Review article on the synthesis of silver nanoparticles from plant extract and its larvicidal activity on the mosquito

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Abstract
Mosquito-vector diseases are spreading worldwide, especially in tropical and sub-tropical countries, as the weather conditions in these countries are favourable for their growth. The larvicide and mosquito repellent are usually used to control the population of the mosquitoes. But using these chemically derived insecticides has made larvae resistant to it. Green-Nanotechnology is recently gaining attention, particularly silver nanoparticles. They are synthesized to control the mosquito population, and since it is synthesised using plant extract, the risk of exposure to any toxic chemical is reduced. This review paper, discuss how silver nanoparticles are synthesized with phytoextract and its effects on the mosquito larval and pupal population. The Silver (Ag) nanoparticles show better larvicidal activity compared to the larvicidal activity of the crude plant extract. Furthermore, it is seen that the dose required to kill half the population of larvae /pupae is less in Green Ag Nanoparticles compared to plant extract.

Keywords: Silver nanoparticles, vector-borne diseases, larvicide, mosquito, green-nanotechnology

Introduction
Among arthropods, mosquitoes transmit the most diseases and are considered to be ‘public enemy no. 1’ by W.H.O [1]. Many life-threatening diseases like malaria, yellow fever, dengue fever, chikungunya, filariasis, encephalitis, West Nile virus infection are caused by mosquitoes [2, 3]. Out of 3500 species, nearly three-fourth is local to the humid tropics and subtropical regions [4]. Temperature, rainfall, and humidity are the factors that affect the development, behaviour, endurance of the mosquitoes, and the spread of disease. Mosquito repellents like N, N-diethyl-meta toluamide (DEET), dimethyl phthalate (DMP), N, N-diethyl mandelic acid amide (DEM), and mosquito insecticide are used to control the mosquito population [5]. It is well known that these repellents are synthetically synthesized, which leads to the formation of mosquito-resistant strains and harms the environment and human health since they contain organophosphates, carbamates, and pyrethroids. Later, microbial insecticides were produced, for example, Bacillus thuringiensis, as control agents, but it was seen that mosquitoes become resistant to them too. Quinine-based drugs like chloroquine and artesunate, which are antimalarial, are very effective but eventually, the mosquitoes become resistant to these drugs. Even though many drugs are developed and are effective against these diseases, the main problem is that mosquitoes become resistant to them [5]. Other methods used to control the mosquito population include Mosquitocidal and mosquito coils. Eighty plant species have been used to synthesize mosquitocide successfully [6]. Fogging for pest control is also used to kill the larvae or the adult mosquitoes. Removing the cause of the mosquito breeding sites where the water gets collected can reduce the chance of disease spreading. Other methods which are still under research are oviposition deterrents. Oviposition deterrent is when we disturb the oviposition of the mosquito. It is well-known that oviposition is essential because it involves visual, olfactory, and tactile signals [7].
The use of predators that feed on the larva and pupa is also one way. *Gambusia* and *Poecilia* have been commonly used for mosquito control [6]. Copepods like *Cyclops vernalis*, *Megacylops formosanus*, *Mesocyclops (M.) aspericornis*, *M. edax*, *M. guangxiensis*, *M. longisetus* and *M. thermocyclopoides* are seen to prey upon the mosquito instar [6].

Nanobiotechnology, especially related to metal nanoparticles, has caught attention because of its cutting edge nature and its application in practically every field of science and technology, including biomedical sciences. Metal nanoparticles are gaining attention because of their catalytic activity, optical properties, electronic properties, antimicrobial activity, and magnetic activity sites for binding [8]. Silver is one of the most commercialized metals for the production of nanoparticles, with the estimation of five hundred tons of silver nanoparticles produced in a year [9].

If we see the history, silver was used in medicine until the discovery of antibiotics in the 1940s. But as the bacteria became resistant to the antibiotics, silver again became a topic of interest. The benefit of using silver was that the microorganism didn’t become resistant to them and hence, used as an anticancer, antifungal, antiparasitic, and antimalarial agent [5].

Ag is antibacterial and toxic to cells. The Ag interacts with the macromolecules of the cell-like proteins and DNA, which break bacterial cell walls, inhibit bacterial cell growth, and disrupt cell metabolism [10].

Initially, they were synthesized using chemicals, but there was a drawback as toxic chemicals like the reducing agents and organic solvents were adsorbed on the nanoparticle's surface. This lead to unfavourable and harmful effects on its treatment. It then led to the scientists using an environmentally friendly method for its production [10].

