 million people get infected with lymphoedema (WHO 2014b). The primary causative agent behind the spread of this dreadful disease is Filarioidea nematodes such as *Wuchereria bancrofti*, which leads to 90% of cases. The parasites such as *Brugia malayi* and *Brugia timori* are spread to humans via different mosquitoes. The vectors for spreading this disease are *Culex* species, such as *Culex quinquefasciatus*, which are believed to be the most common vectors across the semi-urban and urban areas of Asia (Chadee et al. 2002). These mosquitoes not only spread a disease to humans, but mosquitoes also transmit primary pathogen and parasites that dogs and horses are very susceptible to, including dog heartworm, West Nile virus, and Eastern equine encephalitis (WHO 2012). In the current situation, a mosquito vector control strategy is critical and necessary (Benelli 2015a).

The larvae and pupae of Culicidae are targeted through the application of organophosphates, microbial controlling agents and different insect growth-regulating compounds. The indoor residual spraying and insecticide-treated bed nets are few of the strategies which are implicated in controlling the transmission of malaria and arboviruses in tropical countries. However, conventional chemical pesticides tend to exhibit higher toxic impact on the environment, simultaneously affecting human health (Robert and Olson 1989). The second most effective demerit synthetic chemicals are the occurrence of resistance mechanism in the mosquito species (Wattanachai and Tintanon 1999; Liu et al. 2005). Several strategies are being employed to control the population of these dreadful mosquito vectors which can indirectly help in the reduction of the occurrence of different lethal vector-borne diseases. Among the several strategies being employed towards the control of these dreadful vectors, the procedures can be listed as an integrated pest management.

This approach tends to endeavour its application as to prevent the infestation and occurrence of infestation of a pest when necessary. The intelligent selection and use of pest control actions which can ensure the favourable socio-economic and ecological consequences are the prime focus of this system. Through the IPM, several strategies opted towards the supervision of these dreadful vectors responsible for creating the havoc in the society through spreading the devastating diseases. The several strategies being employed for the control of these insect vectors can be categorised into three different categories like chemical methods, biological methods and others. The chemical methods include the application of conventional pesticides and other chemically derived products for controlling the vector population. The biological methods include the use of different biological agents (Bowatte et al. 2013) towards the control of the vector population. In others, the application such as sterile insect technique (SIT) can be implemented (Oliva et al. 2014).

Furthermore, enormous efforts are being carried out towards the improvisation of the insect controlling strategies through the application of botanically driven products (Benelli 2015b and Pavela 2015). Several plants and other herbal extracts, essential oils and other pure compounds have been tested for their applicative potential as an ovicidal, larvicidal, pupicidal and adulticidal agents against the dreadful mosquito vectors (Amer and Mehlhorn 2006a, b; Govindarajan 2010; Benelli et al. 2015a, b, c).

In other perspective, the application of nanotechnological approach towards the control of these dreadful vectors has been an important strategy towards the vector control. The advancement in the nanotechnology provides several methods to counter the problem of increasing the vector-borne diseases. In this context, the application of nanotechnology to formulate nanopesticides with the greener approach is an effective one with lower residual pollution and toxicity to the non-target species and environment.

Conventional pesticides—a primary cause of eco-toxicity

A pesticide may refer to a particular chemically synthesised substance which can be either a fungicide, herbicide, insecticide, nematicide or another chemical constituent used towards the control of unwanted and destructive living organism. The application of these conventional pesticides started since the 1900s, where the humankind is using several forms of pesticides which lead to the accumulation of these hazardous chemicals in the environment. These conventional pesticides can be classified into different forms based upon their chemical moieties like organochlorines, organophosphates, carbamates and pyrethroids. The heavy usage of these conventional pesticides to a broad and unregulated form has created the risk of eco-toxicity in the world. The improvement in the pest control using these chemical compounds cannot be denied, but its irregular application has also effect on the environment and human health.

One of the major demerits in the application of these pesticidal components in the environment is their higher residual pollution (Agnihotri A 1999). An insecticide may have been formulated to have its temporary effect. However, the studies have shown that their residues are found in the air, waterways and ground. The accumulation of these residual pollutants in the environment causes eco-toxicity problem (Bhatnagar 2001).

The residual parts of these pesticides pile up in the atmosphere which affects the quality of air that people breathe. This type of pollution leads to the illness of the humankind through the occurrence of the various forms of air-borne diseases. The same condition prevails in the hydrosphere. Also, the substantial residual part getting washed away from the agricultural lands gets accumulated in the water bodies creating the risk of eco-toxicity. This subsequently becomes harmful for other non-target living system prevailing in the water ecosystem. This residual pollution created due to heavy usage of these harmful pesticides has affected the soil ecosystem. This residual contamination of this pesticidal component into the ground leads to the detrimental impact on the on-target organism prevailing in the ecosystem (Topp et al. 1997; Simon-Sylvestre G, Fournier 1980). Similarly, the nutritional value of crops has been declined due to the accumulation of these chemicals in the soil.

The increasing eco-toxicity due to the usage of these conventional pesticides is an alarm for the humankind to find a different approach for pest management with better efficacy towards the target simultaneously with better eco-safety value. In this context, the approach of the nanotechnology to improvise the condition of the pesticidal application in the day-to-day scenario can become an important step towards the reduction of the eco-toxicity problem.

Pesticidal resistance in mosquito: a major concern and challenge

As recommended by the World Health Organization, several control strategies towards the mosquitoes include IRS. Several other strategies also exist such as the application of long-lasting insecticide-treated bed nets (LLINs) and the obliteration of larvae-breeding sites (WHO 2008). The primary challenge countered during the vector control strategy is the development of resistance towards the insecticides in the mosquitoes (WHO 2007). In recent times, the extensive usage of these pesticides in the agricultural fields (Diabate et al. 2002) also on the bed net treatment (Vulule et al. 1994, Czeher et al. 2008) has resulted in the occurrence of resistance in the mosquito strains. The major resistance in present scenario is seen towards the pyrethroids.

Resistance towards the pyrethroid pesticide is a particular threat to mosquito control since it is the only pesticidal class which is currently applied and is only recommended insecticide for treating bed nets, mainly due to their low toxicity for humans compared to other conventional pesticides (Zaim et al. 2000). The resistance in the mosquitoes towards the insecticide has been determined via two ways: the first approach is through in vivo biological test and the second through the identification of alleles responsible for the resistance mechanism. One of the primary mutations being observed is the kdr mutation which occurs due to the overuse of DDT and pyrethroid. Kdr mutation genotype has been documented to be related to DDT and pyrethroid resistance (Donnelly et al. 2009). *Anopheles gambiae* and *Anopheles funestus* are few of the major malarial vectors for malaria in the African continent (Fontenille et al. 1997); both have earlier been found to have potential resistance to pyrethroid insecticides. The resistance towards the insecticide has been shown to be locally highly variable even inside a country or a region (Vezenegho et al. 2009; Antonio-Nkondjio et al. 2011; Hunt et al. 2011; Balkew et al. 2010). This problem of resistance is a major challenge towards the integrated pest management strategies. The application of green-mediated nanopesticides can serve as a valuable tool to get rid of this resistance threat as well as can become an effective tool towards the control of these dreadful mosquito vectors, creating havoc in the society.

Green synthesis of nanoinsecticide: an incipient tool against mosquito vectors

The advancement in nanotechnology has a potential to revolutionise a wide range of application including drug delivery to pest management approaches (Aurel et al. 2007; Kim et al. 2007; Rai et al. 2009; Amerasan et al. 2015). The synthetic approach of these nanopesticides through plant-mediated manner is beneficial over the other forms of pest management approaches such as chemical and physical methods. A rising number of plant-mediated compounds have been projected for an efficient and quick extracellular formulation of these plant-mediated nanopesticides (e.g. Shankar et al. 2004; Priyadarshini et al. 2012; Ponarulselvam et al. 2012). The efficient mosquitocidal property exhibited by these nanoinsecticides in the field conditions can make these compounds a safer and efficient method to control the mosquito population (Santhoshkumar et al. 2011; Marimuthu et al. 2011; Panneerselvam et al. 2012, 2013; Dinesh et al. 2015; Suresh et al. 2015; Murugan et al. 2015c, b; Benelli 2015c). These plant-derived nanoinsecticides can be subdivided into different forms such as plant-derived nanoparticles and nanoemulsion formulated using the essential oils derived from plant parts.

Plant-mediated nanoparticle with mosquitocidal property

In the recent decade, the increasing numbers of the plant-derived extracts and their metabolites have been applied in the nanoparticle biosynthesis. The rising number of publication which aims at the green synthesis of the nanoparticles is astonishing. A total of 5658 research publications were retrieved from the SCOPUS database which aims at the biological synthesis of the nanoparticles which depicts the application of the biological nanoparticles in vector management. Among the countries worldwide, India and China are the most productive with a majority of the publication in the area of nanoparticle synthesis using greener approach (Rajan et al. 2015).

