Green Synthesis of Silver Nanoparticles from *Ocimum sanctum* against Mosquito Vectors for Malaria and Dengue

A.M. Aswan Ali1, M. Syed Ali1*, P.P. Vijaya1, N.Yogananth1 and M. Munees Prabu2

1PG & Research Department of Biotechnology, Mohamed Sathak College of Arts & Science, Sholinganallur, Chennai-600119, Tamilnadu, India, 2Mohamed Sathak A.J.College of Engineering, Siruseri, Chennai 600119, Tamilnadu, India.

Received April 29, 2014; accepted July 11, 2014

**ABSTRACT**

Vector-borne diseases such as malaria, filariasis, yellow fever, dengue, and Japanese encephalitis are major illnesses in tropical countries. The main objective of this study was to investigate the larvicidal activity of synthesized silver nanoparticles (AgNPs) utilizing aqueous leaf extract of *Ocimum sanctum* against fourth instar larvae of *Aedes aegypti* and *Anopheles stephensi*. The present study was carried out to establish the larvicidal activity of synthesized silver nanoparticles (AgNPs) using leaf aqueous extract of *Ocimum sanctum* against fourth instar larvae of dengue and malaria vector. The larval mortality was observed after different time of exposures. Further, characterization such as XRD and SEM analysis were carried out for the synthesized silver nanoparticles. The mortality values were obtained using the probit analysis.

**KEYWORDS:** *Aedes aegypti*; Dengue; Green synthesis; Larvicidal; Silver particles.

**Introduction**

Mosquitoes are not only the cause of nuisance by their bites but also transmit deadly diseases like malaria, filariasis, yellow fever, dengue, and Japanese encephalitis, which contribute significantly to poverty and social debility in tropical countries, causing millions of death every year (Ravikumar et al., 2011). Malaria and dengue is one of the most widespread infection diseases in the world. The disease is estimated to kill between 1.5 and 2.7 million people every year, most of them African children under 5 years of age. Synthetic insecticides have created a number of ecological problems, such as the development of resistant insect stains, ecological imbalance and harm to mammals (Perkins et al., 2011). Hence there is a constant need for developing biologically active plant materials as larvicides, which are expected to reduce the hazards to human and other organisms by minimizing the accumulation of harmful residues in the environment (Syed Ali et al., 2012a). Malaria is a potentially serious disease caused by parasites called plasmodia. Plasmodia parasites are transmitted between humans by the bite of an infected *Anopheles* mosquito, which can carry the parasites. The remains of the destroyed red blood cells clump together and cause blockages in the blood vessels. This can result in brain damage or kidney damage, which is potentially fatal (Syed Ali et al., 2013c). Dengue fever, also called dengue, is a potentially serious disease caused by a virus. There are four types of dengue virus (DENV 1, 2, 3 and 4) that can cause illness in humans. Which are common and a serious public health threat in warm sub-tropical and tropical areas of the world (WHO, 2009). The most important disease transmitting and nuisance causing mosquitoes belong to the genera *Anopheles, Culex, Aedes, Mansonia, Haemagogus, Sabithes* and *Psorophora*. In India, the various species of *Anopheles, Culex, Aedes* and *Mansonia* are important as carriers of diseases. Malaria, Filariasis, Japanese Encephalitis (JE), Dengue fever and Dengue Haemorrhagic Fever (DHF) are the major mosquito borne diseases in India (Syed Ali et al., 2012a).

Medicinal plants are resources of new drugs. It is estimated there are more than 250,000 flower plant species. Studying medicinal plants helps to understand plant toxicity and protect human and animals from natural poisons. Cultivation and preservation of medicinal plants protect biological diversity. The
medicinal effects of plants are due to metabolites especially secondary compounds produced by plant species (Sarfaraj Hussain, 2012). Among the plants known for medicinal value, the plants of genus Ocimum belonging to family Labiatae are very important for their therapeutic potentials. Ocimum sanctum L (Tulsi), O. gratissimum (Ram Tulsi), O. canum (Dulal Tulsi), O. basilicum (Ban Tulsi), O. kilimandschiricum, O. americanum, O. camphora and O. micranthum are important species of Ocimum family for medicinal value plants (Syed Ali, 2013a). Nanotechnology is research and technology development at the atomic, molecular, or macromolecular levels using a length scale of approximately one to one hundred nanometers in any dimension; the creation and use of structures, devices and systems that have novel properties and functions because of their small size; and the ability to control or manipulate matter on an atomic scale (Syed Ali et al., 2013 a). Present study made on attempted to mosquito larvicidal activity of the synthesized AgNPs from Ocimum sanctum L was carried out against 4th instar larvae of A. aegypti and A. stephensi.