Plants were used to synthesize Ag nanoparticles as it is faster, safer, and lighter, function at low temperature and only moderate and environmentally safe components are required. In addition, Ag nanoparticles weakens the outer membrane and breaks the plasma membrane, which causes a reduction of the intracellular ATP. As Ag has a high affinity towards sulphur and phosphorus, so molecules containing either of them are preferred for the synthesis [11].

Global scenario of diseases caused due to mosquito vector

Out of all the infectious diseases, 17% are vector-borne diseases caused by parasites, bacteria, or viruses. They cause more than 700,000 deaths a year, as reported by the World Health Organization (WHO). Anopheline mosquitoes transmit malaria, and 219 million cases are observed across the globe, with more than 400,000 deaths every year, with most deaths of children under the age of 5. Dengue, transmitted by the Aedes mosquito, affects 96 million people and causes 40,000 deaths every year [12].

Indian scenario of diseases caused due to mosquito vector

Among the South-East Asian countries, India has seen a considerable decrease in malarial cases from 20 million in 2000 to around 5.6 million in 2020. With constant control measures, malarial cases decreased by 28% and deaths by 41% in 2019. But the numbers are still disturbing. In 2019 alone, 338,000 people were affected by malaria and 175,000 people with dengue. India is on the list of the Top 11 countries highly affected by malaria. In 2020 alone, 86% of deaths due to malaria were caused in India in the South-East Asia region [13].

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**Fig 1:** Epidemiological situation of Dengue in India since 2015 (NVBDCP)
Another mosquito-transmitted disease, chikungunya, is also one of the public health concerns in India. After 1973, the virus was observed again in 2005, and there was an outbreak affecting 13 states in India. The no. of cases has increased lately, especially from 2016 to 2017. 64,057 were reported in 2016 in India alone\textsuperscript{[13]}. A study was done from January 2012 to December 2018, in which 7193 confirmed cases of malaria were reported in 22 districts of Punjab\textsuperscript{[13]}. The highest no. of cases was reported in 2013, which was 1760. In 2015, 596 cases were reported, the lowest among all\textsuperscript{[13]}. From 2012 to 2018, 58,729 cases of dengue were registered in Punjab\textsuperscript{[13]}. The numbers notably decreased in 2014 and then increased from 2015 to 2018, with a peak in 2017. Eighty-five deaths have been observed in the last six years, with maximum deaths in 2013 and 2015\textsuperscript{[13]}. Two hundred and twenty-six cases of chikungunya have been reported between 2012 to 2018 in Punjab. The maximum number of cases was observed in 2017\textsuperscript{[14]}. 

Fig 2: Epidemiological situation of Dengue in Maharashtra since 2015

Fig 3: Dengue affected areas since 1991
Epidemiological situation of Chikungunya in India and Maharashtra

The epidemiological profile of Chikungunya fever in India since 2016 indicates a declining phase of confirmed cases of Chikungunya, though suspected cases may be following randomness in the situation. In 2015: 27,533 suspected and only 3342 confirmed cases of Chikungunya. It drastically increased during 2016 with 64057 suspected and 26364 confirmed cases and then followed declining in 2017, 2018 and 2019 with corresponding 67769, 57813, 65217 suspected cases and 12548, 9756 and 9477 confirmed cases of Chikungunya (Figure 5).

The epidemiological profile of Chikungunya fever in Maharashtra State also showed a similar pattern of the declining phase of confirmed cases of Chikungunya from 2016 (Figure 6). In 2015, the suspected and confirmed cases of Chikungunya were 391 and 207, respectively. The corresponding number of suspected cases from 2016 -2019 include 7570, 8110, 9884 and 4382, whereas confirmed cases during the same period 2016-2019 were 2949, 1438, 1009 and 1378 respectively (Source: NVBDCP).
Recent Approaches in Vector Control

Effects of plant extract against the mosquito larvae

Insecticidal properties are observed in the alkaloids, flavonoids, and terpenoids. They are phytochemicals that are found in plants. Plant species, mosquito species, geographical varieties, parts used, extraction methodology used, and the polarity of the solvents used during extraction affects the larvicidal or insecticidal activity of the plant extract [2].
Table 1: Showing plant species, plant parts used for phytoextracts and its impact on mosquito species showing LC$_{50}$ and LC$_{90}$ values.

