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Larvicidal Activity of Silver Nanoparticles synthesized by *Cymbopogon flexuous* to control *Culex quinquefaciatus* (Diptera: Culicidae)

A. Najitha Banu[1]*, Nayem ul Haq [1] and A.M. Raut[2]

School of Bio Engineering & Biosciences[1], School of Agriculture[2], Lovely Professional University, Phagwara, Punjab, India

Corresponding author E-mail: najirila2010@gmail.com

Abstract

Green synthesis of silver nanoparticles (AgNPs) using leaves extract of *Cymbopogon flexuous* (lemon grass) by reduction of silver ions to silver atom from silver nitrate solution has been investigated. The lemon grass synthesized AgNPs were characterized by using UV-vis spectra and Fourier transform infrared spectroscopy (FTIR). After 24 hrs the color change was observed at room temperature. The aqueous solution of $AgNO_3$ with Plant extract showed maximum absorbance at 400 nm. The functional group present in the AgNPs was observed through FTIR, very strong and sharp, strong, peak in the spectrum at 3564.57, 2926.11, 1685.84, 1456.30, 1341.54, 1071.49 , 695.36 are alkane, alkyl halide, esters, ethers, carbonyl and aromatic compounds. The lemon grass AgNPs showed potent larvicidal activity against third instar larvae of *Cx. quienquefasciatus* with 50% mortality at 72 hrs of post treatment.

Keywords:

Culex quinquefasciatus, UV-vis spectra, Lemongrass, green synthesis, silver nanoparticles

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Introduction

Nanotechnology, the young branch of science as a result of increase in industrialization and urbanization, there is an increase in the use of nanoparticles (NPs) and toxicity of nanoparticles rely upon the shape, size, the structure of the particle and aggregations [1-2]. Over worldwide silver nanoparticle products are increasing for the sake of industrial processes, treatments and their importance as an antimicrobial agent [3-4]. The nanoparticle enter into the environment through many ways, either naturally, for example, volcanic dust, glacial ice core or biologically like solid wastes, wastewater effluents, direct release, and accidental spillages [5].

In India, lymphatic filariasis is a general health issue consisting 33% of the total populace of the worldwide [6]. Lymphatic filariasis, caused by *Culex quinquefasciatus*, influences 120 million individuals around the whole world; also, roughly 400 million individuals are in danger of the contracting filariasis, bringing about a yearly monetary loss of US$1.5 billion [7].

The dengue cases have developed drastically in last 10 years. More than 2.5 billion individuals are in danger of dengue. WHO [8] reported there might be 50–100 million dengue cases in the world. In India around 35,066 dengue cases and 216 deaths were accounted in 2012. The most elevated quantities of dengue cases were observed in the nation from 2012 to

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2013, in Tamil Nadu, nine thousand two hundred forty-nine cases and West Bengal six thousand sixty-seven cases. National Vector Borne Disease Control Programme [9] stated, in 2017, the number increased upto 1, 57,220 in India. The present study is planned to evaluate the lemon grass synthesized silver nanoparticles toxicity against *Culex quinquefasciatus.*

Materials and Methods

Sample Collection

Lemon grass *(Cymbopogon flexuous)* was collected from Herbal Garden, Lovely Professional University Phagwara. The leaves of lemon grass were chopped into small pieces and 20 grams of chopped leaves were weighed on weighing machine. The leaves were washed with double distilled water and kept to dry on blotting paper. Leaves of *C. flexuous* (lemongrass) were transferred into dry conical flask by adding 100ml of double distilled water. The flask containing leaves of lemon grass with distilled water was placed on heating mental for 10-12 minutes at 100^{O}C. The prepared extract was permitted to cool and filter through whatman filter paper 1 into cleaned dry beaker.

