

8. Potential of Green Nanoparticles as Mosquitocidal Agents

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Abstract:

Various Diseases including Malaria, Dengue, Filariasis, Chikungunya, West Nile Fever, Japanese Encephalitis etc. are transmitted by Mosquitoes and caused millions of deaths worldwide. Mosquitoes are controlled by using synthetic insecticides but they have various menaces like resistance, resurgence in mosquitoes and harmful impact on non-target organisms including Environment. To cope up with these problems, Biogenic larvicides and mosquito ives are being made using Plants, Algae, Fungi etc. blended with Nanotechnology which is an emerging field in the vector management. In this chapter, we reviewed the Biogenic Nanoparticles synthesis and studied their coquinoidal, larvicidal, ovicidal and poricidal bioassays. Thus, this extensive information can be utilized in the near future for the development of herbal larvicidal formulations and Nano pesticides against vectors that are resistant to insecticides.

Keywords:

Nanoparticles; Green Synthesis; Microorganisms; Plant extracts; Mosquito control

8.1 Green Synthesis:

The creation of nanoparticles can be approached through three methods: chemical, physical and biological. The chemical and biological approaches are together referred to as bottom-up strategy, while the physical approach is known as top-down approach. Green synthesis of nanoparticles is another term for the biological approach [1]. Although physical and chemical methods can produce nanomaterials at a higher rate and well controlled size and shape, but these are proved to be less desirable because they require toxic chemicals, generate a lot of biowaste, and result in significant energy and financial losses. Thus, there is a need for more affordable, biocompatible, and environmentally friendly processes to produce nanomaterials.

The greener way for NPs synthesis offers less expensive, environmentally favorable, and nontoxic methods compared to physical and chemical procedures [2]. Green synthesis of nanoparticles is a sustainable method. The two main objectives of green nanotechnology are to create nanomaterials and products that have no adverse effects on environment or human health, as well as to create nanoproducts that address environmental issues. It makes use of current green chemistry and green engineering principles to create nanomaterials and nanoproducts that don't include any hazardous elements, are made at low temperatures with minimal energy consumption whenever possible, and incorporate lifecycle thinking in every step of the design and engineering process.

Nanoparticles can be easily synthesized by microorganisms such as algae, fungi and bacteria.

8.2 Algae:

Due to their high polymeric molecule concentration, algae have ability to reduce and hyper-accumulate heavy metal ions and change them into more malleable forms. Polysaturated fatty acids, pigments, proteins, minerals and other bioactive substances like antioxidants that function as reducing, capping, and stabilizing agents are commonly found in algae extracts [1]. The salient features of algae like capacity to take in metals and reduce metal ions, low production costs, ability to produce nanoparticles at a large scale, ability to tolerate harsh atmospheric conditions more effectively than other microorganisms, make it more prevalent to use. An algae species reduces the cations collecting them to create NP. They can be produced by either an extracellular or an intracellular process from algal biomass. For biosynthesis of nanoparticles, both alive and dead biomass of algae can be used, therefore these are called bionanofactories [3].

Intracellular Synthesis: Algal biomass must be collected and carefully cleaned with distilled water before it can be used to make intracellular NPs. Following that, metallic solutions like AgNO₃ are applied to the biomass, which consists of living algae. The mixture is then incubated for certain amount of time at a predetermined temperature and PH. To generate the extracted stable nanoparticles, it is lastly centrifuged and sonicated [1].

Extracellular Synthesis: The algal biomass is extracted and washed with distilled water to being utilized in extracellular NP synthesis process. The process uses three methods:

- a) The algal biomass, or dead algae, is dried for a specific length of time. The dried powder is then cleaned with distilled water and filtered.
- b) A cell free extract is obtained by sonicating the algal biomass and distilled water.
- c) The algal biomass is rinsed with distilled water allowed to settle a few hours, and then the final product is filtered. [1]

Mechanism: Algal cell walls include groups and enzymes that combine with the precursors to create complexing agents, which reduces and deposits metal/metal oxide nanoparticles in the ambient environment [4].