| Scientific name of plant species | Plant part | Solvent Used | Mosquito species | Larval instar | LC$_{50}$ | LC$_{90}$ | Ref. |
|---------------------------------|------------|--------------|------------------|--------------|----------|----------|------|
| **Annona squamosa**             | Leaves     | Isoamyl acetate extract | *Aedes aegypti* | I            | 10.29ppm | 31.22ppm | (15) |
|                                 |            |              |                  | II           | 10.72ppm | 32.19ppm |      |
|                                 |            |              |                  | III          | 11.09ppm | 34.14ppm |      |
|                                 |            |              |                  | IV           | 11.77ppm | 35.20ppm |      |
| **Ficus racemosa**              | Bark       | Aqueous extract | *Culex quinquefasciatus* | I            | 10.59ppm | 32.11ppm | (16) |
|                                 |            |              |                  | II           | 11.10ppm | 35.12ppm |      |
|                                 |            |              |                  | III          | 11.90ppm | 37.48ppm |      |
|                                 |            |              |                  | IV           | 12.71ppm | 42.17ppm |      |
| **Cyclamen alpinum**            | Tubers     | Ethanol extract | *Culex pipiens* | I            | 165.3ppm | 42.3ppm | (17) |
|                                 |            |              |                  | II           | 300.7ppm | 262.5ppm |      |
| **Cyclamen miracle**            | Leaves     | Aqueous extract | *Culex pipiens* | I            | 67.72mg/L | 63.7mg/L |      |
|                                 |            |              |                  | II           | 210.73mg/L | 210.73mg/L |      |
|                                 |            |              |                  | III          | 294.8ppm | 247.1ppm |      |
| **Artemisia nilagirica**         | Leaves     | Aqueous extract | *Anopheles stephensi* (24 hours) | I            | 0.722    |         | (18) |
|                                 |            |              |                  | II           | 0.122    |         |      |
|                                 |            |              |                  | III          | 0.381    |         |      |
|                                 |            |              |                  | IV           | 0.224    |         |      |
|                                 |            |              |                  | Pupa        | 0.066    |         |      |
|                                 |            |              |                  | I            | 0.622    |         |      |
|                                 |            |              |                  | II           | 0.122    |         |      |
|                                 |            |              |                  | III          | 0.519    |         |      |
|                                 |            |              |                  | IV           | 0.363    |         |      |
| **Curcuma zedoaria**            | Essential oil | Essential oil | *Culex quinquefasciatus* | I            | 165.3ppm | 42.3ppm | (19) |
|                                 |            |              |                  | II           | 300.7ppm | 262.5ppm |      |
| **Ricinus communis**            | Leaf       | Methanol extract | *Aedes aegypti* | I            | 191.54ppm | 60.68ppm | (20) |
|                                 | Seeds      |              |                  | II           | 185.74ppm | 30.33ppm |      |
|                                 |            |              |                  | III          | 215.04ppm | 43.37ppm |      |
|                                 |            |              |                  | IV           | 241.92ppm | 45.26ppm |      |
| **Moringa oleifera**            | Seeds      | Aqueous extract | *Aedes aegypti* | I            | 151.97ppm | 348.24ppm | (21) |
|                                 |            |              |                  | II           | 185.74ppm | 30.33ppm |      |
|                                 |            |              |                  | III          | 215.04ppm | 43.37ppm |      |
|                                 |            |              |                  | IV           | 241.92ppm | 45.26ppm |      |
| **Carica papaya**               | Leaf extract | Methanol crude (72hrs) | *Dengue virus type 2* | I            | 13.09µg/mL | 13.09µg/mL | (22) |
|                                 |            | Aqueous crude 30min |                  | II           | 182.10µg/mL | 182.10µg/mL |      |
| **Solanum mammosum**            | Fruit      | Aqueous extract | *Aedes aegypti* | I            | 1631.27ppm | 4756.20ppm | (23) |
| **Aquilaria sinensis**          | Essential oil | Essential oil | *Aedes albopictus* | I            | 44.23mg/L | 397mg/L | (24) |
| **Pogostemon cablin**           | Essential oil | Essential oil | *Aedes albopictus* | I            | 1215mg/L | 8762mg/L | (25) |
| **Cassia fistula**              | Fruit pulp | Fruit pulp extract | *Aedes albopictus* | I            | 1215mg/L | 8762mg/L | (25) |
|                                 |            |              |                  | II           | 1458mg/L | 11607mg/L |      |
|                                 |            |              |                  | III          | 1644mg/L | 11589mg/L |      |
|                                 |            |              |                  | IV           | 1948mg/L | 9172mg/L |      |
|                                 |            |              |                  | Pupa        | 3939mg/L | 13972mg/L |      |

DENV-2 NS5 protein

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Effect of silver nanoparticle synthesized from plant extract against mosquito larva

Ag nanoparticles are produced using different methods like physical and chemical. But it is time-consuming and toxic chemicals are used. The methods used are laser ablation, photo-chemical preparation, etc. Alkaloids, flavonoids, and terpenoids are used as phytoconstituents which help in the synthesis as they reduce Ag⁺ ions. Synthesis of Ag nanoparticles by plants is preferred because it is not expensive, environmentally friendly, easy to expand to large-scale processes, and does not need a particular technique [27, 28].

