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Biosynthesis Of Silver Nanoparticles From Formulated Leaf Extracts As An Effective Larvicide On Filarial Vector *Culex Quinquefasciatus*

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ABSTRACT

Phytosynthesis of transition metal silver nanoparticles is gaining importance due to their bio-compatibility, low toxicity, green approach and environment friendly nature. In the present study, silver nanoparticles synthesized from formulated aqueous leaf extracts of *Annona squamosa*, *Azadirachta indica* and *Vitex negundo* against third instar larvae of *Culex quinquefasciatus.* The reduction of silver ions occurred when the silver nitrate solution was treated with formulated aqueous leaf extract. Synthesized silver nanoparticles (AgNPs) were characterized by analyzing the excitation of surface plasmon resonance (SPR) using UV–Vis spectrophotometer at 421.5 nm. The TEM analysis confirmed the average size of the particle as 23.59 nm and are spherical in shape. The XRD spectrum showed the characteristic intense peak 38.28º which confirms that these nanoparticles are high degree of crystalline in nature. Fourier transform infrared spectroscopy (FTIR) of synthesized nanoparticles confirms the presence of alcohol, alkanes, alkenes, amine and alkyl halides compounds. The SEM micrograph of the synthesized AgNPs of aqueous formulated extract was found to be spherical, pentagonal and triangular in structures. The EDX spectrum indicates a strong silver ion signal, including carbon, oxygen, potassium and chloride, which may have raised from the bioactive molecules bound to the surface of the biosynthesized nanoparticles. EDX results exhibited the silver elemental peak at 2.5 to3 keV. In larvicidal bioassay with synthesized AgNPs, highest mortality was observed against *Cx. quinquefasciatus* with LC_{50} and LC_{90} values of 6.12 and 17.19 ppm against 3rd instars larvae. These results suggest that the synthesized AgNPs of formulated aqueous extract have the potential to be used as a supreme environment friendly compound

for the control of the mosquito larvae.

Keywords: *Annona squamosa, Azadirachta indica, Vitex negundo,* Silver Nanoparticles, Larvicidal Activity.

INTRODUCTION

Diseases transmitted by mosquitoes and other insect vectors continue to place a critical burden on the world's poor, particularly in tropical and subtropical areas (WHO, 2008). The most common diseases caused by mosquitoes were dengue fever, chikungunya, malaria, filariasis, and dengue haemorrhagic fever (DHF) (Kovendan and Murugan, 2011).

Nanoparticles have attracted considerable attention owing to their various applications particularly silver nanoparticles, which are reported to possess antifungal, anti-inflammatory and anti-viral activities (Rogers *et al*., 2008). Due to the above reasons the use of biologically synthesized silver nanoparticles for the control of *Culex* mosquito larvae will fulfill the disadvantages of the synthetic insecticides. Nanotechnology is mainly concerned with synthesis of nanoparticles of variable size, shapes, chemical composition and controlled dispersity for human benefits. The most predominant way of using nanoparticles today is made from noble metals, particularly Ag, Pt, Au and Pd. Among the four metals, silver nanoparticles play a significant role in the field of biology and medicine (Shafey, 2020).

The development of reliable green process for the synthesis of silver nanoparticles is an important aspect of current nanotechnology research. Nanoparticles play an indispensable role in drug delivery, diagnostics, imaging, sensing, gene delivery, artificial implants, and tissue engineering (Morones *et al.*, 2005).

There is a growing need to develop clean, non-toxic and environmental friendly (green chemistry) procedures to synthesis and assemble the nanoparticles. Biosynthesis of silver nanoparticlesusing plants, bacteria, fungi and yeast are known to reduce silver ions into silver nanoparticles by both extra and intra-cellular (Subashini *et al*., 2016). Synthesis of silver nanoparticles has been reported by number of methods involving chemical, physical and biological methods.

In the present investigation we have synthesized the AgNPs by using the formulated aqueous leaf extracts of *Annona squamosa*, *Azadirachta indica* and *Vitex negundo* against third instar larvae of *Culex quinquefasciatus.* The formulated leaf extract of synthesized AgNPs has the potential to be used as an ideal ecofriendly approach for the control of mosquito.

MATERIALS AND METHODS

Plant Collection and Authentication

The leaves of *Annona squamosa* (Custard apple), *Azadirachta indica* (Neem tree) and *Vitex negundo* (Chaste tree) were collected from the local area of Walajapet. The leaves were authenticated by Department of Botany, Arignar Anna Government Arts College for Women, Walajapet, Ranipet District, Tamilnadu, India.

Collection of Mosquito Larvae

The mosquito larvae were collected from cemented drains surrounding the college campus of Arignar Anna Govt. Arts College for Women, Walajapet. Third instar larvae of *Culex quinquefasciatus* species were separated and used for the experiments.

Preparation of Plant Powders

Fresh mature leaves of *A. squamosa*, *A. indica* and *V. negundo* were carefully examined and taken. Old leaves, insect damaged, fungus infected leaves were carefully removed. The fresh and healthy leaves were washed with tap water and dried in shade at room temperature (27−31 ºC) for 10 days or until they broke easily by hand. Once completely dried, each plant material was ground to a fine powder using electrical blender and each plant powder was stored at room temperature for further use (Harbone, 1973).