Biological synthesis of silver nanoparticles using *Cymbopogon flexuous* (Lemon grass) leaves

Solution of 1mM Silver Nitrate ($AgNO_3$) was prepared using 1000ml deionized water in the conical flask that was covered with black paper and aluminum foil to prevent photo oxidation. For preparation of silver nanoparticle 1:9 ratio of prepared stock solution of $AgNO_3$ and plant extraction was mixed. This reaction was allowed to be done in dark condition to avoid photo oxidation of AgNPs. The color of $AgNO_3$ solution was crystal clear where as color of plant extract is pale yellow [10].

Purification of AgNPs

The lemon grass Nano sample was then allowed to centrifuge (Remi R-8C., Hyderabad, India) for 10 minutes at 3000 rpm; the sample was then washed thoroughly with distilled water and again centrifuged. The supernatant was then removed and the resulting pellets were dried on heating plate. Scraping of the dried sample was done by sterile blade and was kept in eppendorf tube.

UV- visible spectroscopy analysis of silver nanoparticles

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UV-vis spectroscopy is effective and accurate technique for primary characterization of synthesized nanoparticle. UV-visible spectroscopy was done by Elico double beam-SL-210 Mumbai, at LPU campus Punjab. The UV−visible range of biologically synthesized silver nanoparticles was reported as a function of wavelength using a UV−vis spectrophotometer at 300– 600 nm. Distilled water was used as a reference. Silver nanoparticles spectrum was plotted with the wavelength on the *x*-axis and absorbance on the *y*-axis in the graph

Fourier transforms infrared (FTIR) of silver nanoparticles.

FTIR was performed to identify the major functional groups of the synthesized silver nanoparticle. The technique works on the fact that a chemical substances show selective absorption in the infrared (IR) region and thus IR spectrum of chemical substance is fingerprint for its identification. FTIR measurements were carried out using a FTIR-84005, SHIMADZU, JAPAN by applying the KBr disc technique. In KBr technique solid pellet and potassium bromide are intimately mixed and then passed under high pressure in dye to form a small thick pellet, advantage of KBr pellet is it can be stored for longer period however for some polymers this method is not affluent because they are difficult to grind with KBr.

Phytochemical Analysis

Phytochemical analysis was performed to check the extract of lemon grass does contain following active principles: flavonoids, terpenoids, reducing sugars, saponin, proteins and amino acids

Test of Flavonoids:

Add 5ml of plant extract in 10ml of distilled water and add 5ml of ammonia solution and 1ml of H_2SO_4. Formation of yellow colour reveals the presence of Flavonoids.

Test for Terpenoids:

Add 8ml of plant extract with 10ml of methanol and shake it well. After filtration obtain 5ml of the solution add 2ml of chloroform and mix well then add 3ml of sulphuric acid. Configuration of brown colour indicates the presence of terpenoids.

Test for reducing sugars:

Take 5ml of plant extract with 5ml of distilled water add 1ml of ethanol and 1ml of Fehling solution A and 1ml of Fehling solution, boil the above solution for 10 minutes and

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add aqueous ethanol extract. Colored reaction indicates the presence of reducing sugars.

For Saponin:

Add 1ml of plant extract in 20ml of distilled water and shake it for 15 minutes, formation of foam layer indicates the presence of saponin.

Test for Protein and Amino acids:

Take small quantity of sample in few drops of water and treat it with Million's reagent, the occurrence of red colour indicates the presence of proteins and amino acids. When the same solution was treated with Ninhydrin, purple colour demonstrates the presence of protein and amino acids.

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Mosquito larvicidal bioassay

To check toxicity 10 larvae were added in each cup, three replicates for each test concentration as well as for control. Mosquito larvae were treated with silver nanoparticle, silver nitrate, lemon grass leaf extract and distilled water was used as control. The 16:18 light/dark was maintained with 200^{o}C temperature. Different concentrations 100ppm, 125ppm, 150ppm, 175ppm, 200ppm solution was prepared for AgNPs, $AgNO_3$ and plant sample [11].

Dose response bioassay

Biologically synthesized silver nanoparticle, $AgNO_3$, lemon grass extract exposure to mosquito larvae at different concentrations were prepared for larvicidal activity. Mortality of dead larvae were reported after 24, 48, 72 hours. All results were obtained as mean± standard deviation of the mean values.