Table 2: Showing plant species, plant parts used for Green synthesis of Ag Nano particle and its impact on mosquito species showing LC₅₀ and LC₉₀ values.

| Scientific name of plant species | Plant part | AgNp synthesis using phyto extract | Mosquito species and time of exposure | Larval instar | LC₅₀ | LC₉₀ | Ref |
|---------------------------------|------------|-----------------------------------|--------------------------------------|--------------|------|------|-----|
| Polianthes tuberosa             | Fresh Bud  | Aqueous extract                    | Culex vishnui (24hours)              | III          | 8.25 ppm | 17.99 ppm | [29] |
|                                 |            |                                   | Culex quinquefasciatus (24 hours)    | IV           | 7.46 ppm | 23.26 ppm |     |
|                                 |            |                                   | DENV-2 NS5 protein (24hrs)           | III          | 9.65 ppm | 27.18 ppm | [22] |
|                                 |            |                                   |                                      | IV           | 7.94 ppm | 22.47 ppm |     |
| Carica papaya                   | Leaf       | Methanol extract                   | Dengu virus type 2 (24hrs)           | I            | 2.89 mL  |        | [30] |
|                                 |            | Aqueous extract                    |                                      | IV           | 13.40 mL |        |     |
|                                 |            |                                   |                                      | Pupa         | 25.20 mL |        |     |
| Vinca rosea                     | Leaf       | Aqueous extract                    | Aedes aegypti (24 hours)             | IV           | 4.43 µg/mL | 13.9643 µg/mL | [31] |
| Solanum mammosum                | Fruit      | Aqueous extract                    | Aedes aegypti                        | III          | 0.06ppm | 0.08ppm | [23] |
| Aquilaria sinensis              | Essential oil | Essential oil                    | Aedes albopictus                     | I            | 0.81ppm | 1.72ppm | [24] |
|                                 |            |                                   |                                      | II           | 0.83ppm | 2.33ppm |     |
|                                 |            |                                   |                                      | III          | 1.02ppm | 2.49ppm |     |
|                                 |            |                                   |                                      | IV           | 1.12ppm | 4.36ppm |     |
|                                 |            |                                   |                                      | Pupa         | 0.99ppm | 2.07ppm |     |
| Pogostemon cablin               | Essential oil | Essential oil                    | Aedes albopictus                     | I            | 0.85ppm | 1.79ppm | [24] |
|                                 |            |                                   |                                      | II           | 0.91ppm | 2.26ppm |     |
|                                 |            |                                   |                                      | III          | 1.04ppm | 2.83ppm |     |
|                                 |            |                                   |                                      | IV           | 1.19ppm | 3.43ppm |     |
|                                 |            |                                   |                                      | Pupa         | 0.84ppm | 2.13ppm |     |
| Cassia fistula                  | Fruit pulp | Fruit pulp extract                | Aedes albopictus (24 hours)          | I            | 8.3mg/L  | 51.3mg/L | [23] |
|                                 |            |                                   |                                      | II           | 9.3mg/L  | 47.3mg/L |     |
|                                 |            |                                   |                                      | III          | 12mg/L  | 56mg/L  |     |
|                                 |            |                                   |                                      | IV           | 16.5mg/L | 78mg/L  |     |
|                                 |            |                                   |                                      | Pupa         | 33.1mg/L | 519.3mg/L |     |
| Culex pipiens                   |            |                                   |                                      | I            | 1 mg/L  | 3.7mg/L | [23] |
|                                 |            |                                   |                                      | II           | 2mg/L   | 6.4mg/L |     |
|                                 |            |                                   |                                      | III          | 3.2mg/L | 10.8mg/L |     |
|                                 |            |                                   |                                      | IV           | 3.6mg/L | 20.6mg/L |     |
|                                 |            |                                   |                                      | Pupa         | 4.8mg/L | 32.7mg/L |     |
|                                 |            |                                   |                                      | I            | 1.1mg/L | 4.7mg/L | [23] |
|                                 |            |                                   |                                      | II           | 1.2mg/L | 11.6mg/L |     |
| Plant Latex | Leaf | Aqueous extract | Anopheles stephensi | Aedes aegypti | Culex vishnui | Culex quinquefasciatus | Aedes albopictus | Annona squamosa | Culex quinquefasciatus | Ficus racemosa | Annona glabra | Artemisia nilagirica |
|-------------|------|----------------|-------------------|------------|-------------|----------------------|-----------------|--------------|---------------------|-------------|---------------|------------------|
| Euphorbia milii | Plant latex | Aqueous extract | II 8.76mg/L | 17.11mg/L | 11.52mg/L | 22.75mg/L | 1.49mg/L | 1.20mg/L | 1.35mg/L | 1.02mg/L | 1.38mg/L | 1.25mg/L |
| Jatropha carcas | Plant latex | Aqueous extract | II 8.67mg/L | 17.62mg/L | 11.52mg/L | 22.75mg/L | 1.49mg/L | 1.20mg/L | 1.35mg/L | 1.02mg/L | 1.38mg/L | 1.25mg/L |
| Swietenia mahagoni | Leaf | Aqueous extract | II 8.76mg/L | 17.11mg/L | 11.52mg/L | 22.75mg/L | 1.49mg/L | 1.20mg/L | 1.35mg/L | 1.02mg/L | 1.38mg/L | 1.25mg/L |
| Cadaba indica | Leaf | Aqueous extract | II 8.76mg/L | 17.11mg/L | 11.52mg/L | 22.75mg/L | 1.49mg/L | 1.20mg/L | 1.35mg/L | 1.02mg/L | 1.38mg/L | 1.25mg/L |
| Annona squamosa | Leaves | Isoamyl acetate | II 8.76mg/L | 17.11mg/L | 11.52mg/L | 22.75mg/L | 1.49mg/L | 1.20mg/L | 1.35mg/L | 1.02mg/L | 1.38mg/L | 1.25mg/L |
| Ficus racemosa | Bark | Aqueous extract | II 8.76mg/L | 17.11mg/L | 11.52mg/L | 22.75mg/L | 1.49mg/L | 1.20mg/L | 1.35mg/L | 1.02mg/L | 1.38mg/L | 1.25mg/L |
| Annona glabra | Leaves | Aqueous extract (1:10) | II 8.76mg/L | 17.11mg/L | 11.52mg/L | 22.75mg/L | 1.49mg/L | 1.20mg/L | 1.35mg/L | 1.02mg/L | 1.38mg/L | 1.25mg/L |
| Artemisia nilagirica | Leaves | Aqueous extract | II 8.76mg/L | 17.11mg/L | 11.52mg/L | 22.75mg/L | 1.49mg/L | 1.20mg/L | 1.35mg/L | 1.02mg/L | 1.38mg/L | 1.25mg/L |
Discussion
Nanobiotechnology is gaining attention, and it can be used for various things like drug delivery, imaging, gene delivery, tissue engineering, parasitology, and pest management. Synthesis of silver nanoparticles through plant source is more favourable than the chemical and physical method because it is cheaper, single-step and does not require high pressure and temperature, and most importantly, does not use toxic chemicals, and is environmentally friendly. It has not only affected the environment but also financially to economically backward families. The medical costs for the treatment and the days of labour lost have negatively impacted the families.