Formulation of Plant Extract

All the three plant powders in the ratio of 1:1:1 (each 3.33g) totally 10g was taken for the formulation (Hari and Mathew, 2018).

Bio Synthesis of Silver Nanoparticles

The formulated powder was dissolved in 100 ml of aqueous solvent and the solution was boiled for 5 minutes, cooled and filtered (Abou El-nour *et al.*, 2010). 12 ml of the formulated extract wasmixed with 88 ml of 1 mm $AgNO_3$ solution. The reaction mixture was kept at room temperature. Later the colour change (yellow to brown) was observed to designate the formation of AgNPs (Bhuvaneswari *et al.*, 2016).

Characterization of Silver Nanoparticles

The silver nanoparticles were synthesized and the absorption spectra of the reaction solution were evaluated by Ultraviolet – Visible Spectrophotometer (UV–Vis spectra). The reaction mixture was centrifuged and washed 4 to 5 times with distilled water. To identify the biomolecules, present in the synthesized nanoparticles. Fourier-Transform Infrared Spectroscopy (FTIR) analysis was carried out. The bio-reduced silver solutions have been centrifuged at 10,000 rpm for 15 minutes, and the pellets were washed three times with 20 ml of deionized water. The resulting purified suspensions have been dried and ground with KBr pellets and analysed by FTIR. The FTIR peaks were recorded in the range of 400 - 4000 cm^{-1}. The pellet has been collected and dried in a hot air oven and later subjected to XDL 3000 power X-ray Diffractometer (XRD) which is operated at a voltage of 40 kv and a current of 30 mA. The crystalline nature of air-dried silver nanoparticles was analysed by Philips CM200 with an operating voltage of 20 - 200 kv and the line resolution was 24 Aº. For Transmission Electron Microscope (TEM), the samples were prepared on carbon coated copper grids, the size and morphology of the silver nanoparticles were examined. The dried particles were scanned on a Philips Scanning Electron Microscope (SEM). The presence of elemental silver in the solution mixture was analysed by EDX analysis.

Preparation of Stock Solution for Larvicidal Activity

Stock solution (1000 ppm) was prepared by dissolving 100 mg of formulated biosynthesized AgNPs crude extract in 1 ml acetone and volume raised up to 100 ml with distilled water. From the stock solution 25, 50, 100, 200 and 300 ppm concentrations were prepared with de-chlorinated tap water (Arivoli and Tennyson, 2011).

Mosquito Larvicidal Bioassay

Mosquito larvicidal activity was carried out using biosynthesized AgNPs of aqueous formulated extract. Different concentrations 25 ppm, 50 ppm, 75 ppm, 100 ppm and 150 ppm were prepared in 200 ml of deionized water in plastic cups and to these 20 numbers of third instar larvae of *Cx. Quinquefasciatus* were released. 200 ml water containing 0.1 ml acetone with third instar larvae in a plastic cup served as control. The plastic cups were kept in room temperature. The mortality has been scored after 24 hrs. Dead larvae were removed as soon as possible in order to prevent decomposition. Each treatment was carried with three trials (WHO, 1996)

Statistical Analysis

Probit analysis was applied to get LC_{50} and LC_{90} readings using SPSS statistical data analytical tool (Version11.5).

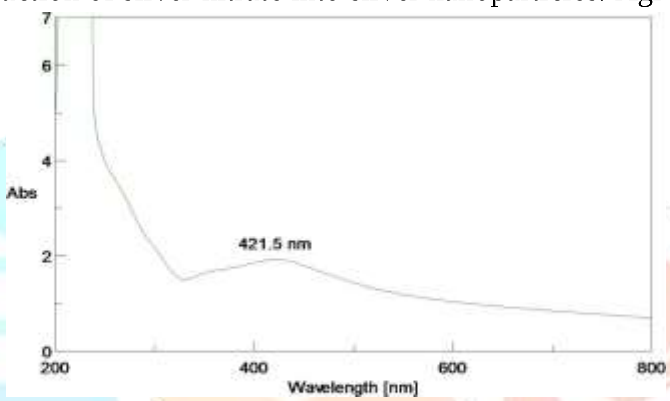
RESULTS

The present study demonstrated silver nanoparticles formation by reduction of the aqueous silver metal ions when exposed to formulated plant extracts and the colour change indicated the formation of nanoparticles. The initial colour was yellow which turned into dark brown after 30 minutes. Biosynthesis of silver nanoparticles has been noticed based on the colour change. Hence characterizations of formulated aqueous plant extract synthesized silver nanoparticles have been done by UV- Vis spectra analysis, FTIR, XRD, TEM, SEM and EDX analysis. Further larvicidal activity has been carried out against third instar larvae of *Culex quinquefasciatus.*

UV–Vis Spectra Analysis

The aqueous silver ions were reduced to silver nanoparticles when added to natural plants aqueous formulated extract. It was observed that the colour of the solution turned from yellow to dark brown in colour after 30 min from the reaction, which indicated the formation of AgNPs. This is a characteristicdue to the Surface Plasmon Resonance (SPR) vibrations of synthesized AgNPs in the reaction medium. The formation of SPR peak was influenced by various factors such as size, shape and particles formed.