Some important nanoparticles synthesized from different algal species are mentioned in Table 8.1.

Table 8.1: Nanoparticles synthesized from Algae

Algae	Metal/Metal Oxide	Reference
<i>Chlorella vulgaris</i>	Silver	[5]
<i>Dunaliella salina</i>	Silver	[6]
<i>Pithophora oedogonia</i>	Silver	[7]
<i>Arthrospira platensis</i>	Zinc oxide	[8]
<i>Sargassum muticum</i>	Zinc oxide	[9]
<i>Ulva fasciata</i>	Zinc oxide	[10]
<i>Ulva lactuca</i>	Iron	[11]
<i>Oscillatoria limnetica</i>	Iron oxide	[12]
<i>Turbinaria</i>	Copper oxide & Copper	[13]
<i>Ulva lactuca</i>	Copper	[14]
<i>Macrocystis pyrifera</i>	Copper oxide	[15]
<i>Chlorella sorokiniana</i>	Gold	[16]
<i>Galaxaura elongata</i>	Gold	[17]
<i>Prasiola crispa</i>	Gold	[18]

8.2 Fungi:

These organisms are capable of breaking down extracellular food and releasing specific enzymes to break down complex compounds into simpler ones that can be absorbed and used as an energy source [19]. Fungi are very diverse and can easily isolated, cultivated, and maintained without requiring a lot of complex equipment. They have a high secretion capacity for enzymes, which facilitates bioreduction and initiates the manufacturing of biocompatible nanoparticles. Additionally, fungi can readily hydrolyze metal ions [20].

Capacity for metal bioaccumulation and tolerance, ease of scaling up, efficient secretors of extracellular enzymes, high wall binding and intracellular metal absorption capabilities are some of the properties of fungi which make it suitable for synthesis of nanoparticles [19].

From fungi, nanoparticles can be synthesized intracellularly or extracellularly.

Intracellular synthesis: When using intracellular synthesis, the metal precursor is introduced to the mycelial culture and ingested by the biomass. As such, following synthesis, the nanoparticles are extracted using a combination of chemical treatment, centrifugation, and filtration to break up the biomass and liberate the nanoparticles [21].

Extracellular Synthesis: In the process of extracellular synthesis, free nanoparticles are formed in the dispersion when the metal precursor is added to aqueous filtrate that only contains the fungal macromolecules. Since there are no steps involved in releasing the nanoparticles from the cells, this approach is most popular. However, to remove the fungal residues and contaminants, the nanoparticle dispersion needs to be purified. This could be done by dialysis, simple filtration, membrane filtration, gel filtration and ultracentrifugation [21]. Some important nanoparticles synthesized from various fungal species are given in Table 8.2.

Table 8.2: Nanoparticles synthesized from Fungal species

Fungus species	Metal/Metal Oxide	Reference
<i>Trichoderma longibrachiatum</i>	Silver	[22]
<i>Aspergillus sydowii</i>	Silver	[23]
<i>Bjerkandera</i>	Silver	[24]
<i>Fusarium oxysporum</i>	Gold	[25]
<i>Aspergillus terreus</i>	Gold	[26]
<i>Aspergillus niger</i>	Gold	[27]
<i>Xylaria acuta</i>	Zinc oxide	[28]
<i>Aspergillus niger</i>	Zinc oxide	[29]
<i>Fusarium oxysporum</i>	Zinc oxide	[30]
<i>Aspergillus terreus</i>	Copper oxide	[31]
<i>Aspergillus niger</i>	Copper	[32]
<i>Macrophomina phaseolina</i>	Copper	[33]
<i>Penicillium oxalicum</i>	Iron	[34]
<i>Fusarium proliferatum</i>	Iron	[35]
<i>Aspergillus flavus</i>	Iron oxide	[36]

8.3 Bacteria:

Since they can effectively reduce heavy metal ions, bacteria are among the greatest options for creating nanoparticles. Bacteria are an excellent choice since they are acclimated to their own environment and can adjust to the initial transmission scenario. Certain bacterial

species have evolved the capacity to respond to external stimuli, such as the toxicity of metals or heavy metal ions, by putting in place intricate defense systems. The process of NPs biosynthesis involves cultivating microorganisms in particular nutritional mediums that contain the appropriate ions. The synthesis can be intracellular or extracellular.