The nanoparticle synthesis through biosynthetic approach often exploits the stabilising and the reducing potential of the plant-driven extracts and metabolites (Govindarajan et al. 2016b, c, d, e). The two most important factors which influence the shape, size and the stability of these biologically driven nanoparticles are the concentration of the plant extract or plant metabolite, and the other is the concentration of the substrate (metal ion) being implicated towards the synthesis (Rajan et al. 2015). These green synthesised nanoparticles can also be explored for mean particle sizes through the use of nanoparticle analysers (Rajan et al. 2015; Murugan et al. 2015b).

Several varieties of nanoparticles exhibiting mosquitocidal properties have been synthesised using plant extracts (Fig. 1). It has been seen that the colour intensity of the plant extract when incubated with the metal on aqueous solution changes from the yellowish or pale brown colour to reddish dark brown which confirms the synthesis of the nanosized metal nanoparticles. The current research studies report the effectiveness of these plant-synthesised nanoparticles as an ovicidal, larvicidal, pupicidal, adulticidal and ovi-deterrents against the dreadful mosquitoes of medical importance. One of the searches conducted on the SCOPUS (May 2017) using 'nanoparticles mosquito' as keywords leads to the retrieval of 236 research papers. The most productive countries in this research area are India followed by the USA. Among the 236 publications, major publications are being published in *Parasitology Research* (Springer), followed by *Environmental Science and Pollution Research* (Springer), *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy* (Elsevier), *Acta Tropica* (Elsevier) and *Applied Materials and Interfaces* (ACS).

Several types of research shed light on the mosquitocidal properties of the metallic nanoparticles (Benelli 2016a, b, c). A research study was carried out to examine the effectiveness of *Sargassum muticum*-synthesised AgNPs towards ovicidal and egg hatchability. The reduction of egg hatchability by 100% was observed after a single exposure to 30 mg/L against the *Anopheles stephensi*, *Aedes aegypti* and *Cx. quinquefasciatus*. Regarding the larvicidal and the pupicidal activity of the nanoparticles, it has been observed that a smaller concentration, i.e. parts per million, of different plant-synthesised metal nanoparticles results in acute toxicity against various mosquito vectors. The published literature from Santhoshkumar et al. (2011) describes that AgNP synthesis using the leaf extract of *Nelumbo nucifera* was lethal to fourth instar larvae of *Anopheles subpictus* (LC_{50} = 0.69 mg/L) and *Cx. quinquefasciatus* (LC_{50} = 1.10 mg/L). Rajakumar and Rahuman (2011) describe the toxicity of AgNP synthesised using leaf extract of *Eclipta prostrata* against the fourth instar of *Cx. quinquefasciatus* (LC_{50} = 4.56 mg/L) and *An. subpictus* (LC_{50} = 5.14 mg/L).

AgNPs synthesised using the aqueous leaf extract from *Citrullus colocynthis* were lethal to the third instar larvae of *Culex pipiens* with an LC_{50} value of 0.5 mg/mL (Shawky et al. 2014). *Morinda tinctoria* leaf extract using acetone was utilised for AgNP fabrication and exhibited its lethal indices

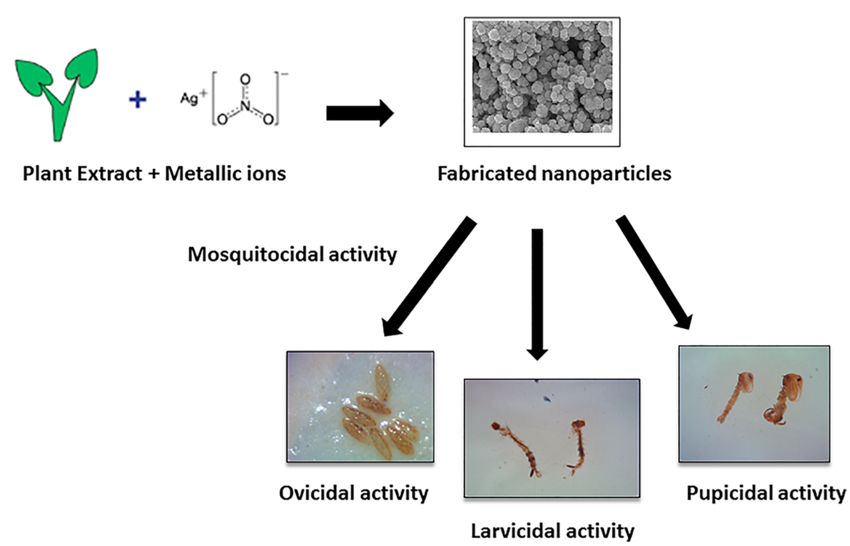

Fig. 1 Schematic representation of the mechanism behind the plant-mediated synthesised nanometric mettalic particles and its effect on the target mosquito (Benelli et al. 2017b)

of LC_{50} of 1.442 mg/L against the third instar larvae of *Cx. quinquefasciatus* (Kumar et al. 2014). Silver nanoparticles fabricated using the aqueous leaf extract of *L. aspera* leaf extract exhibited lethality towards the fourth instar larvae of *Aedes aegypti*, with LC_{50} of 8.5 mg/L (Suganya et al. 2014). *Cymbopogon citratus*-fabricated AuNPs were lethal against *An. stephensi* and *Aedes aegypti.* The LC_{50} of 18.80 mg/L was reported for first instar, 21.32 mg/L for second instar, 25.92 mg/L for third instar and 31.46 mg/L for fourth instar, while pupicidal was reported at 38.32 mg/L. The LC_{50} against the *Aedes aegypti* were 20.27 mg/L for first instar, 23.24 mg/L for second instar, 8.63 mg/L for third instar and 35.09 mg/L for fourth instar, and pupicidal activity was reported at 41.52 mg/L (Murugan et al. 2015b). The aqueous leaf extract obtained from *Azadirachta indica* was examined against the third instar larvae of *Aedes aegypti* and *Cx. quinquefasciatus*. The lethal index LC_{50} were 0.006 and 0.047 mg/L, respectively, for *Aedes aegypti* and *Cx. quinquefasciatus* (Poopathi et al. 2015). Recently, silver nanoparticulates (NPs) fabricated using the seed extract of *M. oleifera* were found to be effective towards the early instars of *Aedes aegypti* as the LC_{50} was found to be 10.24 mg/L for first instar, 11.81 mg/L for second instar, 13.84 mg/L for third instar, 16.73 mg/L for fourth instar and 21.17 mg/L for the pupae. In addition to this, it was observed that these nanoparticles tend to show the growth inhibitory activity of dengue virus, serotype DEN-2 (Sujitha et al. 2015).

Inclusive of all, it can be observed as a general trend that the majority of biologically fabricated metallic nanoparticles have been formulated via exploiting the reducing potential of the plant, algae and seaweed extracts (Murugan et al. 2015a, c; Kalimuthu et al. 2017). The majority of metal NPs were screened against mosquito larval and pupal populations and found to be effective at lower concentrations (Benelli 2016a, d).

Further research was carried out to screen the effectiveness of the metal NPs fabricated using plant extracts against the dreadful mosquito vectors like *Aedes aegypti*, *Aedes albopictus* and *An. stephensi* (Govindarajan et al. 2016f, g, h, i; Murugan et al. 2016c). The third instar larvae were successfully eliminated from the storage water reservoirs in a duration of 72 h after single treatment with *Phyllanthus niruri*, *Mimusops elengi* and *Aloe vera*. It was hypothesised that the mechanism behind the potent mosquitocidal activity may be due its nanometric size which helps the NPs to penetrate into the insect exoskeleton easily. Alongside in the intracellular space, the nanoparticle may bind the sulphur from proteins or phosphorous from the nucleic acid, i.e. DNA, which results in the denaturation of the enzymes and improper functioning of the organelles. Subsequently, the depletion in the membrane permeability and disturbance in the proton motive force leads to the irregular cellular function finally leading to apoptosis or cell death (Rai et al. 2009; Subramaniam et al. 2015).