The UV–Vis absorption spectrometric analysis showed absorbance peak at around 421.5 nm, suggesting bio reduction of silver nitrate into silver nanoparticles. AgNPs stability could be attributed to



capping or stabilizing agents, which contained proteins and other biomolecules presenting the extract into the reaction (Fig. 1).

Fig. 1 UV – Visible Spectrum of Aqueous Formulated Ag NPs Synthesized Plant Extract

FTIR Analysis

The FTIR analysis was carried out to identify the functional groups found on the synthesized silver nanoparticles. The FTIR spectra of synthesized silver nanoparticles using aqueous formulated extract were found to be more distinct and scattered in distribution. In the spectrum, transmittance peaks were at 3300.20, 1633.71, 1352.10, 1076.28, 1043.49 cm-1. The peak values corresponding to the presence of alcohol, alkanes, alkenes, amine and alkyl halides. The spectra absorption band assigned to the stretching vibration of O-H, C=C, C-H, C-N and C-F group respectively (Fig. 2) (Table.1).

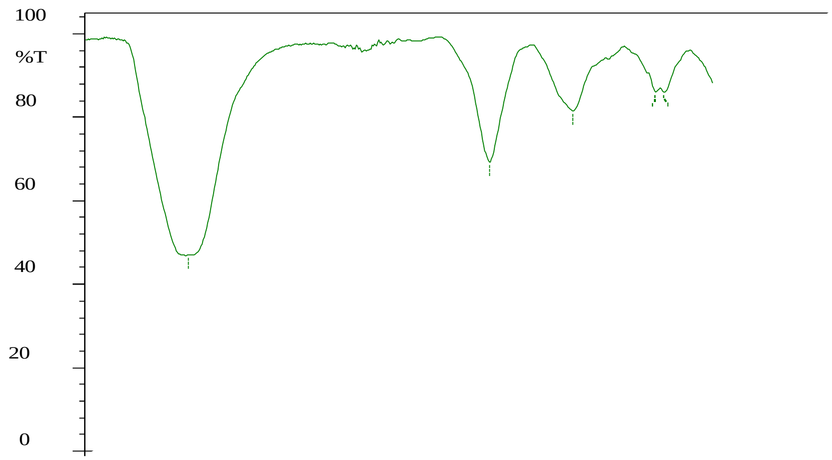


Fig. 2 FT-IR Spectrum of Aqueous Formulated Ag NPs Synthesized Plant Extract.

Table 1: FT-IR Values of Aqueous Formulated Ag NPs Synthesized Plant Extract

Wave number (cm^{-1})	Functional Group	Molecular Motion
3300.20	Alcohol	O-H Stretch
1633.71	Alkanes	C=C Stretch
1352.10	Alkenes	C-H Stretch
1076.28	Amine	C-N Stretch
1043.49	Alkyl halides	C-F Stretch

XRD Analysis

XRD analysis was employed to study the crystalline nature of the AgNPs. The XRD pattern of Ag NPs and peak values at 20 degrees of 38.28º, 44.38º, 64.49º, 77.47º and 81.75º corresponding to (111), (200), (220), (311) and (222) planes of AgNPs. All the degrees of the peaks corresponded to a Face Centered Cubic (FCC) crystalline structure. The intense peak 38.28ºrepresented a high degree of crystallinity (Fig. 3).

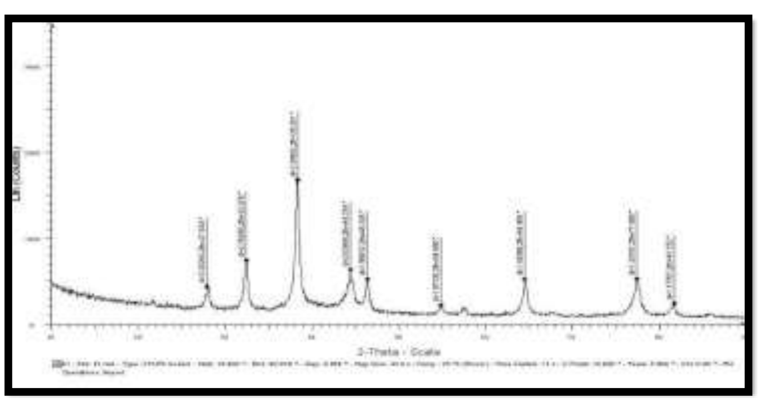


Fig. 3 XRD Spectrum of Aqueous Formulated Ag NPs Synthesized Plant Extract

TEM Analysis

TEM micrograph of synthesized Ag NPs with 10 nm and 200 nm scales. Silver nanoparticles were spherical in structure. The particle size varies from 6.78 to 33.63 nm and the average size of the AgNPs was 23.59 nm (Fig. 4).