Materials and Methods
Biosynthesis of silver nanoparticle
Ocimum sanctum plant was collected from Alwarthiru Nagar, Chennai, Tamil Nadu. The plant material was washed in tap water and then fresh water to remove dusts and other particles. 25 grams of leaves were taken and finely cut into small piece material was washed three times with distilled water and boiled with 100 ml of double sterile distilled water. Separate for 5mins. Reboiled extracts were filtered through whatman no.1 filter paper. The extract was stored into 4 °C for further use. 10 ml of collected filtrate was treated with 90 ml of silver nitrate aqueous solution (0.021 g of AgNO3 powder in 125 ml of double distilled water) and incubated at room temperature for 20-30 minutes, resulting in the formation of brownish black color indicating the synthesis of silver nanoparticles (Syed Ali et al., 2013a & c). The solution was centrifuged with 12,000 rpm for 20 minutes and their pellets were redispersed in sterile distilled water. The centrifugation was repeated thrice to ensure the complete separation of nanoparticles.

Larval rearing culture
To satisfy the need of enormous number of mosquitoes for the day to day bioassays, a colony is essential. The larvae of Aedes aegypti and Anopheles stephensi species were collected by sewage waste water near Mohamed Sathak College of Arts and Science. They were reared indoors at (28 ± 2) °C and 14 hr/10 hr light and dark period cycle. The larvae were fed with powdered mixture of dog biscuits and female mosquitoes were moved into a mosquito cage where the emerging adults were fed with 100 g/l sucrose solution and allowed to blood feed from white mice for 2-3 hr a few days after having a blood meal, the gravid mosquito laid their eggs (Syed Ali, 2012 a, b & c).

Larvicidal activity
The test for larvicidal effect of biosynthesized silver nanoparticle extract derived from Ocimum sanctum plant against Aedes aegypti and Anopheles stephensi was conducted in according with the WHO, 2009 standard method. 50 mg of extract was dissolved with 2 ml of DMSO. Batches of 20 early 4th instar larvae of Ae. aegypti and A. stephensi were transferred to a 50 mL enamol bowls containing various concentrations (100, 150, 200, 250, 300 and 350 mg ml-1). Each experiment was conducted with three replicates and a concurrent control group. A control group consisted of distilled water only. After treatment, symptoms of the treated larvae were observed and recorded immediately without time intervals and no food was offered to the larvae. The larvae were considered dead if, at the end of 24 hours, they showed no sign of swimming movements even after gentle touching with a glass rod, as described in the WHO’s technical report series. Subsequently, the lower concentration of crude extract that had successfully produced more than 50% larval mortality rate was used in a toxicity test on a non-target organism. The percentage of mortality was calculated by with Abbott’s formula (Fradin, 2002).

\[
\text{Percentage of mortality} = \frac{\text{Test mortality} - \text{Control mortality}}{100 - \text{Control mortality}} \times 100
\]

Characterization of biosynthesized nanoparticle
Scanning Electron Microscopic (SEM) analysis was done using AMETEK SEM machine. 1 µg of the sample powder were prepared and placed in the sputter coated on copper stub and then the images of nanoparticles were studied using SEM (JPEG, Model JFC-1600). The purified pellet was dried; mixture of silver nanoparticles was further analyzed with X-ray diffractometer (PAN analytical BV, The Netherlands) operated at a voltage of 40 kV, and a current of 30 mA, with Cu Kα radiation in an 1hr-2hr configuration. In addition, a thin film of sample was also prepared in the cover slip with 100 µl biosynthesized silver nanoparticles solution, and allowed to dry for 5 min, and the slides were analyzed with atomic force microscopy.

Statistical analysis
The average larval mortality data were subjected to probit analysis to calculate LC50, LC95 and 95% fiducial limits of upper confidence limit (UCL) and lower confidence limit (LCL), regression equation. \(R^2\) and analysis variation were calculated using software SPSS. Result with \(P < 0.05\) were considered to be statistically significantly different.