Invertebrate-mediated nanoparticles for mosquito control

Beside the botanicals being used for the nanoparticle synthesis, currently, the usage of invertebrates can be seen. These invertebrates can be exploited for making one pot fabrication of effective mosquitocidal agents. These mosquitocidal agents can serve as a potent larvicidal and pupicidal agent. A recent study by Jaganathan et al. (2016) describes the earthworm (*Eudrilus eugeniae*) mediated nanoparticle synthesis (AgNPs) which were studied for their larvicidal potency against the malarial vector *An. stephensi*. The lethal indices for the particular AgNP against target were found to be 4.8 mg/L for first instar, 5.5 mg/L for second instar, 6.9 mg/L for third instar and 8.5 mg/L for fourth instar. The pupicidal activity was achieved at a concentration of 15.5 mg/L. In addition to the plant-mediated fabrication of nanoparticles, the TiO_2 nanoparticles synthesised using the hydrothermal method exhibited its potency against the *Aedes aegypti* young instars, with lethal indices of LC_{50} ranging from 4.02 mg/L for first instar larvae to 7.52 mg/L for pupae (Murugan et al. 2016a).

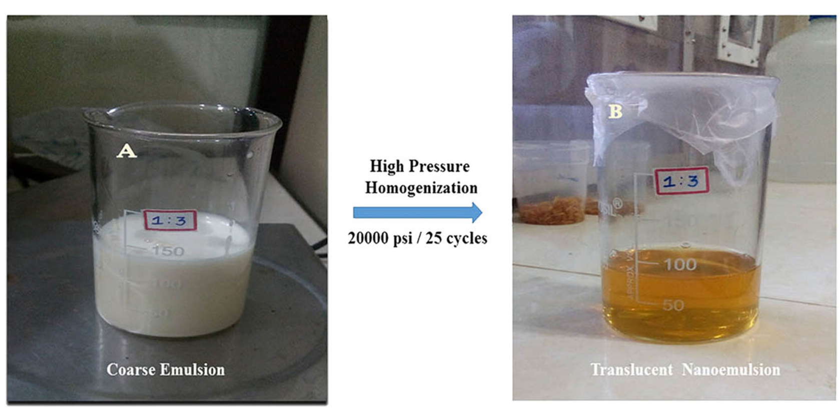

Fig. 2 Formulation of nanoemulsion through high-enegy emulsification process

Essential oil nanoemulsion: a promising tool for mosquito control

The emulsion system which comprises of droplet size in the nanometric scale (20–200 nm) is often termed as mini-emulsions (El-Aasser and Sudol 2004) and nanoemulsions (Nakajima 1997; Sonneville-Aubrun et al. 2004). As the nanoemulsion attains its appropriate size, they appear as transparent or translucent from the naked eye (Fig. 2) and therefore attains the stability against the sedimentation or creaming (Tadros et al. 2004). For these properties, this system is well studied and practically applied in various fields such as chemical, pharmaceutical and cosmetics. Currently, few of the studies described the possible application of these nanoemulsions in the field of insect vector control strategies. The nanoemulsions comprise of an organic and aqueous phase. These systems being the non-equilibrium system cannot be formed spontaneously (Rang and Miller 1999), so they require an external energy for its formation (Binks 1998). The energy is provided to the system either by the chemical potential or through the mechanical devices. The nanoemulsion formulation is so-called as a dispersion or high-energy emulsification method. This is achieved using the high shear stirring, ultrasound generators or through the high-pressure homogenisers. The mechanism behind the formulation of this emulsion system is the available energy input in the shortest time and the uniform flow which leads to a formation of smallest-sized droplets (Walstra 1996).

Ultrasonic emulsification is one of the examples of the high-energy emulsification methods which use the ultrasonic waves to shear the droplets in nonmetric diameter size (Walstra 1996) but has a limitation that it can apply only for small batches. One of the studies carried in the preparation of the polymerised nanoemulsion which upon the formation had the efficiency of the dispersion. This process was strongly dependent on the rate of ultrasonication for different time intervals at various amplitude, which depicted the relation of the organic phase and its time duration, as more hydrophobic the monomer will require a longer time for its emulsification (Landfester et al. 2004). Another form of the high-energy emulsification is the high-pressure homogenisation which works in the energy range of 50–350 MPa (Floury et al. 2003). High kinetic stability and optical transparency of the nanoemulsion compared to the conventional emulsions help them to attain the high degree of stability and aid up the advantage for their possible technological application (Shinoda and Saito 1968). The different kinds of literature and publications on the nanoemulsion shed light on their potential applications. Among all possible application, the application of this nanometric emulsion is a conventional tool to control the vector spreading the terrible diseases. Nanometric emulsion, for example, neem oil nanoemulsion and eucalyptus oil nanoemulsion are few of the examples applied with the larvicidal properties against a larval population.

The presence of the biochemical in these oils provides them with the potency as a larvicidal agent. The presence of various biochemical components like eugenol in basil oil, azadirachtin in neem oil and eucalyptol in eucalyptus oil enhances the larvicidal potency of the nanometric emulsions against *Aedes aegypti* and *Cx. quinquefasciatus* (Veerakumar et al. 2014). The formulation of this nanoemulsion is also one of the types of nanoformulation for the vector control strategy. Various nanotechnologies have been implemented for formulating different nanopesticides, like the formation of the nanoparticle through polymerisation (Pavel 2004), precipitation in an aqueous droplet (Destrée & Nagy 2006), solid lipid nanoparticles (Gasco et al. 2009) and direct solvent evaporation (Margulis-Goshen & Magdassi 2012). Figure 3 describes the possible potential merits of these nanopesticides upon the conventional bulk pesticides, proving the nanopesticide as a potent insecticidal agent with lower residual pollution.

Neem oil nanoemulsion is one of the promising tools for the control of vector population. The anti-ecdysone activity exhibited by the neem oil makes it propitious for this application. The formulation of the neem oil nanoemulsion was carried out using the methodology opted by Anjali et al. (2012). The nanoemulsion was prepared using the neem oil, non-ionic surfactant Tween 20 and Milli-Q water. The coarse emulsion obtained after the combination of oil and aqueous phase was subjected to ultrasonication for the duration of 20 min to achieve the final nanosized emulsion system. The formulated nanoemulsion exhibited the mean hydrodynamic size of 30.12 ± 1.3 nm with the polydispersity index of 0.262. The nanoemulsion system exhibited its noteworthy larvicidal activity against the dreadful filarial vector *Cx. quinquefasciatus*.

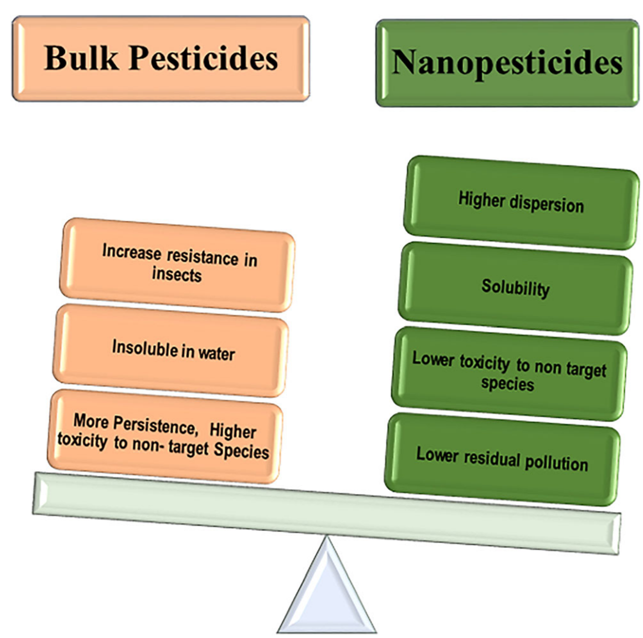

Fig. 3 Pictorial representation of comparative study between the conventional pesticides and nanopesticides

The LC_{50} value for the formed nanoemulsion was found to be 11.75 mg/L for the 24th hour. The nanoformulated emulsion of neem oil with the smallest droplet diameter was efficient than its bulk counterpart, i.e. neem oil. This property of nanosized emulsion system can become an effective alternative for the vector and vector-borne disease control. Similarly, another essential oil that proves its mosquito control activity against the *Cx. quinquefasciatus* is eucalyptus oil nanoemulsion.

According to Sugumar et al. (2014), nanoemulsion prepared using the eucalyptus oil shows an efficient larvicidal property against the larvae of *Cx. quinquefasciatus*. The eucalyptus oil nanoemulsion was formulated using 1:3 ratio of eucalyptus oil/Tween 80 which resulted in the translucent nanoemulsion system. The mean hydrodynamic size of the nanoemulsion was estimated to be 9.4 nm. Later through the estimation of its larvicidal potency, it was found out that this nanosized emulsion also exhibits the efficient larvicidal property. The larvicidal activity exhibited by nanoemulsion was observed at the concentration of 250 mg/L which resulted in 98% mortality within 4 h of treatment.