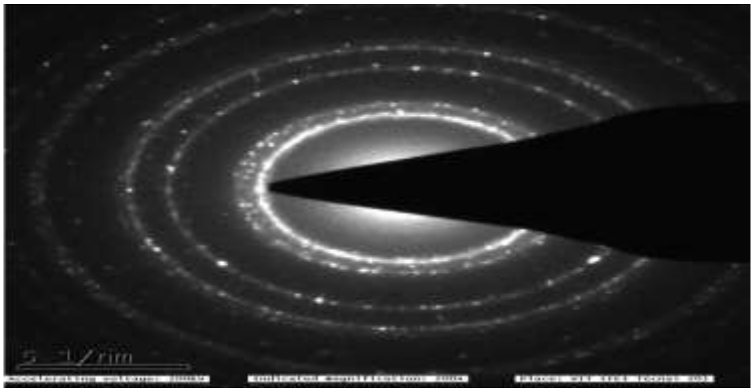


Fig. 4 TEM Image of Aqueous Formulated Ag NPs Synthesized Plant Extract

SEM Analysis

The surface morphology of the synthesized silver nanoparticles in aqueous formulated extract was determined by SEM analysis. The SEM micrograph of the synthesized AgNPs of aqueous formulated extract was found to be spherical, pentagonal and triangular in structures (Fig. 5).

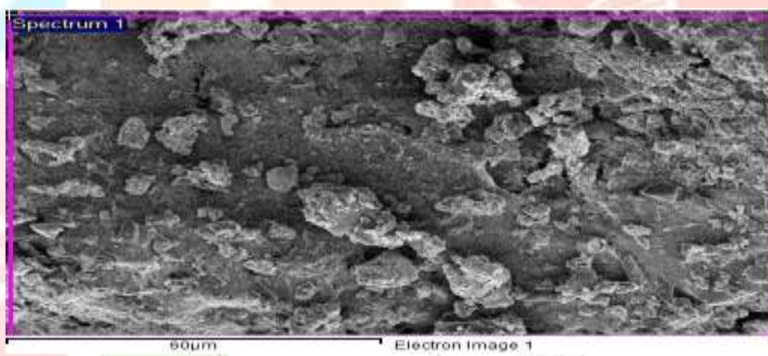


Fig. 5 SEM Image of Aqueous Formulated Ag NPs Synthesized Plant Extract

EDX Analysis

Synthesized silver nanoparticles of aqueous formulated extract were allowed to identify the presence of metallic elemental signals by EDX spectroscopy analysis. The EDX spectrum indicates a strong silver ion signal along with other weak signals, including carbon, oxygen, potassium and chloride, which may have aroused from the bioactive molecules bound to the surface of the biosynthesized nanoparticles. EDX results exhibited the silver elemental peak at 2.5 to 3 keV (Fig. 6) (Table 2).

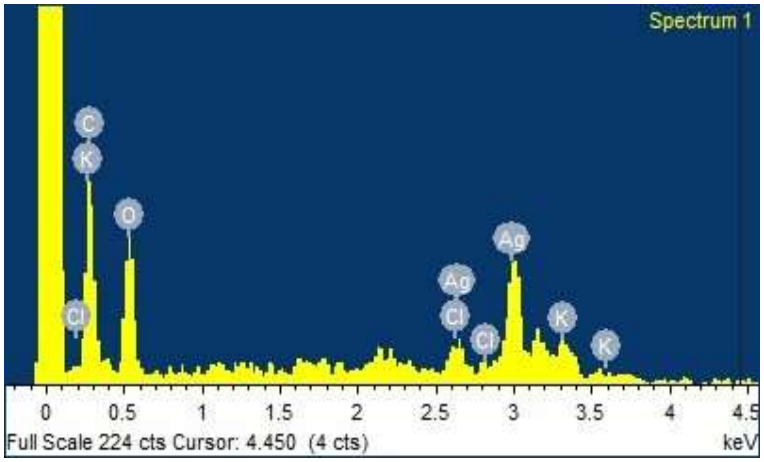


Fig. 6 EDX Spectrum of Aqueous Formulated Ag NPs Synthesized Plant Extract

Table 2: EDX Results of Aqueous Formulated Ag NPs Synthesized Plant Extract

Element	Weight %	Atomic%
C K	4.02	13.82
O K	17.91	46.26
Cl K	4.83	5.64
K K	9.23	9.75
Ag L	64.01	24.53
Total	100.00	100.00

Larvicidal Activity

Larvicidal activity has been carried out against third instar larvae of *Culex quinquefasciatus*. The mortality rate of formulated aqueous extract of synthesized AgNPs of third instar larvae of *Cx. quinquefasciatus* shows 27 ± 2.19, 48 ± 2.86, 58 ± 2.51, 91 ± 2.21, 100 ± 0.00 in the different concentrations of 25, 50, 75, 100 and 150 ppm respectively. The 100 ± 0.00 mortality was observed at 150 ppm concentration after 24 hrs (Table 3).

The lowest rate of mortality 27 ± 2.19 was observed at 25 ppm concentration after 24 hrs. The LC_{50} and LC_{90} value of formulated aqueous extract of synthesized AgNPs indicates 6.12 ppm and 17.19 ppm respectively.

Table 3: Larvicidal activity of synthesized AgNPs using aqueous formulated plant extract against third instar larvae of *Cx. quinquefasciatus* at different concentrations 25 ppm, 50 ppm, 75 ppm, 100 ppm, 150 ppm and control.