Result and Discussion

Collection of sample

Cymbopogon flexuous (Lemon grass) was collected from herbal garden, Lovely Professional University Punjab (Figure 1) and leaves of lemon grass were used to prepare extract which was yellowish in color (Figure 2). Color change occurs in extract when mixed with solution of $AgNO_3$ which detects the preliminary conformation of formation of AgNPs. The colour change from yellow to brown was recorded in an hour after addition of $AgNO_3$. In similar manner other studies also reported the colour change [12]. Sankar *et al*., [13] also suggest the thermal factors, increase in pH and temperature help in rapid synthesis of silver nanoparticle.

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Figure 1: Plant Sample. **Figure 2:** Lemon grass extract.

Synthesis of silver nanoparticle using lemon grass

The yellow colour lemon grass extract (Figure 3a) was mixed with 1mM AgNO3 solution (Figure 3b**).** The colourless 1mM AgNO3, when added with yellow colour extract immediately colour was changed from yellow to brown which indicate the synthesis of AgNPs (Figure 4). Further confirmation was carried out by UV-Vis spectra analysis.

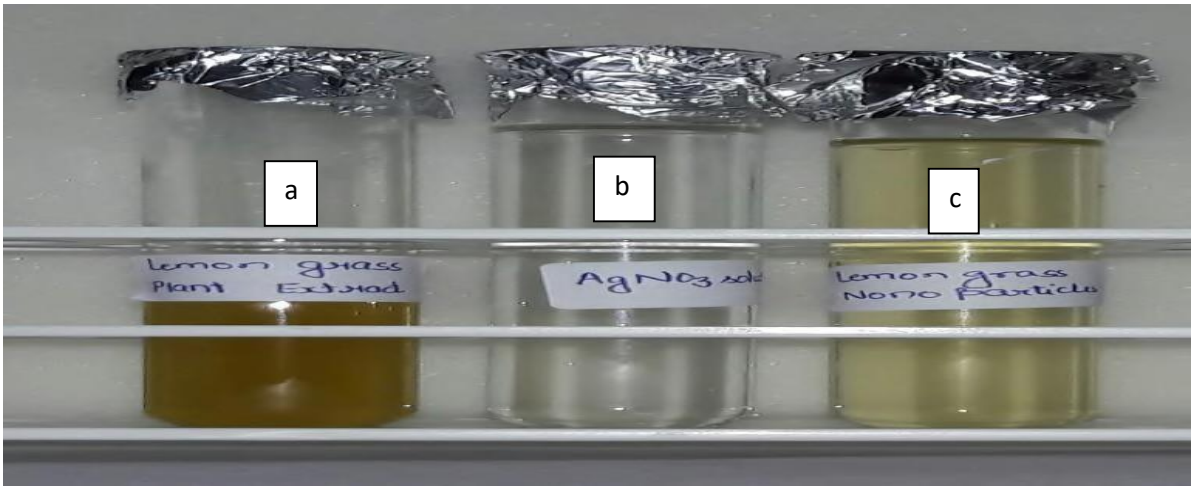

Figure 3: (a) Plant extract (b) AgNO3 (c) mixture of both lemon grass and silver nitrate.

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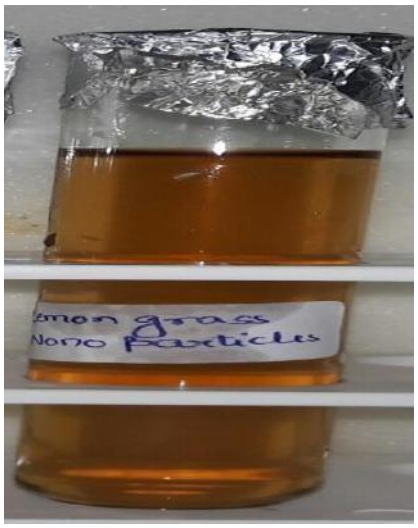


