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Biosynthesis of Silver Nanoparticles from *Allium cepa* Leaf Extract and its Larvicidal Activity against *Culex quinquefasciatus* and *Aedes aegypti*

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Abstract: Biosynthesis of silver nanoparticles was achieved by novel simple green chemistry procedure using *Allium cepa* leaf extract as a reducing and a capping agent. The present study focuses on larvicidal activity of synthesized silver nanoparticles (AgNPs) against *Culex quinquefasciatus* and *Aedes aegypti*. The synthesized AgNPs were characterized by UV-visible spectroscopy and FTIR. This revealed a peak at 414 nm in leaf extract of *A. cepa*, indicating the production of AgNPs. The FTIR spectra of AgNPs exhibited prominent peaks of organic molecules. The synthesized AgNPs from *A. cepa* were highly potent than acetone extract against all the three tested vectors. These results suggest that the synthesized Ag NPs have the potential to be used as an ideal eco-friendly approach for the control of the mosquitoes.

Keywords: *Allium cepa, AgNPs, UV, FITR, Culex quinquefasciatus, Aedes aegypti*

1. INTRODUCTION

Mosquitoes are the principal vector of many vector- borne diseases affecting human beings and other animals. In addition to nuisance, vector-borne diseases cause thousands of deaths per year. India reports 1.48 million malarial cases and about 173deaths:1.4 million suspected and 11,985 confirmed Chikungunya cases; 5,000 *Japanese encephalitis* cases and approximately 1,000 deaths; 383 dengue cases and six deaths during 2006 and 2007 (Kovendan *et al.*, 2012). Mosquito – borne diseases have an economic impact, including loss in commercial and labour output particularly in countries with tropical and subtropical climates; however no parts of the world is free from vector borne disease (Fradin and Day, 2002). Mosquitoes are the major vector for the transmission of Malaria, Dengue fever, Yellow fever, Filariasis, Chikungunya, *Schistasomiasis* and *Japanese encephalitis* (Gubler 1998). Mosquitoes also cause allergic responses in human that include local skin and systemic reactions such as angioedema (Peng et *al.*, 1999). *Culex* mosquitoes are painful and persistent biters and are responsible for filariasis. Lymphatic filariasis is a neglected tropical disease. More than 1.3 billion people in 72 countries worldwide are threatened by lymphatic filariasis, commonly known as elephantiasis. Over 120 million people are currently infected, with about 40 million disfigured and incapacitated by the disease (WHO, 2012).

Plants produce numerous chemicals, many of which have medicinal and pesticidal properties. More than 2000 plant species have been known to produce chemical factors and metabolites of value in pest control programs. Members of the plant families- *Solanaceae*, *Asteraceae*, *Cladophoraceae*, *Labiatae*, *Miliaceae*, *Oocystaceae* and *Rutaceae* have various types of larval, adulticidal or repellent activities against different species of mosquitoes(Shaalan *et al.*, 2005). In addition to the direct use of phytoextracts, in recent days, biosynthesized silver nanoparticles gain momentum as bio control agents against mosquitoes and microbes. In these circumstances, an improvised method using the biologically synthesized silver nanoparticles were evaluated for the destruction of the mosquito

larvae *Aedes albopictus*. Silver nanoparticles are emerging as one of the fastest growing material due to their unique physical, chemical and biological properties, small size and high specific surface area (Sarah *et al*., 2012).

The synthesized silver nanoparticles from *Mimosa pudica* were tested for larvicidal activity against *Anopheles subpictus* and *Culex quinquefasciatus*, which showed increased activity from the synthesized AgNPs, and it was also tested against bacteria and fungi. The formed silver nanoparticles are highly stable and had significant mosquito larvicidal activity (Marimuthu *et al*., 2011). A green approach for the fabrication of stable, bioactive silver nanoparticles using *Hibiscus rosasinensis* leaf extract has been reported. Biologically synthesized silver nanoparticles of *Tinospora cordifolia* were found to produce a high pediculocidal and larvicidal activity. There was no more reports on synthesized AgNPs and its larvididal activity in the literature (Jayaseelan *et al.,* 2011). Vivek *et al*. (2011) have reported the synthesis of bionanoparticles using *Sargassum wightii, Kappaphycus alvarezii* and *Gelidiella acerosa* crude extract respectively. Seaweeds have various phytochemicals including Carbohydrates, Alkaoids, Steroids, Phenols, Saponins and Flavonoids act as a reducing agents of silver. A rapid reduction of the silver ions occurred when the silver nitrate solution was contacted with geranium leaf extract. Studies have indicated that biomolecules like protein, phenols and falvonoids not only play in reducing the ions to the nano sizes, but also play an important role in the capping of the nanoparticles (Raja *et al*., 2012). Thus it has being expensively exploited for these properties, here in the present study we have used the plant source *Allium cepa* and AgNPs on Larvicidal against *Ae. aegypti* and *Cx. Quinquefasciatus*.