Extract	Con. (ppm)	24 h Mortality (%)	LC50 UCL-LCL (ppm)	LC90 UCL-LCL (ppm)	r^2
Synthesized AgNPs	150 100 75 50 25 Control	100 ± 0.00 91 ± 2.21 58 ± 2.51 48 ± 2.86 27 ± 2.19 0 ± 0.00	6.12 8.19-4.36	17.19 23.1-17.19	0.89

Control- nil mortality; Significant at $p < 0.05$ level; LC_{50} - Lethal concentration that kills 50% of the exposedlarvae;

LC_{90} – that kills 90% of the exposed larvae; UCL- upper confidence limit; LCL - lower confidence limit.

DISCUSSION

In the present study, aqueous silver nitrate plant extract formulated solution indicated change of colour. The appearance of dark brown colour may be due to the excitation of Surface Plasmon Resonance (SPR) of the synthesized AgNPs which have been confirmed by several researchers Rajkumar and Rahuman, 2011; Vijayaragavan *et al.*, 2012; Karuppiah and Rajmohan, 2013; Basavegowda and Rok Lee, 2014; Mansour and Robabeh 2014. According to Noginov *et al.*, 2007; Vijayakumar *et al.*, 2014; Bhuvaneswari *et al.*, 2016, the NPs nature, size and shape were very sensitive to SPR phenomenon, due to the interparticle distance and the surrounding media. In the present study UV-Vis spectrum of green synthesized AgNPs maximum peak at 421.5 nm have been observed which predicted the formation of AgNPs. Shannahan *et al.* (2013) have reported the formation of AgNPs was attributed to hydrophilic and hydrophobic interaction, which prevents the particles from aggregation by inter-molecular force.

FTIR analysis indicated clear peaks at 3300.20, 1633.71, 1352.10, 1076.28, 1043.49 cm-1. The peak values corresponded to functional groups such as alcohol, alkanes, alkenes, amine and alkyl halides confirmed their presence in AgNPs. They have been denoted as possible biomolecules responsible for stabilizing, capping and reducing agents by the AgNPs. Results of the present study is similar to the studies of Cho *et al.*, 2005; Vijayaraghavan *et al.*, 2012; Srinivasan *et al.*, 2014; and Nataya *et al.*, 2018.

The XRD analysis of synthesized AgNPs of formulated plant extract presented five major peaks (111), (200), (220), (311) and (222), in the present study that are in the Bragg's reflection for a given crystal lattice plane of silver nanocrystals. The XRD pattern with sharp Bragg's peaks confirmed the crystalline cubic nature of the AgNPs. The XRD pattern of pure silver ions is known to display peaks at 2^{θ} = 7.9º, 11.4º, 30.38º and 44º (Gong *et al.*, 2007). Similar XRD patterns of AgNPs synthesized from seed extract of *Medicago sativa* showed distinct diffraction peaks at 38.32º, 44.48º, 64.68º, and 77.64º corresponding to the respective (111), (200), (220) and (311) crystalline planes have been observed by Lukman *et al.* (2011). Murali Krishna *et al.* (2016) have been reported that this crystalline cubic nature of biosynthesized AgNPs has been reported by many researchers.

TEM micrograph of the present study visualized the shape and measured the diameter of the AgNPs. TEM micrograph indicated the AgNPs between 10 nm and 200 nm scales. The present study revealed spherical AgNPs crystals and the average size of AgNPs was 23.59 nm is in accordance with Aguilare *et al.*, 2011; Halder *et al.*, 2013; Thomas *et al.*, 2014; Soni and Prakash, 2014; Bhuvaneswari *et al.*, 2016. The average size of the AgNPs has been reported as 24 nm by Bhuvaneswari *et al.* (2016) which is in accordance to our present study.

The SEM image of synthesized AgNPs showed a spherical, pentagonal and triangular in shape, which proved the natural character of silver. This result is consistent with the shape of the SPR peak in the UV/Vis spectra. The noble metallic silver nanocrystals that mainly display a regular absorption peak around 2.5 – 3 keV, because of surface plasmon resonance. Globular shape of natural silver has been reported by Shameli *et al.*, 2012; Basu *et al.*, 2016; Moldovan *et al.*, 2018; Gaal *et al.*, 2018; Sutthanont *et al.*, 2018.

The EDX pattern of synthesized AgNPs also showed strong signals of the silver element along with weak oxygen and carbon peaks. However, the C and O elements might come from biomolecules bound to the surface of AgNPs. 2.5 – 3 keV peaks of silver element of the present study are in accordance with similar reports of Rokade *et al.*, 2017; Fouad *et al.*, 2018. Green Silver nanoparticles have been synthesized using *Azadirachta indica* (Tripathi *et al.*, 2009). Arjunan *et al.* (2012) reported the synthesized median lethal concentration (LC_{50}) of stable AgNPs using *Annona squamosa* leaf broth that killed fourth instar larvae of *Ae. aegypti*, *Cx. quinquefasciatus* and *An. stephensi*.

In the present study larvicidal activity of synthesized silver nanoparticle using formulated aqueous extract against third instar larvae of *Cx. quinquefasciatus* have been carried out. The mortality rate of formulated aqueous synthesized AgNPs against third instar larvae of *Cx. quinquefasciatus* shows 27 ± 2.19, 48 ± 2.86, 58 ± 2.51, 91 ± 2.21, 100 ± 0.00% in different concentrations of 25, 50, 75, 100 and 150 ppm respectively. The 100 ± 0.00% mortality was observed at 150 ppm concentration after 24 hrs. The lowest rate of mortality 27 ± 2.19% was observed at 25 ppm concentration after 24 hrs. The LC_{50} and LC_{90} values of formulated aqueous synthesized AgNPs indicates 6.12 ppm and 17.19 ppm respectively.