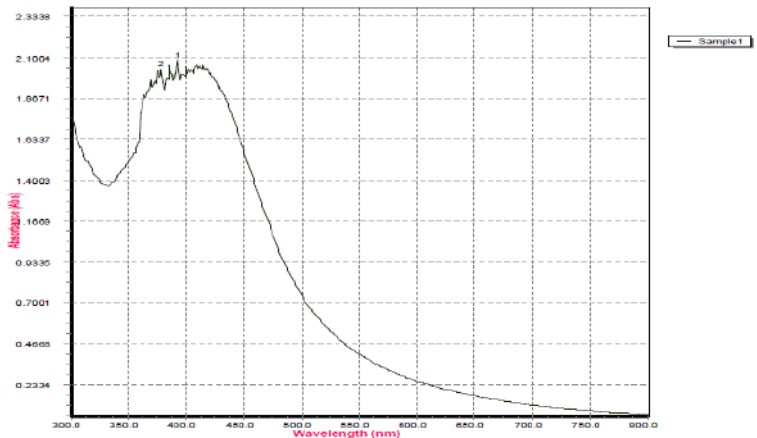
Figure 4: Colour change of plant extract on addition of AgNO3 within hour

Silver Nanoparticle Characterization

UV-Vis spectroscopy is basic technique to confirm the formation of silver nanoparticle. The presence of functional groups was determined by Fourier transform infrared spectroscopy.

UV–Visible Spectrophotometric analysis

To confirm the bio-reduction of silver ions (Ag2+) into metallic silver nanoparticles by lemongrass leaf extract, brown solution of synthesized silver nanoparticle was analyzed by UV-Vis spectroscopy. Reaction mixture showed absorbance peak at 400 nm. Surface Plasmon Resonance (SPR) peaks observed at 400nm confirmed that leaf extract has potential to reduce Ag ions into Ag nanoparticles. Premasudha *et al*., [14] suggested visualization of additional absorption peaks may be due to the presence of many participating organic compounds that



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can interact to reduce the silver ions (Figure 5)

Figure 5: Ultraviolet-visible absorption of silver nano particle synthesized from lemon grass .

Fourier transforms infrared (FTIR) of silver nanoparticles.

FTIR analysis was used to analyze the functional group present in the lemon grass. Capping and stabilizing agent present in the extract was reason for the synthesis of silver nanoparticle. FTIR spectrum resolution for synthesized silver nanoparticle revealed sharp absorbance between 500 -4000 cm^{-1}

The FTIR analysis spectrum presented sharp absorbance between 500 and 4000 cm^{-1} for the synthesized silver nanoparticles, peaks in the spectrum at 3564.57, 2926.11, 1685.84, 1456.30, 1341.54, 1071.49 , 695.36 are alkane, alkyl halide, esters, ethers, carbonyl and aromatic compounds.

The FTIR analysis spectrum show sharp absorbance between 440 and 4000 cm^{-1} for the synthesized nanoparticles. The peaks in the spectrum at 722.37, 1050.28, 1315.50, 1416.13, 1684.88, 2925.15, and 3741.06cm^{-1} are alkynes, alchol, amide, alkyl halides esters, ethers, and carbonyl or aromatic compounds. Along plant extract was not showing the visible functional group, but after synthesis of AgNPs all functional groups are highly visible in the spectra. It was clearly mentioned that these functional groups are responsible for synthesis of AgNPs (Table 1 and Figure 6).

S.NO	Vibration Type/ Functional Group	Observed Peak	Visible Intensity
1	Stretch, H-bonded O-H	3564.57	Strong, sharp
2	Stretch C-H	2926.11	Strong
3	Stretch C=O	1685.84	Strong
4	Stretch ring C=C	1456.30	Medium-strong sharp
5	Stretch C-F	1341.54	Vary sharp

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6	Sym & asym. stretch=C-O-C	1071.49	Medium small
7	Bend =C-H	695.36	Strong