The larvicidal activity exhibited by the nanoemulsion against the vector causing the dreadful diseases can be attributed to the essential oil components. These components such as azadirachtin, eucalyptol, etc. consist of chemical constituents like terpene hydrocarbons such as monoterpenes and sesquiterpenes and oxygenated compounds such as phenols, alcohols, aldehydes, ketones, esters, lactones, ethers and oxides. This distinct variety of kinetically stabilised formulation comprises the two different phases, which are immiscible. According to Solans et al. (2005), this nanoemulsion attains the translucent property with smaller droplet diameter of 20–200 nm, therefore attaining bluish aspect (Forgiarini et al. 2000). These nanosized emulsions of essential oils tend to attain the insect control property (Wang et al. 2007). Figure 4 describes the mechanism behind the efficient property of the essential oil nanoemulsion as a larvicidal agent. The chemical components present in the nanoemulsion, for example, azadirachtin present in neem oil, attain the growth inhibitory property. The azadirachtin tends to have action on the ecdysone hormone, which is a key hormone for the growth of insects. The nanometric size of the nanoemulsion enhances its specificity and target delivery resulting in more effectiveness than bulk pesticides. The unregulated growth hormone tends to stop the mounting of the insects, consequently leading to their mortality.

Nanopermethrin is the water dispersible powder form of permethrin. This nanoformulation is achieved using the solvent evaporation of the o/w microemulsion consisting of pesticide in an organic phase. The composition of the obtained o/w microemulsion was permethrin as an active ingredient, *n*-butyl acetate and sec-butyl alcohol as an organic solvent, ammonium glycyrrhizinate and soybean lecithin as a surfactant and water. The obtained microemulsion is further lyophilized at −95 °C and <1 mbar for 24 h which resulted in water dispersible nanopowder along with surfactants. The nanoformulated permethrin powder consisted of permethrin (13%), AG (29.5%), SbPC (29.5%) and sucrose (28%) by weight. Permethrin in its NP form exhibited amorphous nature as reported by Anjali et al. (2010). The mean hydrodynamic size of the obtained nanopermethrin is found to be 151 ± 27 nm. Formulated nanopermethrin exhibits a potent larvicidal property against various dreadful species of mosquitoes. Similarly, the NP has exerted the improved efficacy

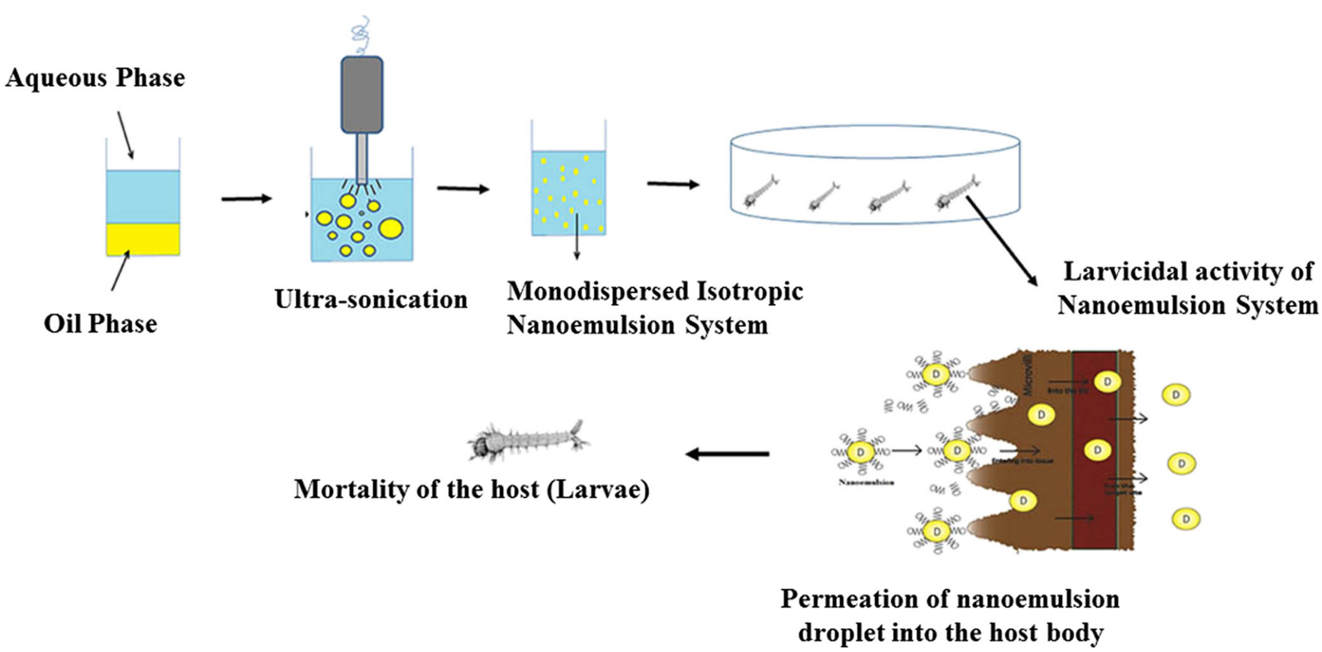

Fig. 4 Schematic representation of the mechanism behind the nanoemulsion effect on the target mosquito species

against the adult mosquitoes as compared to its bulk form. The median knockdown values of NP (60 min) for *Culex tritaeniorhynchus*, *Cx. quinquefasciatus* and *Aedes albopictus* were found to be 0.98 × 104, 1.17 × 104 and 0.05 × 103 mg/L (Balaji et al. 2015; Mishra et al. 2017).

Non-target effects of the green nanopesticides

Currently, extensive efforts are being carried out to study the possible toxic behaviour of these nanopesticides prevalent in the mosquito and other pest specific ecosystems (e.g. Oberdorster et al. 2006; Park et al. 2014; Baun et al. 2008 and Fabrega et al. 2011). However, limited experimental studies have been carried out to the shed the light on the toxicity profile of these nanometric pesticides towards the non-target species prevalent in target ecosystem. Moderate studies are available which describes the possible acute toxicity of AgNPs towards the non-target species such as *Poecilia reticulata* fishes after the 48th hour of exposure did not exhibit any significant toxicity on the fishes (Patil et al. 2012a, b). Subarani et al. (2013) describe the biosafety of *Vinca rosea* synthesised AgNP against *P. reticulata*, where the exposure of LC_{50} concentration to the fishes till 72nd hour did not possess any toxic induced damage. Haldar et al. (2013) describe the non-toxic behaviour of AgNP fabricated using dried green fruits of *Drypetes roxburghii* against *P. reticulate*, where the treatment of the host organism with LC_{50} concentration did not lead to any significant toxicological response. The bio-safe property of these essential oil nanoemulsions makes them a noteworthy insect control agent.

Sugumar et al. (2014) describe the bio-safety property of the eucalyptus oil nanoemulsion against the *Rhizobium leguminosarum*. The tested strain exhibited no zone of inhibition depicting the eco-safe feature of the nanoemulsion. Similarly, the neem oil nanoemulsion exhibited the bio-safe property against the fingerlings of *Labeo rohita* and *Enterobacter ludwigii* which is a beneficial bacterial isolate from the paddy rhizome (Mishra et al. 2014, 2016). Therefore, this study describes that the nanopesticide can be an efficient tool with an eco-safe property and can be applied safely for the control of the vector creating the havoc among humans through the spread of the deadly diseases.

The extensive application of the nanometric materials towards the control of these dreadful mosquito vectors is one of the successful strategies (Table 1). The increasing pollution of the environment due to the accumulation of the conventional pesticides has become a major concern. The implementation of these nanometric pesticides either in the form of metallic nanoparticles derived from the plant source (Benelli et al. 2017a; Govindarajan and Benelli 2016a, b, c; Govindarajan et al. 2016a) or the nanoemulsion from the essential oils

Table 1 Versatile forms of the nanopesticides with its applicative measures against the different dreadful vector mosquitos' species