Raman *et al.* (2012) have reported biosynthesis of AgNPs using aqueous leaf extract of *Pithecellobium dulce* have showed larvicidal activity against the *Cx. quinquefasciatus*. Soni and Prakash (2014) have reported larvae of *Cx. quinquefasciatus* were found highly susceptible to the synthesized AgNPs when the aqueous extract of bark of *Cinnamon zeylanicum* was used. Marimuthu *et al.* (2011), studied larvicidal activity of synthesized AgNPs using *Mimosa pudica* leaf extract against the larvae of *Cx*. *quinquefasciatus* (LC_{50}=13.90 ppm).

Rajakumar and Rahuman (2011), reported larvicidal activity of synthesized AgNPs using aqueous extract of *Eclipta protrasta* against larvae of *Cx. quinquefasciatus* and the lethal values were LC_{50} = 4.56 mg/l and LC_{90} = 13.4 mg/l. Ramanibai and Velayutham (2016), reported highest mortality of synthesized AgNPs tested against first to fourth instar of *Ae*. *aegypti* (LC_{50} = 2.50, 2.78, 3.02, 3.05 µg/ml; LC_{90} = 8.28, 7.47, 8.13, 8.74 µg/ml) and *Cx. quinquefasciatus* (LC_{50} = 2.75, 3.00, 3.21, 3.48 µg/ml; LC_{90} = 6.45, 7.72, 11.06, 12.83 µg/ml), at 10 µg/ml, respectively. Morejon *et al.* (2018) reported highest mortality in the larvae of *Ae*. *aegypti* using the AgNPs of *Ambrosia arborescence* aqueous plant extract.

In the present study 100% mortality have been observed in the formulated aqueous synthesized AgNPs against the third instar larvae of *Cx. quinquefasciatus* after 24 hrs in 150 ppm concentration. The LC_{50} and LC_{90} values 6.12 and 17.19 µg/ml have been observed. Interestingly our present study results have been consistent with the several research reports. Toxicity of aqueous biosynthesized AgNPs showed increase in the lethality at low concentrations in comparison with plant extracts by themselves. Plant extracts individually and formulated showed highest mortality at 300 ppm concentration, when compared to the formulated aqueous biosynthesized AgNPs. Halder *et al.* (2013) and Rawani *et al.*(2013) have reported that this biosynthesized AgNPs have no toxic effect on other non-target species suchas insects and fishes suggesting only it as toxic to mosquito larvae.

The physiological basis for the toxicity of plant synthesized AgNPs has been suggested that a key factor is their ability to permeate through the invertebrate exoskeleton and penetrate into the insect's cells, where they bind macro molecules such as proteins and DNA altering the structure. Though several research have been done and going on in the green synthesized nanoparticles against mosquito larvae using different solvents keeping in mind the vector borne diseases caused by them, exact mechanism by which AgNPs induce their toxic effect on the larvae of mosquito is yet to be identified. Since, the long-term use of pesticides and insecticides have disturbed our ecosystem, scientists and industrialists were pushed to solve this problem by plant products to avoid bioaccumulation. Though the metal silver is tiny in size, long term exposure of AgNPs to the target species and non-target species have to be extensively workout for the safety of our ecosystem. The tiny size of silver, have been explained by the researchers/ scientist/ industries, not only in the biological control of insect vectors but also in drug delivery.

CONCLUSION

Vector management is one of the major issues due to the capacity of resistance against the usual insecticide. Synthesis of silver nanoparticles has the potential to be utilized as a good, eco- friendly approach for the control of mosquito population. The silver nanoparticle itself was not toxic to mosquito larvae has no mortality was seen in the control group. The augmentation of toxicity of formulated plant extracts by AgNPs to mosquito larvae is probably due to the tiny size of AgNPs. Since the biosynthesized AgNPs is globular in shape, crystalline in nature, 23.59 nm in size. Invention of nanometals using synthesized AgNPs of formulated plant extract could provide a new product to control the mosquitoes by replacing the synthetic larvicidal product.

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REFERENCES

Abou El-nour, K.M., Eftaiha, A., Al-Warthan, A., Ammar, R.A. 2010. Synthesis and applications of silver nanoparticles. *Arabian J.Chem*. 3(3):135–140.

Aguilare, M.A.M., Martin, E.S.M., Arroyo, L.O., Portillo, G.C., and Espindola, E.S.J. 2011. Synthesis and characterization of silver nanoparticles: effect on phytopathogen *Colletotrichum gloespori- oides*. *Nanopart*. *Res*. 13(6): 2525– 2532.

Arivoli, S., and Tennyson, S. 2011. Larvicidal and adult emergence inhibition activity of *Abutilon indicum* (Lin.) (Malvaceae) leaf extracts against vector mosquitoes (Diptera: Culicidae). *Journal of Biopeticides.* 4(1): 27-35.

Arjunan, N.K., Murugan, K., Rejeeth, C., Madhiyazhagan, P., and Barnard, D.R. 2012. Green Synthesis of Silver Nanoparticles for the Control of Mosquito Vectors of Malaria, Filariasis, and Dengue. *Vector-Borne and Zoonotic Dis.*12 (3): 262-268.