Either by active transport, endocytosis, ion channels, or lipid membrane penetration, metal ions reach the bacterial cell. Capping, bioreducing, and trapping different NPs are steps in the intracellular manufacturing. The processes of bioreduction, particle capping, and enzyme secretion comprise extracellular synthesis. Extracellular synthesis is preferred over intracellular approaches due to ease of low-flow and purification or downstream and purification procedures [37].

Some bacterial mediated common nanoparticles are listed in Table 8.3.

Table 8.3: Nanoparticles synthesized from Bacterial species

Bacteria species	Metal/Metal Oxide	Reference
<i>Bacillus cereus</i>	Silver	[38]
<i>Lactobacillus</i>	Silver	[39]
<i>Escherichia coli</i>	silver	[40]
<i>Bacillus circulans</i>	Iron oxide	[41]
<i>Pseudomonas aeruginosa</i>	Iron oxide	[42]
<i>Lawsonia inermis</i>	Iron oxide	[43]
<i>Lactobacillus plantarum</i>	Zinc oxide	[44]
<i>Pseudomonas aeruginosa</i>	Zinc oxide	[45]
<i>Aeromonas hydrophila</i>	Zinc oxide	[46]
<i>Pseudomonas fluorescens</i>	Copper	[47]
<i>Pseudomonas silesiensis</i>	Copper	[48]
<i>Bacillus marisflavi</i>	Gold	[49]
<i>Pseudomonas aeruginosa</i>	Gold	[50]

Synthesis from Plant Extracts:

Plants are referred to as low maintenance, low-cost chemical factories found in nature. Due to the fact that even minute amounts of heavy metals are hazardous, plants have shown great promise for both heavy metal detoxification and accumulation, which could help solve the issue of environmental contaminants [51]. Using leftover plants or food to make nanomaterials is arguably the most fascinating and environmentally responsible method for green synthesis.

Generally, the plant or leftovers go through a procedure to extract certain chemicals from them [52]. The synthesis of nanoparticles using plant extracts has certain advantages over other biological synthesis methods, like microorganisms, since it can be accomplished through intricate processes of maintaining microbe cultures. Also, this synthesis is preferable over other biosynthetic methods including much faster kinetics. Because of the superior phytochemicals that plants produce, main sections of plants including the fruit, leaf, stem, and root have been employed extensively for green synthesis of nanoparticles [51].

There are various methods for handling and plant materials. A particular set of molecules must be extracted using each preparation technique in order to assess the physiochemical characteristics of those molecules, including their solubility and thermostability [53]. The portion of the plant that must be used for synthesis in order to create nanoparticles can be rinsed and boiled in distilled water. After squeezing, filtering and adding the appropriate solutions for the nanoparticles we wish to synthesize, the colour of the solution changes, revealing the production of the nanoparticles, which we can separate [51].

Plant extracts contain flavonoids, terpenoids, phenols, proteins, glycosides, and polysaccharides. These bioactive compounds have functional components that serve as stabilizers and reducers for precursors of nanoparticles [52].

Some important nanoparticles synthesized from plant extracts along with plant part are illustrated in the Table 8.4.