Nanopesticide	Source	Lethal indices (LC_{50}) 24 h	Host vector insect	References
AgNP	*E. eugeniae* (earthworm)	8.5 mg/L	*An. stephensi*	Jaganathan et al. (2016)
AgNP	*C. colocynthis*	0.5 mg/L	*Cx. pipiens*	Shawky et al. (2014)
AgNP	*M. tinctoria*	1.4 mg/L	*Cx. quinquefasciatus*	Kumar et al. (2014)
AgNP	*L. aspera*	8.5 mg/L	*A. aegypti*	Suganya et al. (2014)
AgNP	*N. nucifera*	0.69 mg/L 1.10 mg/L	*An. subpictus* *Cx. quinquefasciatus*	Santhoshkumar et al. (2011)
AgNP	*E. prostrata*	4.56 mg/L 5.14 mg/L	*Cx. quinquefasciatus* *An. subpictus*	Rajakumar and Rahuman (2011)
AgNP	*Z. diphylla*	12.5 mg/L 13.4 mg/L 14.6 mg/L	*An. subpictus* *A. albopictus* *Cx. tritaeniorhynchus*	Govindrajan et al. (2016g)
AgNP	*C. chinense*	11.1 mg/L 12.3 mg/L	*A. albopictus* *Cx. tritaeniorhynchus*	Govindrajan et al. (2016e)
AgNP	*A. indica*	31.5 mg/L 38.08 mg/L	*A. subpictus* *Cx. tritaeniorhynchus*	Govindrajan et al. (2016h)
AuNP	*C. citratus*	25.92 mg/L 8.36 mg/L	*An. stephensi* *A. aegypti*	Murgan et al. (2015b)
TiO_2 NP	Hydrothermal method	5.6 mg/L	*A. aegypti*	Murgan et al. (2016b)
Leaf extract	*A. elaeignoidea*	246.4 mg/L 207 mg/L	*Cx. quinquefasciatus* *An. stephensi*	Benelli et al. (2017a)
Neem nanoemulsion	Neem oil (*A. indica*)	11.5 mg/L	*Cx. quinquefasciatus*	Anjali et al. (2012)
Eucalyptus nanoemulsion	Eucalyptus oil	250 mg/L	*Cx. quinquefasciatus*	Sugumar et al. (2014)
Nanopermethrin	Permethrin	0.117 mg/L	*Cx. quinquefasciatus*	Anjali et al. (2010)
Nanopermethrin	Permethrin	0.051 mg/L	*Cx. tritaeniorhynchus*	Mishra et al. (2016)

(Pavela 2015) can be helpful in reducing the risk of the eco-toxicity (Pavela and Benelli 2016). Through these studies, it gives a clear visualisation that these metallic nanopesticides and nanometric emulsions prepared from the essential oils pose a significant activity towards the control of the dread vectors which are responsible for havoc in the society. The few of the mechanistic approach that describes the efficient property of these nanopesticides as an effective tool for vector control can be attributed to their nanometric size. The size of these nanometric pesticides below 100 nm makes their passage inside the cells to be easier. Further, the easier penetration into the host cells makes their application specific as different nanopesticides pose a different mechanism of the hindrance. When it comes to metallic nanoparticles, these nanopesticides lead the hindrance at the molecular level which targets the DNA/RNA leading to host mortality. Subsequently, the nanometric emulsion system prepared using the different essential oils have resulted in mortality in the host vector species through obstructing the hormonal system which leads to the growth inhibition further leading to death. Lastly, the nanopesticides prepared from the conventional pesticides through use bio-surfactants has their different mechanism, where it leads to blockage in the sodium potassium gate channel finally resulting in paralysis and host death.

This mechanistic behaviour with the add-on nanometric features makes these pesticidal components as a potent weapon which can be applied towards the regulation of the dreadful vector species. Further, the enhanced property of the nanopesticides such as proper hydro-dispersity compared to the conventional pesticides. In the case of a conventional pesticide, the usage of organic solvents for proper dispersion increases the toxicity of bulk pesticides. The second benefit of nanopesticide is higher target specificity which it is effective at lower concentration compared to conventional pesticides. The first important characteristic of these nanometric pesticides is its lower residual pollution with its eco-safe property towards the non-target species.

Conclusion

Nanometric pesticides such as plant-mediated nanoparticles, nanomodified pesticides from the synthetic form of conventional pesticides and the nanoemulsion from the essential oils are one of the efficient tools to control the vector population of the mosquitoes. The hydro-immiscibility of these nanopesticides in nature significantly improvises their pesticidal life. It also improves their specificity of these nanometric pesticides towards the target organisms. Nanoformulation requires a lower concentration of its active ingredient and also the usage of the plant-mediated synthesis, capping with the biological surfactants or emulsifiers, tends to make these nanopesticide propitious to the environment. The removal of the volatile organic solvent from the pesticidal formulation improves its bio-safe property and makes it a 'greener' strategy for the vector control. These nanopesticides, therefore, can become a potent tool towards the control of dreadful mosquito vector population which is creating havoc in the society. The nanometric form with additional properties of effectiveness at lower concentration and target specificity with lower residual pollution in environment enhances the efficiency of these pesticides, when compared to conventional pesticides. This strategy can serve as a critical component in the effective implementation towards becoming a solution for resistance in the mosquitoes also making it a vital part in integrated vector management technique.

Acknowledgements We acknowledge the Vellore Institute of Technology for providing the laboratory space and facilities.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

Agnihotri A (1999) Pesticide: safety evaluation and monitoring. Indian Agricultural Research Institute, Division of Agricultural Chemicals

Antonio-Nkondjio C, Fossog BT, Ndo C, Djantio BM, Togouet SZ (2011) *Anopheles gambiae* distribution and insecticide resistance in the cities of Douala and Yaounde (Cameroon): influence of urban agriculture and pollution. Malar J 10:154: 154

Amer A, Mehlhorn H (2006a) Larvicidal effects of various essential oils against Aedes, Anopheles, and Culex larvae (Diptera, Culicidae). Parasitol Res 99:466–472

Amer A, Mehlhorn H (2006b) Repellency effect of forty-one essential oils against Aedes, Anopheles and Culex mosquitoes. Parasitol Res 99:478–490

Amerasan D, Nataraj T, Murugan K, Madhiyazhagan P, Panneerselvam C, Nicoletti M, Benelli G (2015) Myco-synthesis of silver nanoparticles using Metarhizium anisopliae against the rural malaria vector Anopheles culicifacies Giles (Diptera: Culicidae). J Pest Sci. doi:10.1007/s10340-015-0675-x

Anjali C, Khan SS, Margulis-Goshen K, Magdassi S, Mukherjee A, Chandrasekaran N (2010) Formulation of water-dispersible nanopermethrin for larvicidal applications. Ecotox environ safe 73: 1932–1936

Anjali C, Sharma Y, Mukherjee A, Chandrasekaran N (2012) Neem oil (*Azadirachta indica*) nanoemulsion—a potent larvicidal agent against *Culex quinquefasciatus*. Pest Manag Sci 68:158–163

Aurel Y, Jan G, Paul VL, Thijs W, Stephan WFM, Van H, Tom AM, Beumer TA, Robert R, Wijn RR, Rene G, Heideman RG, Vinod S, Johannes S, Kanger JS (2007) Fast ultrasensitive virus detection using a young interferometer sensor. Nano Lett 7:394–397

Balaji A, Mishra P, Kumar RS, Ashu A, Margulis K, Magdassi S, Mukherjee A, Chandrasekaran N (2015) The environmentally benign form of pesticide in hydrodispersive nanometric form with improved efficacy against adult mosquitoes at low exposure concentrations. Bull Environ Contam Toxicol 95:734–739

Balkew M, Ibrahim M, Koekemoer LL, Brooke BD, Engers H (2010) Insecticide resistance in Anopheles arabiensis (Diptera: Culicidae)

