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Efficacy of Nanoparticles as a research tool to control Mosquito vector: A review

Vareesh Baghela and *Neetu Kachhwaha

Department of Zoology,
University of Rajasthan,
JAIPUR (RAJASTHAN)-302001, INDIA
*Corresponding Author
E-mail: drneetu2011@gmail.com

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ABSTRACT

Nanotechnology has revolutionized a wide array of disciplines with its explicit applications in various important fields related to human health and environment. Utilization of nanoparticles to control mosquito menace is one of the most important output of nano research. Mosquitoes are a threat to the worldwide population as they serve as vectors for a variety of disease-causing organisms. They are responsible for numerous diseases like malaria, dengue, chikungunya, yellow fever, West Nile fever, Japanese encephalitis, *etc.* Conventional control methods use various chemicals and physical agents which are unable to fulfill the current demand of mosquito control strategy. From the decades, the application of these chemicals are causing a continuous harm to biotic and abiotic components of the ecosystem. Therefore, recent studies are focusing on the novel and safe nanotool that are proving a boon to control measures against mosquito population.Plant mediated nanoparticles approach has shown impressive results in the field of mosquito control biology.Green synthesis of copper, zinc and silver nanoparticles have shown their toxic effects against mosquito.The review provides an insight of green revolution of rapidly growing nano research as a green nanotool control tactics to minimize the damage caused due to mosquito borne diseases.

Figure : 00 References : 54 Table : 00
KEY WORDS : Characterization, Green synthesis, Insecticidal bed nets, Mosquito, Nanotechnology, Nanotool, Vector-borne diseases.

Introduction

A tiny mosquito is the potential vector of life-threatening disease to the worldwide population such as malaria, dengue, chikungunya, yellow fever, West Nile fever, Japanese encephalitis. Mosquito-borne diseases have vital economic and health effects, contributing widely or a greater disease load, hunger, poverty, deaths, and social frailty mainly in tropical and subtropical poor countries. Outbreaks of these diseases emphasize the need of potential, eco-friendly, bio-degradable, economic and alternative control method to check mosquito population[3,53]. Malaria is an important parasitic disease and a major cause of death, especially in tropical and subtropical regions. Dengue fever is another common infection that is a menace in Southeast Asian countries including India, which is caused by *Aedes* species of mosquito among which *A. aegypti* and *A. albopictus* are the main vectors[8]. India has faced major Chikungunya outbreak in the year 2006 where about thousands of cases have been reported in a very short tenture[15]. Lymphatic filarias is also referred as elephantiasis, is another menace that has been transmitted by more than one species of mosquitoes that particularly belongs to the four principal genera—*Anopheles, Mansonia, Culex* and *Aedes.* Japanese encephalitis (JE) which is a mosquito-borne viral encephalitis infection that is caused by a virus, closely related to other viral diseases like dengue, yellow fever, and West Nile viruses. JE is the prominent reason for viral encephalitis in Asiatic countries that has been encountering about 68,000 clinical cases every year[9]. Conventional techniques of mosquito control like use of insecticides have not been proven effective due to resistance developed in mosquitoes against them. Recent studies have shown that mosquitoes have developed behavioural, metabolic, target- site and knock down resistances against conventional insecticides[29]. There is a need to investigate newer approaches for mosquito control.

Nanotechnology has grown as an important field of modern research with its potential applications in medicine.Efficacy of metal nanoparticles prepared by green synthesis method against mosquitoes are being

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explored[25]. The biosynthesis of plant-based nanoparticles is beneficial in comparison to chemical and physical methods, because plant mediated nanoparticles biosynthesis is a single-step process, cheap, does not require much inputs such as high energy consumptions, use of high temperature and pressure and uses very less toxic chemicals[40].

For the effective and efficient manufacturing of green fabricated metal nanoparticles, plant-based compounds have been identified[36], which resulted in recognizable mosquitocidal properties, not only in the laboratory conditions but also in-field conditions[4].

1. Plant-based biosynthesis and characterization of mosquitocidal nanoparticles: -

Plant extracts and metabolites are also used in the production of metal nanoparticles because of their reducing and stabilizing potentials. There are various factors on which the performance of nano formulation depends against target mosquitoes.Two major ingredients of the nano formulation are metal ions and source plant extracts. The key factor on which the efficacy depends is concentrations of these ingredients used to prepare these nanoparticles[36].

Characterization techniques are used to confirm successful preparation of desired nanoparticles. Transmission electron microscopy, scanning electron microscopy, UV visualization spectroscopy,Fourier transform infrared spectroscopy,X-ray diffraction studies and energy-dispersive X-ray spectroscopy are mainly used characterization technique.Mosquitocidal nanoparticles that were made using plant extracts showed that when UV visualization spectroscopy detect the change in intensity of color that can be observed when plant extracts mix with the metal ion solution.