Basavegowda, N., and Rok Lee, Y. 2014. Synthesis of silver nanoparticles using *Satsuma mandarin* (*Citrus unshiu*) peel extract: a novel approach towards waste utilization. *Mater*. *Lett*. 109: 31–33.

Basu, S., Maji, P., and Ganguly, J. 2016. Rapid green synthesis of silver nanoparticles by aqueous extract of seeds of *Nyctanthes arbor-tristis*. *Appl. Nanosci*. 6: 1–5.

Bhuvaneswari, R., Xavier, R.J., and Arumugam, M. 2016. Larvicidal property of green synthesized silver nanoparticles against vector mosquitoes (*Anopheles stephensi* and *Aedes aegypti*). *Journal of King Saud University – Science.* 28: 318–323.

Cho, K.H., Park, J.E., Osaka, T., and Park, S.G. 2005. The study of antimicrobial activity and preservative effects of nano silver ingredient. *Electrochim. Acta.* 51: 956–960.

Fouad, H., Hongjie, L., Hosni, D., Wei, J., Abbas, G., Gaal, H., and Jianchu, M. 2018. Controlling *Aedes albopictus* and *Culex pipienspallens* using silver nanoparticles synthesized from aqueous extract of *Cassia fistula* fruit pulp and its mode of action. *Artif. Cells Nanomed. Biotechnol*. 46: 558–567.

Gaal, H., Foud, H., Mao, G., Tian, J., and Jianchu, M. 2018. Larvicidal and pupicidal evaluation of silver nanoparticles synthesized using *Aquilaria sinensis* and *Pogostemon cablin* essential oils against dengue and Zika virus vector *Aedes albopicts* mosquito and its histopathological analysis. Artif. Cells Naomed. *Biotechnol.* 46: 1171-1179.

Gong, P., Li. H., He. X., Wang, K., Hu, J., Tan, W., Zhang, S., and Yang, X. 2007. Preparation and antibacterial activity of Fe3O4 @ Ag nanoparticles. *Nanotechnology*. 18: e285604.

Haldar, K.M., Haldar, B., and 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.

Harbone, J.B. 1973. Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis. 3rd ed. Chapman and Hall London: Cambridge Univesity Press, p. 279.

Hari, I. and Mathew, N. 2018. Larvicidal activity of selected plant extract and their combinations against the mosquito vectors *Culex quinquefasciatus* and *Aedes aegypti. Environmetal Science and Pollution Research.* 25(9): 9176-9185.

Karuppiah, M., and Rajmohan, R. 2013. Green synthesis of silver nanoparticles using *Ixora coccinea* leaves extract. *Mater. Lett*. 97:141–143.

Kovendan, K., and K. Murugan. 2011. Effect of medicinal plants on the mosquito vectors from the different agro-climatic regions of Tamil Nadu, India. *Adv Environ Biol.* 5.2: 335-344.

Lukman, A. I., Gong, B., Marjo, C. E., Roessner, U., & Harris, A. T. 2011. Facile synthesis, stabilization, and anti-bacterial performance of discrete Ag nanoparticles using *Medicago sativa* seed exudates. *Journal of colloid and interface science*. 353(2): 433-444.

Mansour, G.M., and Robabeh, D. 2014. Plant mediated green synthesis and antibacterial activity of silver nanoparticles using *Crataegusdo uglasii* fruit extract. *Ind. Eng. Chem.* 20: 739–744.

Marimuthu, S., Rahuman, A. A., Rajakumar, G., Santhoshkumar, T., Kirthi, A. V., Jayaseelan, C., & Kamaraj, C. 2011. Evaluation of green synthesized silver nanoparticles against parasites. *Parasitology research*, *108*: 1541-1549.

Moldovan, B., Sincari, V., Perde-Schrepler, M., and David, L. 2018. Biosynthesis of silver nanoparticles using *Ligustrum ovalifolium* fruits and their cytotoxic effects. *Nanomaterials* (Basel). 8: 627.

Morejon, B., Pilaquinga, F., Domenech, F., Vega, D.G., Debut, A. & Neira, M. 2018. Larvicidal activity of silver nanoparticles synthesized using extracts of *Ambrosia arborescence* (Asteraceae) to control *Aedes aegypti* L. (Diptera: Culicidae). *Journal of Nanotechnology*. 2: 1-8.

Morones, J. R., Elechiguerra, J. L., Camacho, A., Holt, K., Kouri, J. B., Ramírez, J. T., & Yacaman, M. J. 2005. The bactericidal effect of silver nanoparticles. *Nanotechnology*, *16*(10), 2346.

Murali Krishna, I., Bhagavanth Reddy, G., Veerabhadram, G., and Madhusudhan, A. 2016. Eco-friendly green synthesis of silver nanoparticles using *Salmalia malabarica*: Synthesis, characterization, antimicrobial, and catalytic activity studies. *Appl. Nanosci*. 6: 681–689.

Nataya S., Attrapadung, S., and Nuchprayoon, S. 2019. Larvicidal Activity of synthesized Silver Nanoparticles from *Curcuma zedoaria* Essential Oil against *Culex quinquefasciatus*. *Insects*. 10: 27.