from villages in central, northern and south west Ethiopia and detection of kdr mutation. Parasit Vectors 3:40
Baun A, Hartmann NB, Grieger K, Kusk KO (2008) Ecotoxicity of engineered nanoparticles to aquatic invertebrates: a brief review and recommendations for future toxicity testing. Ecotoxicology 17:387–395
Benelli G (2015a) Research in mosquito control: current challenges for a brighter future. Parasitol Res:2801–2805
Benelli G (2015b) Plant-borne ovicides in the fight against mosquito vectors of medical and veterinary importance: a systematic review. Parasitol Res 114:3201–3212
Benelli G (2015c) Plant-synthesized nanoparticles: an eco-friendly tool against mosquito vectors? Springer International Publishing Switzerland, H. Mehlhorn (ed.), Nanoparticles in the fight against parasites—parasitology research monographs, doi:10.1007/978-3-319-25292-6_8 **(ISSN: 2192-3671)**
Benelli G (2016a) Plant-mediated biosynthesis of nanoparticles as an emerging tool against mosquitoes of medical and veterinary importance: a review. Parasitol Res 115(1):23–34. doi:10.1007/s00436-015-4800-9
Benelli G (2016b) Green synthesized nanoparticles in the fight against mosquito-borne diseases and cancer—a brief review. Enzym Microb Technol 95:58–68
Benelli G (2016c) Plant-mediated synthesis of nanoparticles: a newer and safer tool against mosquito-borne diseases? Asia Pac J Trop Biomed 6:353–354
Benelli G (2016d) Spread of Zika virus: the key role of mosquito vector control. Asian Pacific Journal of Tropical Biomedicine 6:468–471
Benelli G, Bedini S, Cosci F, Toniolo C, Conti B, Nicoletti M (2015a) Larvicidal and ovi-deterrent properties of neem oil and fractions against the filariasis vector *Aedes albopictus* (Diptera: Culicidae): a bioactivity survey across production sites. Parasitol Res 114:227–236
Benelli G, Bedini SFG, Cosci F, Cioni PL, Amira S, Benchikh F, Laouer H, Di Giuseppe G, Conti B (2015b) Mediterranean essential oils as effective weapons against the West Nile vector *Culex pipiens* and the Echinos to ma intermediate host *Physella acuta*: what happens around? An acute toxicity survey on non-target mayflies. Parasitol Res 114:1011–1021
Benelli G, Murugan K, Panneerselvam C, Madhiyazhagan P, Conti B, Nicoletti M (2015c) Old ingredients for a new recipe? Neem cake, a low-cost botanical by-product in the fight against mosquito-borne diseases. Parasitol Res 114:391–397
Benelli G, Govindarajan M, Senthilmurugan S, Vijayan P, Kadaikunnan S, Alharbi NS, Khaled JM (2017a) Fabrication of highly effective mosquito nanolarvicides using an Asian plant of ethno-pharmacological interest, Priyangu (Aglaia elaeagnoidea): toxicity on non-target mosquito natural enemies Environmental science and pollution research, 1-11
Benelli G, Pavela R, Maggi F, Petrelli R, Nicoletti M (2017b) Commentary: making green pesticides greener? The potential of plant products for nanosynthesis and pest control. J Cluster Sci, 1-8
Bhatnagar V (2001) Pesticides pollution: trends and perspectives. ICMR Bull 31:87–88
Binks BP (1998) Modern aspects of emulsion science. Royal Society of Chemistry
Bowatte G, Perera P, Senevirathne G, Meegaskumbura S, Meegaskumbura M (2013) Tadpoles as dengue mosquito (*Aedes aegypti*) egg predators. Biol Control 67:469–474
Breman J (2001) The ears of the hippopotamus: manifestations, determinants, and estimates of the malaria burden. AmJTropMedHyg 64:1–11
Chadee DD, Williams SA, Ottesen EA (2002) Xenomonitoring of *Culex quinquefasciatus* mosquitoes as a guide for detecting the presence or absence of lymphatic filariasis: a preliminary protocol for mosquito sampling. Ann Trop Med Parasitol 96:47–53
Czeher C, Labbo R, Arzika I, Duchemin JB (2008) Evidence of increasing LeuPhe knockdown resistance mutation in *Anopheles gambiae* from Niger following a nationwide long-lasting insecticide-treated nets implementation. Malar J 7:189
Destrée C, Nagy J (2006) Mechanism of formation of inorganic and organic nanoparticles from microemulsions. Adv Colloid Interf Sci 123:353–367
Diabate A, Baldet T, Chandre F, Akoobeto M, Guiguemde TR et al (2002) The role of agricultural use of insecticides in resistance to pyrethroids in *Anopheles gambiae* s.l. in Burkina Faso. Am J Trop Med Hyg 67:617–622
Dinesh D, Murugan K, Madhiyazhagan P, Panneerselvam C, Nicoletti M, Jiang W, Benelli G, Chandramohan B, Suresh U (2015) Mosquitocidal and antibacterial activity of green-synthesized silver nanoparticles from *Aloe vera* extracts: towards an effective tool against the malaria vector *Anopheles stephensi*? Parasitol Res 114:1519–1529
Donnelly MJ, Corbel V, Weetman D, Wilding CS, Williamson MS et al (2009) Does kdr genotype predict insecticide-resistance phenotype in mosquitoes? Trends Parasitol 25:213–219
El-Aasser MS, Sudol ED (2004) Miniemulsions: overview of research and applications. JCT Res 1(1):21–31
Fabrega J, Luoma SN, Tyler CR, Galloway TS, Lead JR (2011) Silver nanoparticles: behaviour and effects in the aquatic environment. Environ Int 37:517–531
Floury J, Desrumaux A, Axelos MAV, Legrand J (2003) Effect of high pressure homogenisation on methylcellulose as food emulsifier. J Food Eng 58:227–238
Fontenille D, Lochouarn L, Diagne N, Sokhna C, Lemasson JJ (1997) High annual and seasonal variations in malaria transmission by anophelines and vector species composition in Dielmo, a holoendemic area in Senegal. Am J Trop Med Hyg 56:247–253
Forgiarini A, Esquena J, González C, Solans C (2000) Studies of the relation between phase behavior and emulsification methods with nanoemulsion formation. Progr Colloid Polym Sci 115:36–39
Gasco MR, Priano L, Zara GP (2009) Solid lipid nanoparticles and microemulsions for drug delivery: the CNS. Prog Brain Res 180:181–192
Govindarajan M (2010) Larvicidal efficacy of *Ficus benghalensis* L plant leaf extracts against *Culex quinquefasciatus* Say, *Aedes aegypti* L and *Anopheles stephensi* L (Diptera: Culicidae). Eur Rev Med Pharmacol Sci 14:107–111
Govindarajan M, Benelli G (2016a) Facile biosynthesis of silver nanoparticles using *Barleria cristata*: mosquitocidal potential and biotoxicity on three non-target aquatic organisms. Parasitol Res 115:925–935
Govindarajan M, Benelli G (2016b) A facile one-pot synthesis of eco-friendly nanoparticles using Carissa carandas: ovicidal and larvicidal potential on malaria. Dengue and Filariasis Mosquito Vectors Journal of Cluster Science:1–22
Govindarajan M, Benelli G (2016c) One-pot green synthesis of silver nanocrystals using Hymenodictyon orixense: a cheap and effective tool against malaria, chikungunya and Japanese encephalitis mosquito vectors? RSC Adv 6:59021–59029
Govindarajan M, Hoti S, Benelli G (2016a) Facile fabrication of eco-friendly nano-mosquitocides: biophysical characterization and effectiveness on neglected tropical mosquito vectors. Enzym Microb Technol 95:155–163
Govindarajan M, Hoti S, Rajeswary M, Benelli G (2016b) One-step synthesis of polydispersed silver nanocrystals using *Malva sylvestris*. Parasitol Res 115:2685–2695
Govindarajan M, Khater HF, Panneerselvam C, Benelli G (2016c) One-pot fabrication of silver nanocrystals using *Nicandra physalodes*: a novel route for mosquito vector control with moderate toxicity on non-target water bugs. Res Vet Sci 107:95–101

Govindarajan M, Nicoletti M, Benelli G (2016d) Bio-physical characterization of poly-dispersed silver nanocrystals fabricated using Carissa spinarum: a potent tool against mosquito vectors. J Clust Sci 27: 745–761

Govindarajan M, Rajeswary M, Hoti S, Murugan K, Kovendan K, Arivoli S, Benelli G (2016e) Clerodendrum chinense-mediated biofabrication of silver nanoparticles: mosquitocidal potential and acute toxicity against non-target aquatic organisms. J Asia Pac Entomol 19:51–58

Govindarajan M, Rajeswary M, Hoti S, Nicoletti M, Benelli G (2016f) Facile synthesis of mosquitocidal silver nanoparticles using Mussaenda glabra leaf extract: characterisation and impact on non-target aquatic organisms. Nat Prod Res 30:2491–2494

Govindarajan M, Rajeswary M, Muthukumaran U, Hoti SL, Khater HF, Benelli G (2016g) Single-step biosynthesis and characterization of silver nanoparticles using Zornia diphylla leaves: A potent eco-friendly tool against malaria and arbovirus vectors. J Photochem Photobiol B 161:482–489

Govindarajan M, Rajeswary M, Veerakumar K, Muthukumaran U, Hoti S, Benelli G (2016h) Green synthesis and characterization of silver nanoparticles fabricated using Anisomeles indica: mosquitocidal potential against malaria, dengue and Japanese encephalitis vectors. Exp Parasitol 161:40–47

Govindarajan M, Rajeswary M, Veerakumar K, Muthukumaran U, Hoti S, Mehlhorn H, Barnard DR, Benelli G (2016i) Novel synthesis of silver nanoparticles using *Bauhinia variegata*: a recent eco-friendly approach for mosquito control. Parasitol Res 115:723–733

Haldar KM, Haldar B, Chandra G (2013) Fabrication, characterization and mosquito larvicidal bioassay of silver nanoparticles synthesized from aqueous fruit extract of putranjiva, Drypetes roxburghii (Wall.) Parasitol Res 112:1451–1459

Hunt RH, Fuseini G, Knowles S, Stiles-Ocran J, Verster R (2011) Insecticide resistance in malaria vector mosquitoes at four localities in Ghana, West Africa. Parasit Vectors 4:107