Table 1: FTIR functional groups present in silver nanoparticle

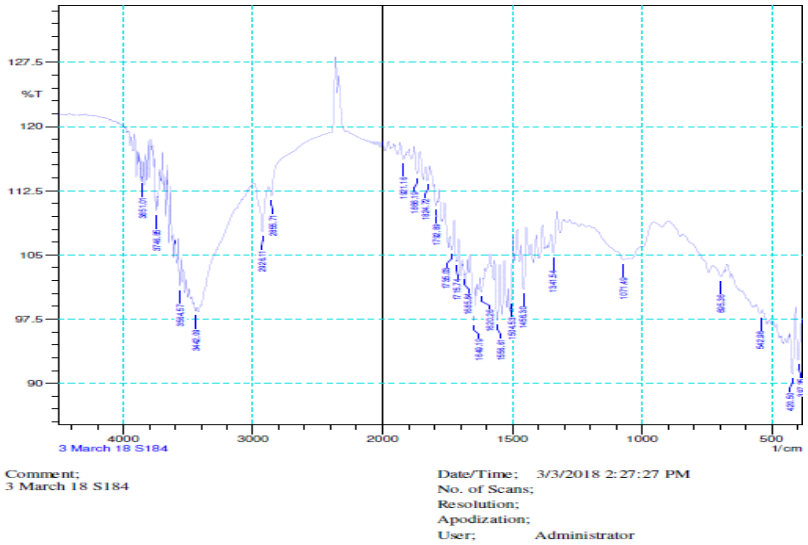

Figure 6: FTIR spectra of synthesized silver nanoparticle

The FTIR analysis spectrum show sharp absorbance between 440 and 4000 cm−1 for the synthesized nanoparticles. The peaks in the spectrum at 722.37, 1050.28, 1315.50, 1416.13, 1684.88, 2925.15, and 3741.06cm−1 are alkynes, alchol, amide, alkyl halides esters, ethers, and carbonyl or aromatic compounds. Along plant extract was not showing the visible functional group,but after synthesis of AgNPs all functional groups are highly visible in the spectra. It was clearly mentioned that these functional groups are responsible for synthesis of AgNPs. Hydroxyl and phenolic groups help in reduction and carboxyl group can help in shape directioning functionality, the result obtain by other studies also reported the same [15].

Phytochemical Analysis

The phytochemical analysis of the lemon grass showed the presence of Flavonoids,

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Terpenoids and Reducing Sugar, while there was the absence of Saponin, Proteins and Amino acids (Table 2). Phytochemical analysis of lemon grass showed the presence of flavonoids, terpenoids and reducing sugars while it showed absence of saponin, proteins and amino acids this conformed to the work of Umar *et al.*, [16] reported the presence of the phytochemicals in the lemongrass extracts.

TEST	RESULT
Flavonoid test	+
Terpenoids	+
Reducing sugar	+
Saponin	-
For protein and Amino Acids	
Ninhydrin test	-
Million's reagent test	-

Table 2: Preliminary Phytochemical screening of lemon grass

Bioassay

Toxicity effect of AgNPs, AgNO3 and lemon grass extract was observed on mosquito larvae which are target organism.

Concentration (ppm)	**Mortality (%)**		
	Plant extract	**$AgNO_3$**	**AgNPs**
100	1.33±0.57	4.00±1.00	6.66±1.54
125	4.00±1.00	6.66±1.54	8.00±1.00
150	6.66±1.15	8±1.00.00	9.33±0.57
175	8.00±1.00	9.33±0.57	14.66±0.57

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200	9.33±1.52	12.00±0.00	16.00±0.00
Control	0.00±0.00	0.00±0.00	0.00±0.00

%percentage±standard deviation

Table 3: Larvicidal activity of AgNPs, $AgNO_3$, plant extract on *Cx. quinquefasciatus* after 24 hours

Mosquito larvae treated with AgNPs, $AgNo_3$ and lemon grass extract at different concentration 100ppm, 125ppm, 150ppm, 175ppm, 200ppm respectively. In third instar larvae of *Cx. quinquefasciatus* at 100ppm 14.66% mortality was obtained at 24hrs of post treatment when treated with AgNPs, 9.33 and 5.33% mortality was obtained by treated with $AgNO_3$, plant extract respectively. There was no mortality observed in control setup. While increase in concentration from 100pmm-200pmm mortality was 13.33% in AgNPs, 26.66% in $AgNO_3$ and 22.66% in Plant extract increased (Table 3).