Changes in color intensity result in the change in the absorbance of the test solution and hence, comparing the results of spectroscopy with the standards, provide the information of prepared nanoparticles. Absorption maxima changes with the concentration of metal ions as well as reaction time. Size of nanoparticles is inversely proportional to the width of energy gap and because of this, the absorption peaks shifted towards higher energy[10]. The phenomenon of Surface plasmon resonance might be responsible for the color shift that arrives as it causes excitation in metal ions. The synthesized metal nanoparticles could contain free electrons as a result of simultaneous electronic vibrations of nanoparticles in resonance with light wave[32], which are responsible for an SPR absorption band. The biogenesis of nanoparticles can also be validated using UV-visible spectroscopy.For example, silver nanoparticle shows a peak at 3 keV, that is due to the phenomenon of surface plasmon resonance, that was very unique for absorption of metallic Agnano crystallites[42]. SEM and TEM help to see the shape and size of nanoparticles. Normally, the shape of nano particles that has been synthesized using plant source may be cubic, spherical, rod-like, or triangular while nanoparticles that has been synthesized using neem leaves, were spherical and flat in shape (size 5–35 nm)[43].

2. Different nanoparticle formulations against mosquito:

Carbon-dot silver nanohybrid particles that has been prepared with Solanum tuberosum, examined against mosquito species *Culex quinquefasciatus* and *Anopheles stephensi* were found highly effective against immatures of both mosquito species. These nanohybrid particles resulted into deformation of larval bodies, which has been confirmed by SEM, while another examination that was conducted using electro diffraction X-ray technique, proved that silver got deposited in the tissues, which itself shows that lethality due to the presence of nano-silver toxicity at the cellular level. The HR-TEM revealed distortions in the cuticle and cellular organization[47].

Now a days, researchers have reported that green-synthesized nanoparticles given to mosquito in early instars have substantial effects. According to some scientists, exposing fourth instar *Aedes aegypti* larvae to Ag nanoparticles produced from a rhizome (*Hedychium coronarium*) resulted in the death of epithelial cells of midgut, and deformation of apical membranes[23]. The nanoparticles synthesized using Polystyrene can also have its significant impact as it interferes with cytochrome enzymes which are involved in drug metabolism, such as isoenzymes of P450,CYP2A1, CYP2D6, CYP3A4, CYP2C9 and that have been expressed in cells of insects in Baculosomes, results in restricting the enzymatic activity of CYP450 isoenzymes in Baculosomes[12].

Silica nanoparticles have potential to be used as a control measure[1]. It has been observed that silica nanoparticles act as physio-sorbed for the cuticular lipids of insects, that causes distortion, resulting in the death of the test insect, a mechanism was found to be similar to the diatom that has been used for control of foodstuff pests[24].

Several histological and morphological abnormalities in *Ae. Aegypti*IIIrd instar larvae was observed when these organisms were exposed to Zinc oxide (ZnO) nanoparticles prepared using the *Lobelia leschenaultiana* leaf extract, that showed the shrinkage in the abdominal region, loss of lateral hairs, thoracic shape change, midgut

damage, anal gills, and accumulated ZnO nanoparticles in the abdomen as well as the thorax[2].

3. Effect of plant-based nanoparticles on mosquito vectors:

Whole of the process includes the nanoparticles synthesis with the help of plant extract and their commercial application as an alternative control measure against mosquitoes involves the following steps:

3.1 Nano formulation preparation:

In green synthesis of nanoparticles, active agent obtained from leaf, bark, root or any other plant part can be used to reduce and stabilize the metal nanoparticle. A worker[5] in 2018 used the leaf powder of *Acacia caesia* plant. Before grinding leaves to powder,they were properly cleansed and rinsed with tap water. Aqueous extract was prepared using 50 g of the leaf powderand0.5 l distilled water which was continuously stirred. The suspension was left for three hours after which it was filtered[5]. Similar work was done by others using the mangrove plant, *Rhizophora mucronate* to test its efficacy against *Aedes aegypti* and *Culex quinquefasciatus*[16]. Other workers used leaf extracts of *Heliotropium indicum* for preparation of silver nanoparticles[8]. There are numerous researches and works conducted by many scientists by using different plant species or even different parts of the same plant species that may give variable results.