Jaganathan A, Murugan K, Panneerselvam C, Madhiyazhagan P, Dinesh D, Vadivalagan C, Aziz AT, Chandramohan B, Suresh U, Rajaganesh R, Subramaniam J, Nicoletti M, Higuchi A, Alarfaj AA, Munusamy MA, Kumar S, Benelli G (2016) Earthworm-mediated synthesis of silver nanoparticles: a potent tool against hepatocellular carcinoma, pathogenic bacteria, Plasmodium parasites and malaria mosquitoes. Parasitol Int 65:276–284

Jensen M, Mehlhorn H (2009) Seventy-five years of ResochinÂ® in the fight against malaria. Parasitol Res 105(3):609–627

Kalimuthu K, Panneerselvam C, Chou C, Tseng L-C, Murugan K, Tsai K-H, Alarfaj AA, Higuchi A, Canale A, Hwang J-S (2017): Control of dengue and Zika virus vector *Aedes aegypti* using the predatory copepod *Megacyclops form*osanus: synergy with *Hedychium coronarium*-synthesized silver nanoparticles and related histological changes in targeted mosquitoes. **Process Safety and Environmental Protection**

Kim JS, Kuk E, Yu KN, Kim JS, Park SJ, Lee HJ, Kim SH, Park YK, Park YH, Hwang CY, Kim YK, Lee YS, Jeong DH, Cho MH (2007) Antimicrobial effects of silver nanoparticles. Nanomed Nanotechnol Biol Med 3:95–101

Kumar KR, Nattuthurai N, Gopinath P, Mariappan T (2014) Synthesis of eco-friendly silver nanoparticles from Morinda tinctoria leaf extract and its larvicidal activity against *Culex quinquefasciatus*. Parasitol Res 114:411–417

Landfester K, Eisenbla¨tter J, Rothe R (2004) Preparation of polymerizable miniemulsions by ultrasonication. JCT Res 1:65–68

Liu H, Xu Q, Zhang L, Liu N (2005) Chlorpyrifos resistance in mosquito *Culex quinquefasciatus*. J Med Entomol 42:815–820

Margulis-Goshen K, Magdassi S (2012) Organic nanoparticles from microemulsions: formation and applications. Curr Opin Colloid Interface Sci 17:290–296

Marimuthu S, Rahuman AA, Rajakumar G, Santhoshkumar T, Kirthi AV, Jayaseelan C, Bagavan A, Zahir AA, Elango G, Kamaraj C (2011) Evaluation of green synthesized silver nanoparticles against parasites. Parasitol Res 10:2212–2224

Mehlhorn H, Al-Rasheid KA, Al-Quraishy S, Abdel-Ghaffar F (2012) Research and increase of expertise in arachno-entomology are urgently needed. Parasitol Res 110:259–265

Mishra P, Balaji A, Dhal P, Kumar RS, Magdassi S, Margulis K, Tyagi B, Mukherjee A, Chandrasekaran N (2017) Stability of nano-sized permethrin in its colloidal state and its effect on the physiological and biochemical profile of Culex tritaeniorhynchus larvae. Bulletin of entomological research, 1-13

Mishra P, Balaji A, Swathy J, Paari AL, Kezhiah M, Tyagi B, Mukherjee A, Chandrasekaran N (2016) Stability assessment of hydro dispersive nanometric permethrin and its biosafety study towards the beneficial bacterial isolate from paddy rhizome. Environ Sci Pollut Res 23:24970–24982

Mishra P, Jerobin J, Thomas J, Mukherjee A, Chandrasekaran N (2014) Study on antimicrobial potential of neem oil nanoemulsion against *Pseudomonas aeruginosa* infection in *Labeo rohita*. Biotechnol Appl Bioc 61:611–619

Murrell S, Wu SC, Butler M (2011) Review of dengue virus and the development of a vaccine. Biotechnol Adv 29:239–247

Murugan K, Aruna P, Panneerselvam C, Madhiyazhagan P, Paulpandi M, Subramaniam J, Rajaganesh R, Wei H, Saleh Alsalhi M, Devanesan S, Nicoletti M, Syuhei B, Canale A, Benelli G (2015a) Fighting arboviral diseases: low toxicity on mammalian cells, dengue growth inhibition (in vitro) and mosquitocidal activity of Centroceras clavulatum-synthesized silver nanoparticles. Parasitol Res. doi:10.1007/s00436-015-4783-6

Murugan K, Benelli G, Panneerselvam C, Subramaniam J, Jeyalalitha T, Dinesh D, Nicoletti M, Hwang JS, Suresh U, Madhiyazhagan P (2015b) *Cymbopogon citratus*-synthesized gold nanoparticles boost the predation efficiency of copepod *Mesocyclops aspericornis* against malaria and dengue mosquitoes. Exp Parasitol 153:129–138

Murugan K, Benelli G, Suganya A, Dinesh D, Panneerselvam C, Nicoletti M, Hwang JS, Mahesh Kumar P, Subramaniam J, Suresh U (2015c) Toxicity of seaweed-synthesized silver nanoparticles against the filariasis vector *Culex quinquefasciatus* and its impact on predation efficiency of the cyclopoid crustacean *Mesocyclops longisetus*. Parasitol Res 14:2243–2253

Murugan K, Vadivalagan C, Karthika P, Panneerselvam C, Paulpandi M, Subramaniam J, Wei H, AlThabiani A, Saleh Alsalhi M, Devanesan S, Nicoletti M, Paramasivan R, Parajulee MN, Benelli G (2015d) DNA barcoding and molecular evolution of mosquito vectors of medical and veterinary importance. Parasitol Res. doi:10.1007/s00436-015-4726-2

Murugan K, Dinesh D, Kavithaa K, Paulpandi M, Ponraj T, Saleh Alsalhi M, Devanesan S, Subramaniam J, Rajaganesh R, Wei H, Suresh K, Nicoletti M, Benelli G (2016a) Hydrothermal synthesis of titanium dioxide nanoparticles: mosquitocidal potential and anticancer activity on human breast cancer cells (MCF-7). Parasitol Res 115:1085–1096

Murugan K, Wei J, Alsalhi MS, Nicoletti M, Paulpandi M, Samidoss CM, Dinesh D, Chandramohan B, Paneerselvam C, Subramaniam J (2016b) Magnetic nanoparticles are highly toxic to chloroquine-resistant Plasmodium falciparum, dengue virus (DEN-2), and their mosquito vectors. Parasitol Res:1–8

Nakajima H (1997) Microemulsions in cosmetics. In: Solans C, Kunieda H (eds) Industrial applications of microemulsions. Marcel Dekker, New York, pp 175–197

Oberdorster E, Zhu S, Michelle Blickley T, McClellan-Green P, Haasch ML (2006) Ecotoxicology of carbon-based engineered nanoparticles: effects of fullerene (C60) on aquatic organisms. Carbon 44: 1112–1120

Oliva CF, Damiens D, Benedict MQ (2014) Male reproductive biology of Aedes mosquitoes. Acta Trop 132S:S512–SS19

Panneerselvam C, Murugan K, Kovendan K, Mahesh Kumar P (2012) Mosquito larvicidal, pupicidal, adulticidal, and repellent activity of *Artemisia nilagirica* (Family: Compositae) against *Anopheles stephensi* and *Aedes aegypti*. Parasitol Res 111:2241–2251

Panneerselvam C, Murugan K, Kovendan K, Mahesh Kumar P, Subramaniam J (2013) Mosquito larvicidal and pupicidal activity of *Euphorbia hirta* Linn. (Family: Euphorbiaceae) and *Bacillus sphaericus* against *Anopheles stephensi* Liston. (Diptera: Culicidae). (Diptera: Culicidae). Asian Pac J Trop Med 6:102–109

Park J, Kim S, Yoo J, Lee JS, Park JW, Jung J (2014) Effect of salinity on acute copper and zinc toxicity to *Tigriopus japonicus*: the difference between metal ions and nanoparticles. Mar Pollut Bull 85:526–531

Patil CD, Borase HP, Patil SV, Salunkhe RB, Salunke BK (2012b) Larvicidal activity of silver nanoparticles synthesized using *Pergularia daemia* plant latex against *Aedes aegypti* and *Anopheles stephensi* and non-target fish *Poecillia reticulata*. Parasitol Res 111:555–562

Patil CD, Patil SV, Borase HP, Salunke BK, Salunkhe RB (2012a) Larvicidal activity of silver nanoparticles synthesized using *Plumeria rubra* plant latex against *Aedes aegypti* and *Anopheles stephensi*. Parasitol Res 110:1815–1822