Table 8.4: Nanoparticles synthesized from plant extracts

Plant species	Plant part	Metal/Metal Oxide	Reference
<i>Catharanthus roseus</i>	Leaves	Silver	[54]
<i>Eugenia roxburghii</i>	Leaves	Silver	[55]
<i>Abelmoschus esculentus</i>	Flower	Silver	[56]
<i>Tagetes erecta</i>	Flower	Silver	[57]
<i>Rubus ellipticus</i>	Root	Silver	[58]
<i>Tectona grandis</i>	Seed	Silver	[59]
<i>Hibiscus rosa-sinensis</i>	Leaves, stem and flower	Zinc oxide	[60]
<i>Cassia fistula & Melia azedarach</i>	leaves	Zinc oxide	[61]
<i>Senecio chrysanthemoides</i>	Leaves	Zinc oxide	[62]
<i>Calendula officinalis</i>	Flower	Zinc oxide	[63]

Plant species	Plant part	Metal/Metal Oxide	Reference
<i>Nyctanthes arbor-tristis</i>	Flower	Zinc oxide	[64]
<i>Rubus fairholmianus</i>	Root	Zinc oxide	[65]
<i>Carica papaya</i>	Leaf	Iron oxide	[66]
<i>Phoenix dactylifera</i>	Leaf	Iron	[67]
<i>Hibiscus rosa-sinensis</i>	Flower	Iron oxide	[68]
<i>Punica granatum</i>	Seeds	Iron oxide	[69]
<i>Ziziphus zizyphus</i>	Leaf	Gold	[70]
<i>Moringa oleifera</i>	Leaf	Gold	[71]
<i>Mentha longifolia</i>	Leaf	Gold	[72]
<i>Jatropha integerrima</i>	Flower	Gold	[73]
<i>Aegle marmelos, Eugenia jambolana & Annona muricata</i>	Fruit	Gold	[74]
<i>Alstonia scholaris</i>	Leaf	Copper	[75]
<i>Celastrus paniculatus</i>	Leaf	Copper	[76]
<i>Krameria sp.</i>	Root	Copper	[77]
<i>Nigella sativa</i>	Seed	Copper	[78]

8.4 Mosquito Control:

Many fatal infections like malaria, dengue, filariasis, chikungunya, zika, etc are spread by mosquitoes. Thus, controlling mosquitoes is crucial to eliminate these diseases. Mosquitoes can be controlled at any stage of their life cycle-egg, larva, pupa or adult-depending on the circumstances. A promising use of nanoparticles in mosquito management has been identified. Due to their growing popularity as sustainable, eco-friendly and affordable mosquito control agent, nanoparticles have been used more frequently in recent years. These are easily synthesized using environmentally friendly techniques. Numerous studies have demonstrated the larvicidal effectiveness of nanoparticles against mosquitoes. Silver nanoparticles are most frequently employed due to their affordability and availability.

8.4.1 Ovicidal Activity:

Many nanoparticles are able to kill the eggs of the mosquitoes. Ovicidal activity of silver nanoparticles produced by extract of *Sida acuta* plant against eggs of *Anopheles stephensi* (malarial vector), *Aedes aegypti* (dengue vector), and *Culex quinquefasciatus* (filariasis vector) was studied. Egg rafts and eggs of younger age groups had a low hatchability rate after exposure to greater concentrations of the extract, while those of older age groups

exhibited a high hatchability rate when subjected to lower concentrations of the extract [79]. In a study according to percentage of ovicidal activity *Anopheles stephensi* was more vulnerable to the activity of silver nanoparticles synthesized by *Bacillus marisflavi*, followed by *Culex quinquefasciatus* and *Aedes aegypti* [80]. The ovicidal activity of biosynthesized AgNPs from *Aspergillus niger* strain at concentration of 25 and 30 ppm caused 100% egg mortality [81].

8.4.2 Larvicidal Activity:

Various nanoparticles have emerged as the effective larvicides for the mosquitoes. ZnO nanoparticles mediated by *Phaseolus vulgaris* aqueous extract exhibit greater larvicidal activity against *Aedes aegypti* and *Anopheles stephensi* in a 24-hour period than CuO nanoparticles. The investigation additionally demonstrated that *Aedes aegypti* was more susceptible to *Phaseolus vulgaris*- mediated CuO and ZnO nanoparticles at a given dose than *Anopheles stephensi*. Further, the low quantities of biosynthesized CuO and ZnO nanoparticles have larvicidal activity, making them perhaps the most cost effective, ecologically friendly, and naturally produced biocontrol agent for vector mosquitoes [82].