Results
In the present study made an attempt on larvicidal activity of biosynthesized silver nanoparticles from
**O. sanctum** against *Ae. aegypti* and *An. Stephensi*, *Ae. aegypti* mosquito larvae showed minimum concentration of maximum activity of LC₅₀ value 55.1 ± 7.7 l and LCL-UCL = 52.3-55.9 in 200 mg/ml. The regression equation of biosynthesized nanoparticles of *O. sanctum* extracts for ⁴th instar larvae. Moreover, The Chi-square values were significant at p ≤ 0.05 level. The statistical data R² = 0.99, R² = 0.987 and confidence limit also calculated (Table 1) respectively.

Table 2 showed minimum concentration of maximum activity level of LC₅₀ value (52 ± 4.3), LCL-UCL = 51.6-53.7 in 150 mg/ml. The regression equation of biosynthesized nanoparticles of *O. sanctum* extracts for ⁴th instar larvae *An. stephensi*. Moreover, The Chi-square values were significant at p ≤ 0.05 level. The statistical data R² = 0.89, R² = 0.987 and confidence limit. The morphology of the silver nanoparticles was determined by scanning electron microscopy.

Figure 1 showed the SEM image of bio-synthesized silver nanoparticles. From the image it can be seen that the spherical morphology of silver nanoparticles is randomly distributed with average range > 16to18 nm in diameter. Additionally, the results (Fig. 4) of the XRD analysis showed sharp peaks with various 20 intense degree values (37.10°, 47.66°, 63.97° and 70.01°) and these results are corresponds to the standard [JCPDS No.89-3722 (111), (200) and (220)] planar values, and the formation of the sharp peaks might be due to the stabilization of the synthesized nanoparticles by the reducing agents of the *O. sanctum* leaf extracts, and thus confirming the crystallization of the surface of the silver nanoparticles (Cho et al., 2005).

**TABLE 1**

Larvicidal activity of *Ocimum sanctum* against *Aedes aegypti*

<table>
<thead>
<tr>
<th>S. no.</th>
<th>Concentration (%)</th>
<th>LC₅₀(mg/ml)</th>
<th>R²</th>
<th>P ≤ 0.05</th>
<th>LCL – UCL</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>100</td>
<td>33.3 ± 7.8</td>
<td>32.2 – 35.8</td>
<td>1.89</td>
<td>2.81</td>
</tr>
<tr>
<td>2.</td>
<td>150</td>
<td>40 ± 6.3</td>
<td>39.31 – 41.5</td>
<td>1.12</td>
<td>1.89</td>
</tr>
<tr>
<td>3.</td>
<td>200</td>
<td>55.1 ± 7.7</td>
<td>52.3 – 55.9</td>
<td>0.90*</td>
<td>0.032*</td>
</tr>
<tr>
<td>4.</td>
<td>250</td>
<td>63.1 ± 3.5</td>
<td>61.21 – 63.9</td>
<td>3.81</td>
<td>1.43</td>
</tr>
<tr>
<td>5.</td>
<td>300</td>
<td>67.0 ± 9.5</td>
<td>65.1 – 68.2</td>
<td>2.91</td>
<td>2.02</td>
</tr>
<tr>
<td>6.</td>
<td>350</td>
<td>91.6 ± 78.6</td>
<td>94.2 – 92.3</td>
<td>3.12</td>
<td>4.01</td>
</tr>
</tbody>
</table>

LCL means lower confidence level and UCL-upper confidence level.

*Significant at P < 0.05 level – Analysis variation between lethal dose and concentration.