In this review paper, we observed the larvicidal activity of plant extract and Ag nanoparticles synthesized from plant extract against the larva and pupa of the mosquitoes when exposed to different period of time. We can see from the results obtained by other researchers that the amount of Ag nanoparticles required to kill 50 and 90% of the larva (and in some cases, pupa) is less than the amount of the plant extract used for the same purpose. Since the amount required is less and is synthesized by plants, one doesn’t need to worry about the toxic effects and their harmful effects on the environment, animals, and humans. It hasn’t been reported that there were any non-target effects. The lethal dose used to kill the mosquito larva has a negligible impact on the aquatic environment. Not only that, but we can also observe that larvicidal activity is different for different plant species, the part of the plant used, the solvent used for extraction, time of exposure, the mosquito species. Hence, Ag nanoparticles as a biocontrol is an excellent step to keep the population of mosquitoes under control without worrying about any harmful effects on the environment and other living organisms.

Conclusion
The increase in the cases of mosquito-borne diseases led to the production of chemical insecticides, wiping out the breeding ground, or using indoor residual spraying. Later, these chemically synthesized products did more harm to the environment and living organisms. New strains were formed because these parasites became resistant to these insecticides, which led to the scientist synthesizing a new insecticide. This was a never-ending cycle. A novel idea of using silver nanoparticles as a biocontrol is an excellent step to keep the population of mosquitoes under control without worrying about any harmful effects on the environment and other living organisms.
energy to synthesize [42]. This can solve the significant problem that those chemically synthesized insecticides cause. With no harm to the environment and minimal dosage requirement, the population of the mosquitoes can be controlled [43]. But still, study needs to be done in this field to accomplish a green future.

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