High concentrations of Ag nanoparticles synthesized from apple extracts have a quick effect on the population of vector mosquitoes, indicating that this synthesis may be more effective and sustainable method of vector control. The study showed that after 100 minutes, all of the larvae of *Aedes aegypti* were successfully removed by the AgNPs-RT, while the AgNPs-T took 160 minutes [83]. It was discovered that the silver nanoparticles synthesized by *Pseudomonas sp.*, *Bacillus sp.*, *Lactobacillus sp.* were extremely harmful to *Aedes aegypti* larvae in their third instar. The probit analysis of microorganism- synthesized silver nanoparticles revealed that, at 2 hours exposure, *Lactobacillus sp.* had the lowest lethal concentration to kill *Aedes aegypti* vector, followed by *Bacillus sp.* and *Pseudomonas sp.* [84]. In a study, natural larvicides, eugenol, clove essential oil, cinnamonaldehyde, and cinnamon essential oils were utilized against *Anopheles stephensi*. To increase their efficacy, nanoliposomes were created, this increased their potency by roughly 2-12-fold. The nanoliposomes were also applied as natural larvicides against other medically important mosquitoes [85].

Zinc Sulphide nanoparticles have also emerged as an approach to control *Aedes aegypti*. After being treated with ZnS nanoparticles, the larvae's bodies shrank and suffered damage, which was indicative of their morphological changes [86].

The effective killing of *Aedes aegypti* at concentration of 6.5 ppm of copper sulfide-based hybrid nanoemulsions of eucalyptus oil without harming non targeted organisms proved copper sulfide hybrid nanoemulsions as the sustainable larvicidal agent for *Aedes aegypti* [87].

8.4.3 Pupical Activity:

The nanoparticles can also kill the pupas of common mosquitoes. The silver nanoparticles, photosynthesized by aqueous leaf filtrate of *Artemisia nilagirica*, a powerful pupical agent against the development stages of *Anopheles stephensi* and *Aedes aegypti* resulted in 100% mortality of pupa of *Aedes aegypti*. It is also fast, efficient, and environmentally acceptable process for a large-scale production [88]. Silver nanoparticles synthesized using *Euphorbia hirta* have also shown effective pupical activity against *Anopheles stephensi* [89]. The silver nanoparticles produced by *Phyllanthus niruri* showed great efficacy against the pupae of *Aedes aegypti* [90].

8.4.4 Adulticidal Activity:

A few researches have been conducted to note the adulticidal activity of nanoparticles against the mosquitoes. Silver nanoparticles were produced using plant leaf extract from *Feronia elephantum*, and their adulticidal effectiveness against adult *Anopheles stephensi*, *Aedes aegypti* and *Culex quinquefasciatus* was assessed and found that for three mosquito species, the artificially created AgNPs from *F. elephantum* were far more hazardous than the unprocessed leaf aqueous extract [91]. Silver nanoparticles synthesized using bacterial strains of *Listeria monocytogenes*, *Bacillus subtilis* and *Streptomyces anulatus*, found the susceptibility of adults of *Anopheles stephensi* to the nanoparticles [92]. Adult *Aedes aegypti* were exposed to the vapor of burning AgNPs based coils produced from *Aspergillus niger* strain, which resulted in a decrease in the proportion of unfed individuals [81]. In the adulticidal experiments against *Aedes aegypti*, the silver nanoparticles synthesized from *Phyllanthus niruri* confirmed good results [90].

Gold nanoparticles synthesized from *Couroupita guianensis* showed good efficacy against the adults of *Anopheles stephensi* [93].

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