2. MATERIALS AND METHODS

2.1 Study material: *Allium cepa*, a leaf extract (Fig.1) was chosen for the present study.

2.2 Sample collection: *Allium cepa* was reared in the Botanical garden of Vivekananda College up to flowering stage. The leaves were removed and it was washed with tap water and rinsed with distilled water. The cleaned leaf material was dried in shade at room temperature and stored for further use.

Fig.1. Experimental plant *Allium cepa*

2.3 Preparation of acetone extract: The dried leaves were used to prepare the extract adopting the procedure described by Satyavani *et al*. (2011). 25gms of the dried material was powdered mechanically using electrical stainless steel blender. The powder was mixed with 250ml acetone and boiled (boiling point range 55.5-56.5^0 C) in Soxhlet apparatus for 8hrs. The extract collected was stored at 4^{0}C for further use.

2.4 Synthesis and purification of silver nanoparticles: For synthesis of silver nanoparticles, 10ml acetone extract of *Allium cepa* was added to 90ml 1mM solution of silver nitrate in 250ml conical flask and kept at room temperature for 1hr. The primary detection of synthesized silver nanoparticle was carried out in the reaction mixure by observing the colour change of the medium. The silver nanoparticle solution thus obtained was purified by repeated centrifugation at 5,000rpm for 20mts. The supernatant was discarded and the pellet was dissolved in double distilled water. The silver nanoparticles were confirmed by colour change (Satyavani *et al*., 2011).

2.5 Characterization of silver nanoparticles: The produced nanoparticles were subjected to UV-Vis spectroscopy analysis, and Fourier Transform Infrared Radiation (FTIR) spectroscopy analysis at Madurai Kamaraj University.

2.5.1 UV-Vis absorbance spectroscopy analysis: The samples used for analysis were diluted with 2ml of double distilled water and subsequently measured by the UV-Vis spectroscopy at regular time intervals by using a quartz cuvette with water as a

reference (Rajesh *et al.*, 2009), UV-Vis spectroscopy analysis of silver nanoparticles produced were carried out as a function of bioreduction time at room temperature on ELICO spectrophotometer at a resolution of 1nm. The UV-Vis spectrometric readings were recorded at a scanning speed of 200-800nm.

2.5.2 Fourier transform infrared radiation spectroscopy analysis: To remove free biomass residue (or) compound that is not the capping ligand of the nanoparticles, the residue solution of 100ml after reaction was centrifuged at 5,000rpm for 10mts. The supernatant was again centrifuged at 10,000rpm or 60mts, and the pellet was obtained. This was followed by redispersal of silver nanoparticle pellets into 1 ml deionized water. Thereafter, the purified suspension was freeze dried to obtain dried powder. Finally, the dried silver nanoparticles were analyzed by FTIR analysis (Vivek *et al.*, 2011).

2.6 Larvicidal bioassay: The eggs and egg rafts of *Ae .aegypti* and *Cx. quinquefasciatus* were procured from Centre for Research in Medical Entomology (ICMR), Madurai, India. The larvae were kept in enamel tray containing distilled water. They were maintained and reared in the laboratory as per method adopted by Kamaraj *et al.* (2009) and all experiments were carried out at room temperature. Larvae were fed with yeast powder and dog biscuits. The larvicidal activity was assessed by following the procedure of WHO (1996) with modifications as per the method described by Rahuman *et al.* (2000). Twenty five number of 3rd instar larvae of *Ae. aegypti* and *Cx. Quinque-fasciatus* were transferred separately from culture being maintained in the laboratory to the 250 ml beaker containing the 100 ml of desired concentration of plant extracts and AgNPs. The control was set up with dechlorinated tap water. The Moribund larvae were counted after 24h of exposure and the percentage mortality was recorded for the average of four replicates.

2.6.1 Statistical analysis: Statistical analysis such as LC_{50}, 95% confidential limit and chi square values were calculated by using EPA Probit analysis programme version 1.5.

3. RESULTS

3.1 Biosynthesis of Silver Nanoparticles: The change in colour was noted by visual observation in the silver nitrate solution incubated with *Allium cepa* leaf extract (Fig.2).

Fig.2. Colour change during the bioreduction of $AgNO_3$ into AgNPs using plant extract: before synthesis (left) and after synthesis (right).