Noginov, M.A., Zhu, G., Bahoura, M., Adegoke, J., and Small, C. 2007. The effect of gain and absorption on surface plasmos in metalnanoparticles. *App. Phys*. 86: 455–460.

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

Raman, N., Sudharsan, S., Veerakumar, V., Pravin, N., Vithiya, N. 2012. *Pithecellobium dulce* mediated extra-cellular green synthesis of larvicidal silver nanoparticles. *Spectrochim Acta A Mol Biomol Spectrosc*. 96:1031–1037

Ramanibai, R., and Velayutham, K. 2016. Synthesis of silver nanoparticles using 3,5-Di- t-butyl-4-hydroxyanisole from *Cynodon dactylon* against *Aedes aegypti* and *culex quinquefaciatus*. *Journal of Asia-Pacific Entomology*.19: 603-609.

Rawani, A., Ghosh, A., & Chandra, G. 2013. Mosquito larvicidal and antimicrobial activity of synthesized nano-crystalline silver particles using leaves and green berry extract of *Solanum nigrum* L.(Solanaceae: Solanales). *Acta Tropica*. 128(3): 613-622.

Rogers, J.V., Parkinson, C.V., Choi, Y.W., Speshock, J.L., Hussain, S.M. 2008. A preliminary assessment of silver nanoparticles inhibition of monkey pox virus plaque formation. *Nanoscale Res Lett*. 3:129–133

Rokade, A.A., Kim, J.H., Lim, S.R., Yoo, S.I., Jin, Y.E., Park, S.S., and Novel, A. 2017. Green Synthesis of Silver Nanoparticles Using *Rubuscrata egifolius* Bge Fruit Extract. *J. Cluster Sci.* 28: 2017–2026.

Shafey, A.M.E. 2020. Green synthesis of metal and metal oxide nanoparticles from plant leaf extracts and their applications: A review. *Green Processing and Synthesis.* 9.1: 304-339.

Shameli, K., Ahmad, M.B., Zamanian, A., Sangpour, P., Shabanzadeh, P., Abdollahi, Y., and Zargar, M. 2012. Green biosynthesis of silver nanoparticles using *Curcuma longa* tuber powder. *Int. J. Nanomed*. 7: 5603–5610.

Shannahan, J.H., Lai, X., Ke, P.C., Podila, R., Brown, J.M., and Witzmann, F.A. 2013. Silver nanoparticle protein corona composition in cell culture media. *PLOS One.* 8(9): e74001.

Soni, N., and Prakash, S. 2014. Microbial synthesis of spherical nanosilver and nanogold for mosquito control. *Annals of Microbiology*. 64(3): 1099-1111.

Soni, N., and Prakash, S. 2014. Silver nanoparticles: A possibility for malarial and filarial vector control technology. *Parasitology Research*. 113(11): 4015–4022.

Soni, N., and Prakash, S., 2014. Green Nanoparticles for Mosquito Control. The *Scientific World Journal*. 496362: 6.

Srinivasan, B., Muthukumaraswamy, S., and Mohanraj, J. 2014. Biosynthesis of silver nanoparicles from mangrove plant *Avicennia marina* extract and their potential mosquito larvicidal property. *Journal of Prasit. Dis.* 40: 991-996.

Subashini, K., Jeyasankar, A., Ramesh, N., and Sivakami, R. 2016. Larvicidal activity of Silver Nanoparticle synthezed by the leaf extracts of *Azadirachta indica* against *Culex quinquefasciatus* (Say) (Diptera :Culicidae). *Int. Jrnl. of Zool. Stud.* 1(6), 07-11.

Sutthanont, N., Attrapadung, S., & Nuchprayoon, S. 2018. Chemical composition and larvicidal activity of essential oils of *Curcuma zedoaria* against the deltamethrin resistant of *Culex quinquefasicatus*. *American Journal of Trop Med & Hygiene*. 99(4): 42.

Thomas, S., John Britto, S., Mathew, S., and Mani, B. 2014. Evaluation of antibacterial potential of Silver nanoparticles (SNPS) Produced using rhizome extract of *Hedychium*. *Int. J. Pharm. Pharm. Sci*. 6 (10): 92-95.

Tripathi, A., Chandrasekaran, N., Raichur, A.M., and Mukherjee, A. 2009. Antibacterial applications of silver nanoparticles synthesized by aqueous extract of *Azadirachta indica* (Neem) leaves. *J. Biomed. Nanotechnol.* 5: 93-98.

Vijayakumar, P.P.N., Pammi, S.V.N., Pratap, K., Satyanarayana, K.V.V., and Shameem, U. 2014. Green synthesis and characterization of AgNPs using *Boerhavia diffusa* plant extract and their antibacterial activity. *Ind. Crops Prod*. 52: 562-556.

Vijayaraghavan, K.S.P., Nalini, K., Udhaya Prakash, N., and Madhankumar, D. 2012. Biomimetic synthesis of silver nanoparticles by aqueous extract of *Syzygium aromaticum*. *Mater. Let*. 75: 33-35.

WHO. 2008. WHO position statement on integrated vector management. *Weekly Epidemiol Rec*. 83: 177-181.

World Health Organization. 1996. The World Health Report, Geneva.