Pavel FM (2004) Microemulsion polymerization. J Disper Sci Technol 25:1–16

Pavela R (2015) Essential oils for the development of eco-friendly mosquito larvicides: a review. Ind Crop Prod 76:174–187

Pavela R, Benelli G (2016) Essential oils as ecofriendly biopesticides? Challenges and constraints. Trends Plant Sci 21:1000–1007

Ponarulselvam S, Panneerselvam C, Murugan K, Aarthi A, Kalimuthu K, Thangamani S (2012) Synthesis of silver nanoparticles using leaves of *Catharanthus roseus* Linn. G. Don and their antiplasmodial activities. Asian-Pacif J Trop Biomed 2:574–580

Poopathi S, De Britto LJ, Praba VL, Mani C, Praveen M (2015) Synthesis of silver nanoparticles from *Azadirachta indica*—a most effective method for mosquito control. Environ Sci Pollut Res 22:2956–2963

Priyadarshini AK, Murugan K, Panneerselvam C, Ponarulselvam S, Hwang JS, Nicoletti M (2012) Biolarvicidal and pupicidal potential of silver nanoparticles synthesized using *Euphorbia hirta* against *Anopheles stephensi* Liston (Diptera: Culicidae). Parasitol Res 111: 997–100

Rai M, Yadav A, Gade A (2009) Silver nanoparticles as a new generation of antimicrobials. Biotechnol Adv 27:76–78

Rajakumar G, Rahuman A (2011) Larvicidal activity of synthesized silver nanoparticles using *Eclipta prostrata* leaf extract against filariasis and malaria vectors. Acta Trop 118:196–203

Rajan R, Chandran K, Harper SL, Yun SI, Kalaichelvan PT (2015) Plant extract synthesized nanoparticles: an ongoing source of novel biocompatible materials. Ind Crop Prod 70:356–373

Rang MJ, Miller CAC (1999) Spontaneous emulsification of oils containing hydrocarbon, nonionic surfactant, and oleyl alcohol. J Colloid Interface Sci 209:179–192

Robert LL, Olson JK (1989) Susceptibility of female *Aedes albopictus* from Texasto commonly used adulticides. JAmMosqControlAssoc 5:251–253

Santhoshkumar T, Rahuman AA, Rajakumar G, Marimuthu S, Bagavan A, Jayaseelan C, Zahir AA, Elango G, Kamaraj C (2011) Synthesis of silver nanoparticles using *Nelumbo nucifera* leaf extract and its larvicidal activity against malaria and filariasis vectors. Parasitol Res 108:693–702

Shankar S, Rai A, Ahmad A, Sastry M (2004) Rapid synthesis of Au, Ag and bimetallic Au core-Ag shell nanoparticles using neem (*Azadirachta indica*) leaf broth. J Colloid Interface Sci 275:496–502

Shawky AM, Abdulall AK, Rabeh MA, Abdellatif AO (2014) Enhanced biocidal activities of Citrullus colocynthis aqueous extracts by green nanotechnology. Int J Appl Res Nat Prod 7:1–10

Shinoda K, Saito H (1968) The effect of temperature on the phase equilibria and the types of dispersion of the ternary system composed of water, cyclohexane, and nonionic surfactant, J. Colloid Interface Sci. 26 70–74

Simon-Sylvestre G, Fournier J-C (1980) Effects of pesticides on the soil microflora. Adv Agron 31:1–92

Solans C, Izquierdo P, Nolla J, Azemar N, Garcia-Celma M (2005) Nanoemulsions. Curr Opin Colloid Interface Sci 10:102–110

Sonneville-Aubrun O, Simonnet J-T, L'Alloret F (2004) Nanoemulsions: a new vehicle for skincare products. Adv Colloid Interf Sci 108–109:145–149

Subarani S, Sabhanayakam S, Kamaraj C (2013) Studies on the impact of bio synthesized silver nanoparticles (AgNPs) in relation to malaria and filariasis vector control against *Anopheles stephensi* Liston and *Culex quinquefasciatus* Say (Diptera: Culicidae). Parasitol Res 112: 487–499

Subramaniam J, Murugan K, Panneerselvam C, Kovendan K, Madhiyazhagan P, Mahesh Kumar P, Dinesh D, Chandramohan B, Suresh U, Nicoletti M, Higuchi A, Hwang JS, Kumar S, Alarfaj AA, Munusamy MA, Messing RH, Benelli G (2015) Eco-friendly control of malaria and arbovirus vectors using the mosquitofish *Gambusia affinis* and ultra-low dosages of *Mimusops elengi*-synthesized silver nanoparticles: towards an integrative approach? Environ Sci Pollut Res. doi:10.1007/s11356-015-5253-5

Suganya G, Karthi S, Shivakumar MS (2014) Larvicidal potential of silver nanoparticles synthesized from *Leucas aspera* leaf extracts against dengue vector *Aedes aegypti*. Parasitol Res 113:1673–1679

Sugumar S, Clarke S, Nirmala M, Tyagi B, Mukherjee A, Chandrasekaran N (2014) Nanoemulsion of eucalyptus oil and its larvicidal activity against *Culex quinquefasciatus*. Bull Entomol Res 104:393–402

Sujitha V, Murugan K, Paulpandi M, Panneerselvam C, Suresh U, Roni M, Nicoletti M, Higuchi A, Madhiyazhagan P, Subramaniam J, Dinesh D, Vadivalagan C, Chandramohan B, Alarfaj AA, Munusamy MA, Barnard DR, Benelli G (2015) Green-synthesized silver nanoparticles as a novel control tool against dengue virus (DEN-2) and its primary vector *Aedes aegypti*. Parasitol Res. doi: 10.1007/s00436-015-4556-2

Suresh U, Murugan K, Benelli G, Nicoletti M, Barnard DR, Panneerselvam C, Mahesh Kumar P, Subramaniam J, Dinesh D, Chandramohan B (2015) Tackling the growing threat of dengue: *Phyllanthus niruri*-mediated synthesis of silver nanoparticles and their mosquitocidal properties against the dengue vector *Aedes aegypti* (Diptera: Culicidae). Parasitol Res 114:1551–1562

Tadros TF, Izquierdo P, Esquena J, Solans C (2004) Formation and stability of nano-emulsions. Adv Colloid Interface Sci 108:109 303–109 318

Topp E, Vallaeys T, Soulas G, van ELSAS J, Trevors J, Wellington E (1997) Pesticides: microbial degradation and effects on microorganisms. Modern soil microbiology:547–575

Veerakumar K, Govindarajan M, Rajeswary M, Muthukumaran U (2014) Low-cost and eco-friendly green synthesis of silver nanoparticles using Feronia elephantum (Rutaceae) against *Culex quinquefasciatus*, Anopheles stephensi, and Gasco MR, Priano L, Zara GP (2009): Solid lipid nanoparticles and microemulsions for drug delivery: the CNS. Prog Brain Res 180, 181-192 *Aedes aegypti* (Diptera: Culicidae). Parasitol Res 113:1775–1785

Vezenegho SB, Brooke BD, Hunt RH, Coetzee M, Koekemoer LL (2009) Malaria vector composition and insecticide susceptibility status in Guinea Conakry, West Africa. Med Vet Entomol 23:326–334

Vulule JM, Beach RF, Atieli FK, Roberts JM, Mount DL (1994) Reduced susceptibility of Anopheles gambiae to permethrin associated with

the use of permethrin-impregnated bednets and curtains in Kenya. Med Vet Entomol 8:71–75
Walstra P (1996) Emulsion stability. In: Becher P (ed) Encyclopedia of emulsion technology. Marcel Dekker, New York, pp 1–62
Wang L, Li X, Zhang G, Dong J, Eastoe J (2007) Oil-in-water nanoemulsions for pesticide formulations. J Colloid Interface Sci 314:230–235
Wattanachai P, Tintanon B (1999) Resistance of *Aedes aegypti* to chemical compounds in aerosol insecticide products in different areas of Bangkok, Thailand. Commun Dis J 25:188–119
WHO (2007) Insecticide-treated mosquito nets: a WHO position statement. Global malaria programme. Geneva, World Health Oraganization
WHO (2008) World malaria report 2008. World Health Oraganization, Geneva
WHO (2012) Handbook for integrated vector management. World Health Organization
WHO (2014a) Malaria. Fact sheet no. 94. World Health Organization, Geneva
WHO (2014b) Lymphatic filariasis. Fact sheet no. 102. World Health Organization, Geneva
WHO (2015) Dengue and severe dengue. Fact sheet no. 117, Geneva, World Health Organization
Zaim M, Aitio A, Nakashima N (2000) Safety of pyrethroid-treated mosquito nets. Med Vet Entomol 14: 1– Geneva