**TABLE 2**

Larvicidal activity of *Ocimum sanctum* against *Anopheles stephensi*

<table>
<thead>
<tr>
<th>S. no.</th>
<th>Concentration (%)</th>
<th>LC₅₀(mg/ml)</th>
<th>R²</th>
<th>P ≤ 0.05</th>
<th>LCL – UCL</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>100</td>
<td>42.0 ± 6.3</td>
<td>41.2 – 43.1</td>
<td>1.12</td>
<td>0.99</td>
</tr>
<tr>
<td>2.</td>
<td>150</td>
<td>52 ± 4.3</td>
<td>51.6 – 53.7</td>
<td>0.98*</td>
<td>0.05*</td>
</tr>
<tr>
<td>3.</td>
<td>200</td>
<td>67 ± 4.4</td>
<td>65.1 – 63</td>
<td>1.35</td>
<td>1.23</td>
</tr>
<tr>
<td>4.</td>
<td>250</td>
<td>68 ± 2.8</td>
<td>67.6 – 69.3</td>
<td>1.81</td>
<td>2.02</td>
</tr>
<tr>
<td>5.</td>
<td>300</td>
<td>73.0 ± 9.7</td>
<td>72.8 – 74.0</td>
<td>2.08</td>
<td>2.12</td>
</tr>
<tr>
<td>6.</td>
<td>350</td>
<td>79.6 ± 0.2</td>
<td>78.0 – 79</td>
<td>2.11</td>
<td>2.89</td>
</tr>
</tbody>
</table>

LCL means lower confidence level and UCL-upper confidence level.

*Significant at P < 0.05 level – Analysis variation between lethal dose and concentration.
Discussion

Silver nanoparticles find use in many antibacterial applications, the action of this metal on microbes is not fully known. It has been hypothesized that silver nanoparticles can cause cell lysis or inhibit cell transduction. There are various mechanisms involved in cell lysis and growth inhibition. There are many ways depicted in various literatures to synthesize silver nanoparticles. These include physical, chemical, and biological methods. The physical and chemical methods are numerous in number, and many of these methods are expensive or use toxic substances which are major factors that make them ‘not so favored’ methods of synthesis. An alternate, feasible method to synthesize silver nanoparticles is to employ biological methods of using microbes and plants (Syed Ali et al., 2013c). The use of natural product chemistry coupled with nanotechnology that reduces mosquito populations at the larval stage can provide many associated benefits to vector control. Since silver nanoparticles are considered to be potential agents for various biological applications including antimicrobial, its application as a mosquito larvicidal agent was investigated. Syed Ali et al., 2012a, the bark extract of *Avicennia marina* showed maximum larvicidal activity against the 4th instars larvae of *Ae. aegypti*, followed by the leaf extract of *Excoecaria agallocha*. Similarly, silver synthesis of nanoparticles of *O. sanctum* extract showed larvicidal activity against *Ae. aegypti* and *An. stephensi*. It reveals that, silver particles extract of *O. sanctum* showed various ranges of larvicidal activities of the maximum percentage in minimum concentration of larvicidal activity.

*O. sanctum* leaf extract showed mosquito larvicidal properties (Singh et al., 2003). *O. sanctum* (LC50 = 68.84, 81.56 and 38.39 ppm) and the methanol leaf extract of *R. nasutus* (LC50 = 68.07, 94.43 and 73.40 ppm) were highly effective against the larvae of *Ae. aegypti* and *C. quinquefasciatus*, respectively (Kamaraj et al., 2008). In this present studies support the results observed that *O. sanctum* act as potent larvicide as well as disrupting the growth of larvae. High larval mortality (80-100%) was noticed in mixture treatment, *V. negundo*, *Z. officinalis* and *O. sanctum* which may be due to the chemical constituents present in leaf and seed extracts that arrest the metabolic activities of larvae.

The mechanism which causes the death of larvae could be ability of nanoparticles to penetrate through the larval membrane. The silver nanoparticles in the intracellular space can bind to sulfur containing proteins or to phosphorus containing compounds like DNA, leading to the denaturation of some organelles and enzymes (Syed Ali et al., 2014b). Subsequently the disturbance in proton motive force causes loss of cellular function and finally cell death.

Dengue and malaria are effectively managed through a combination of vector control, drugs and management of clinical illness. There are numerous cases of insecticide resistance reported for *Aedes* and *Anopheles* species. The emergence of mosquito species resistant to insecticides, widely used in malaria and dengue control, has the potential to impact severely on the control of these disease vectors. In this present study made attempt larvicidal activities of different mosquito larvicidal extract of silver nanoparticles from *O. sanctum*.

Acknowledgements

The authors are thankful to the authorities of Mohamed Sathak College of Arts and Science, Chennai for providing required facilities.

References


**Address correspondence to:** M. Syed Ali, PG & Research department of Biotechnology, Mohamed Sathak College of Arts & Science, Sholinganallur, Chennai-600119, Tamilnadu, India.

E-mail: syedmicro555@gmail.com