Concentration	**% mortality**		
in ppm	Plant Extract	$AgNO_3$	AgNPs
100	5.33±1.00	9.33±0.57	14.66±0.00
125	10.66±1.15	14.66±1.00	18.66±0.57
150	15.99±0.57	17.33±0.57	21.33±0.00
175	20.00±1.00	21.33±1.00	30.66±1.00
200	22.66±1.15	26.66±0.57	33.33±1.54
control	0.00±0.00	0.00±0.00	0.00±0.00

%percentage±standard deviation

Table 4: Larvicidal activity of AgNPs, $AgNO_3$, plant extract on *Cx. quinquefasciatus* after 48 hours

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Mortality after 48 hour period was shown in table 4. The 50.66% mortality was observed at 200ppm, 42.66%, 38.66% respect to $AgNO_3$, plant extract. In case of lower concentration like 100, 125, 150, 175ppm the mortality rate for AgNPs was 23, 31, 35, 46% respectively. Mortality is direct proportional to concentration, while concentration is increased mortality also increased.

Concentration	**% mortality**		
in ppm	Plant Extract	$AgNO_3$	AgNPs
100	13.33±1.00	18.66±0.57	23.99±0.57
125	19.99±0.57	25.32±0.57	31.99±0.57
150	27.99±1.00	29.33±0.00	35.99±0.57
175	34.66±0.57	35.99±0.57	46.66±1.00
200	38.66±1.00	42.66±0.00	50.66±0.57
Control	0.00±0.00	0.00±0.00	0.00±0.00

%percentage±standard deviation

Table 5: Larvicidal activity of AgNPs, $AgNO_3$, plant extract on *Cx. quinquefasciatus* after 72 hours

After 72 hour exposure mortality was observed at 100pm, 125ppm, 150ppm, 175pp and 200ppm concentrations by treatment with AgNPs, AgNo3,and lemon grass extract. At 100ppm 23.99% mortality was obtained in *Cx. quinquefasciatus* on exposure of AgNPs whereas 18.66% and 13.33% mortality was observed in $AgNO_3$ and plant extract respectively. However, no mortality was observed in control. While increase in concentration from 100pmm-200pmm mortality also increased from 23 to 50% (Table 5).

Mosquito larvae were exposed to AgNPs, $AgNO_3$, lemon grass extract at different concentrations and was found to exhibit mosquito larvicidal activity at different time

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intervals.Through worldwide control of mosquito is a serious concern as it is the disease carrying vector cause deadly disease like malaria and dengue. To avoid mosquito biting better strategy needs to involve at larval stage from biological source because it don't cause too much effect on non-target organism. High mortality was observed by Kalimuthu *et al.*, [17] in larvae of *Ades aegypti* on exposure of seaweed extract of *Gracilaria firma* and in our result larvae of *Culex quinquefasciatus* also showed high mortality on exposure of *Cymbopogon flexuous* mediated silver nanoparticle Result obtained by Sareen *et al*., [18] AgNPs synthesized from aqueous leaf extract of *Hibiscus rosasinensis* effectively inhibit the population of larvae of *Aedes albopictus*. AgNPs of leaf of *Pongamia pinnata* for mosquito control show moderate larvicidal effect [19].

Conclusion

In the present study, the green synthesis of silver nanoparticles using aqueous leaves extract lemon grass was attempted. The physical properties of nanoparticles were characterized using relevant techniques. Further, synthesized nanoparticles were tested against *Cx. quinquefasciatus* third instar larvae. The data represented in this study contributes to a novel approach of nano-based insecticides as an alternative to chemical pesticides. However, further studies are needed to fully characterize the morphological structure and mechanisms of synthesis of AgNPs.

Acknowledgements

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