3.2 Synthesis and characterization of nanoparticles:

Nanoparticle solution is mixed with the extracted compounds from the plants for the synthesis of required nanoparticles. Ninety milliliters of 1 mM $AgNO_3$ solution and 10 milliliters of the *Acacia caecia* leaf extract was used for the preparations of silver nanoparticles[5]. The synthesis of colloidal Ag NPs causes a change in color from yellow to brown. UV-Visible spectrophotometry, FTIR spectroscopy, EDX, TEM, and SEM were used to characterize the composition, size, and morphology of silver nanoparticles[5].

Some researchers used UV-based spectrophotometer in wavelength range of 200-900 nm for the characterization of silver nanoparticles[21] and cubic cobalt tetraoxide($Co_3O_{4)}$ nanoparticles, mostly used techniques were high-resolution transmission electron microscopy, field emission scanning electron microscopy,X-ray diffraction[11].

3.3 Rearing of mosquito:

Some studies conducted the rearing of three mosquitoes species that has been contamination-free for several generations in the form of pure line breeding[17]. These species of mosquitoes were reared at 28±2°C, with 70–85% Relative Humidity. For feeding of these organisms a mixture of yeast powder together with biscuits (used for dog feed)were considered[17,18]. Various extrinsic and intrinsic factors affected survival of *Aedes* mosquito[22]. Some workers reared mosquitoes at anaverage temperature of 28 °C (± 2 °C) and 80% (± 5%) of relative humidity with continuous light and dark period in 12:12 hours proportion[54].

3.4 Exposure to nanoparticles and observations:

Efficacy of nanoparticles can be tested against eggs, larva, pupa or adult stage of mosquitoes:

3.4.1 Efficacy against eggs:

Ovicidal experiments were carried to test the effect of nanoparticles on the eggs of mosquito so as to examine the lethality of nanoparticles on them. Following the experiment[49], 100 eggs were selected to be treated at six different concentration ranges between 150 to 900 ìg per ml in 150 ìg per ml increments of the leaf extract that has been prepared, with the equal number of concentrations of silver nitrate, ranging between 15 to 90 ìg per ml in 15 ìg per ml increments and were tested against different mosquito species *Anopheles subpictus, Aedes albopictus* and *Culex tritaeniorhynchus*[5]. Eggs were exposed to the nanoparticle formulations for about 24 hours and then counted with the help of the microscope. After the treatment, eggs were moved to the plastic containers and were examined for hatching. At 60, 75, and 90 µg/ml, AgNPs completely inhibited egg hatchability among all the three vectors[5].

3.4.2 Efficacy against larvae:

Nano formulation prepared using the extract of *Acacia* leaves in five different doses, from 60 to 300 ìg $ml^{"1}$ together with 60 ìg $ml^{"1}$ increments and AgNPs (five doses, ranging from 5 to 25 ìg $ml^{"1}$, in 5 ìg $ml^{"1}$ increments) reported impressive results against III^{rd} instar larvae. Mortality rate of larvae were assessed after 24 hrs of exposure and for every test control groups correspondingly, five repetitions were done that contained $AgNO_3$ and distilled water[5,18]. Larvicidal experiments against different mosquito species using laboratory prepared silver nanoparticles with leaf extract of *Acacia* showed better efficacies. Highest sensitivity was reported against *Anopheles subpictus* which was followed by *Aedes albopictus* and least sensitivity was found against *Culex tritaeniorhynchus*[5]. Silver nanoparticles blended with *Nelumbo nucifera* leaf extract were found toxic againstIV^{th} instars of *Anopheles subpictus* and *Culex quinquefasciatus.* LC_{50} value for *Anopheles subpictus* was found to be 0.69 ppm and LC_{50} value for Culex *quinquefasciatus* was found to be 1.10 ppm[41]. Efficacy of AgNP blended with *Mimosa pudicaleaf* extract against larvae of *Culex quinquefasciatus* and *Anopheles subpictus,* with LC_{50} value of 11.73 and 13.90 mg/lwere observed respectively[28]. Silver nanoparticles were

synthesized using *Eclipta prostata* leaf extract and its toxicity was demonstrated towards IVth instar larvae of *Anopheles subpictus* (LC_{50}=5.14 mg/l) and *Culex quinquefasciatus* (LC_{50}=4.56 mg/l)[35].

3.4.3 Efficacy against adults:

Adulticide experiments with green silver nanoparticles have shown remarkable results[19]. Experiment was performed on twenty female mosquitoes of different species which were kept in a plastic container. Mosquitoes were exposed to the nano formulation for one hour. In the adulticidal assay, adulticidal activity was observed highest against *Anopheles subpictus* with anLD_{50} value of 18.66 ìg/ml which was followed by *Aedes albopictus* with an LD_{50} value of 20.94 ìg/ml and *Culex tritaeniorhynchus* with an LD_{50} value of 22.63ìg/ml[21].

3.5 LLINs:

LLINs (Long-Lasting Insecticidal Nets) for controlling mosquito populations were also initiated, examined, and marketed. The utility of anti-mosquito textiles such as insecticide-installed and nets that are mosquito repellent have been documented as an effective technique in reducing the spread of mosquito-borne diseases in human communities[45].

The use of nanoparticles in the production of mosquito repellent fabrics has also been studied. In one such study conducted by renowned researchers, in which the ionic jellification approach was used to make nanoparticles loaded with *Vitex negundo* leaf extract[38] while the other principle that was using cation-induced controlled jellification of alginate was used to prepare *Alginate nanoparticles*[14].

Hence, in recent trends, nanoparticles are being studied widely for the control of the mosquito population.

Discussion

Several greenmetal-based nanoparticles have already been synthesized for mosquito control which also demonstrate and elaborate about various biophysical characteristics of these synthesized nanoparticles. These characteristics of nanoparticles are highly influenced by the botanicals (plant based resources) which act as reducing agents[6,30,33,46,48]. However, knowledge about their mode of action is scarce[7]. Although, the larvicidal and adulticidal potential[5] of these nano synthesized mosquitocidal have been demonstrated[50,51]. The larvicidal activity of AgNPs prepared using *Pithecellobium dulce* showed outstanding results. *Culex quinquefasciatus* which is a Filariasis vector, was targeted with dosages (LC_{50} = 21.56 mg l”1) and an effective larvicidal activity was seen[37]. The impact of nanoparticles towards non-target mosquito predators[5] has also been evaluated[5,17,26,31,34]. A study talks about the little biotoxicity of *Malva sylvestris*- synthesized AgNPs over aquatic two different dosages on two non-targeted different organisms *i.e.,* G. affinis (LC_{50} = 1044.52 ìg/ml) and D. indicus (LC50 = 813.16 ìg/ml)[20].

Ag NPs prepared with the bark and leaf extract of *Azadirachta indica* were used to access the larvicidal, pupicidal, and adulticidal potential of nanoparticles against *Culex quinquefasciatus*. There was complete larval mortality after an exposure of 30 minutes. Exposure to pupae of *Culex quinquefasciatus* showed value of LC_{50} as 4 ìg ml”1. Exposing adult mosquitoes for 4 hours, showed LC_{50} values of 1.06 ìL cm”250. Ag NPs find its way to the epithelial membrane of midgut of larvae, causing inactivation of enzymes and generation of peroxides leading to the death of the cell[6]. Silver nanoparticles prepared using extracts of *Solanum nigrumwas* also proved effective and showed LC_{50} values of 1.33, 1.56, 1.59 ppm for dried leaves and berries against the malaria causing species *Anopheles stephensi*[39]. An experiment with the larvicidal activity of AgNPs synthesized from *S. acuta* leaves against *Anopheles stephensi* showing LC_{50} value of 21.92 ìg ml”1, Culex quinquefasciatuswith LC_{50} value of 26.13 ìg ml”1 and *Aedes aegypti* with LC_{50} value of 23.96 ìg ml”1,52. Larvicidal effectiveness of the leaf extract of *Morinda tinctoria* and prepared AgNPs was assessed towards the *Culex quinquefasciatus* larvae. Values of LC_{50} was found to be 1.442 ppm reported effectiveness against *Culex quinquefasciatus*[27].

Conclusion

Mitigation in deaths being caused by diseases spread due to mosquitoes is necessary.Some studies are showing that there are some similarities in the symptoms of two different diseases; the dengue and COVID-2019[13].

Various control tactics have been found on account of various researches being done. In very early researches, scientists relied on the use of chemicals to control the mosquito population. Mosquito repellents and sprays had also been formulated.Pyrethroids was one of the most commonly used chemicals used in the form of sprays to control adult mosquitoes in outbreaks but development of resistance against them caused another problem. Indoor residual spraying (IRS) has also been a key tool in fight against the dreadful malaria as the National Malaria Control Program since 1949. Use of Insecticide-treated nets (ITNs)in the program was also common in 1980. However, the more use of chemical compounds for mosquito control raises the problem of insecticidal resistance in the targeted vector population. In recent trends, researchers are focusing on more advanced tactics. One of such techniques involves the use of plant-based nanoparticles against the mosquito. This technique

is gaining importance as conventional techniques have not been proving very much effective in dealing with this wrathful insect. Green synthesis of nanoparticles with the help of plant products is low-cost, single-step technique and eco-friendly approach. Biologically synthesized metal nanoparticles are significant in showing high effectiveness against mosquito vectors and can be used as a potential control agent for mitigating mosquito-borne diseases.

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