The colour of the extract was changed to reddish brown in 1hrs at 37^0C due to the formation of silver nanoparticle by the reduction of the silver metal ions by the extract of *Alliu cepa*. Control sample silver nitrate without *Allium cepa* extract did not shown any change in colour.

3.2 UV-Vis spectroscopy analysis: The reduced silver was subjected to analysis by the UV-Vis spectrophotometer. Absorption spectra of AgNPs formed in the reaction media has absorbance peak at 414 nm (Fig.3).

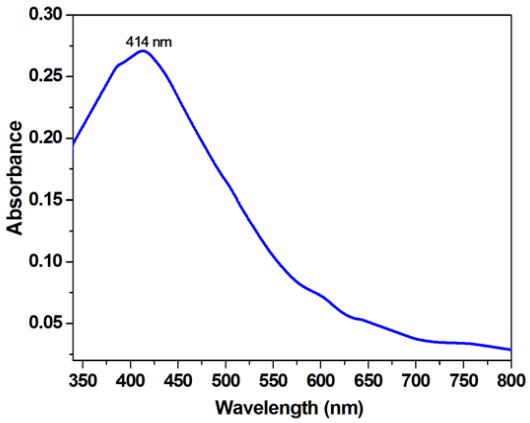

Fig 3. UV-Vis spectra of silver nanoparticles synthesized by treating *Allium cepa* leaf extract with 1mM silver nitrate solution.

Broadening of peak indicated that the particles are polydispersed. The frequency and width of the surface Plasmon absorption depend on the size and shape of the metal nanoparticles as well as the dielectric constant of metal itself and surrounding medium.

3.3 Fourier transform infrared radiation spectroscopy analysis: FTIR measurement was carried out to identify the possible biomolecules responsible for the reduction of silver ions and capping agent of bio reduced silver nanoparticles by *Allium cepa*. The FTIR spectrum of synthesized silver nanoparticles shows peak at 3435cm-1, 2085cm-1, 1639cm-1, 1369cm-1, 1226cm-1(Fig 4).

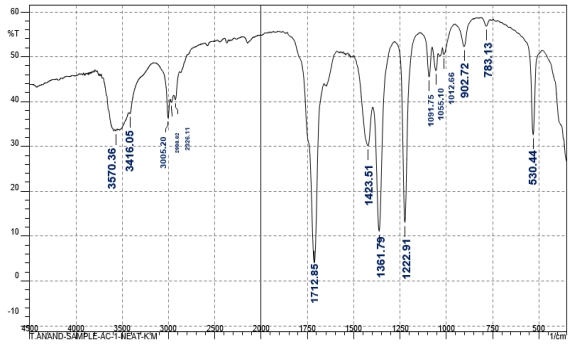

Fig 4. FTIR spectra silver nanoparticles synthesized using *A. cepa* leaf extract with 1mM silver nitrate solution

The peak at 3435cm-1 shows the presence of hydrogen bonded hydroxyl (-OH) group. The peak at 2085cm-1 indicates the presence of benzene rings. The peak at 1639cm-1 shows the presence of C=O stretching bands of the carboxylic acid. The peaks at 532cm-1 indicate the presence of alkyl halides. These records indicate the presence of silver ions formed due to the bioreduction and their stabilization as nanoparticles. The results revealed that the capping ligand of the AgNPs may be hydroxyl (-OH), Benzene ring, carboxylic acid (C=O) and alkyl halides.

3.4 Larvicidal activity of leaf extract and synthesized silver nanoparticles: In the present study was carried out to establish the larvicidal activity of synthesized silver nanoparticles (AgNPs) using leaf extract of Allium cepa against third instar larvae of *Ae. aegypti and Cx. quinquefasciatus*. Table 1 reveals that the LC_{50} values for *Allium cepa* leaf extract and silver nanoparticles as 11.716, 8.088ppm and 3.631, 1.442ppm of *Ae. aegypti* and *Cx. quinquefasciatus* respectively. Chi-square value was significant at p<- 0.05 level.

Table1: Larvicidal activity of leaf extract of *Allium cepa* against *Ae.aegypti*

Time Duration	LC50 value(ppm)	95% Confidential limit		Chi-square χ^2	S.E	Slope
		LCL	UCL			
24 hours	79.59	68.69	92.22	3.07	0.536	2.873
48 hours	65.78	39.01	110.94	18.19	0.853	2.911
72 hours	43.18	22.39	83.29	31.82	0.818	2.691

Table2: Larvicidal activity of leaf extract synthesized silver nanoparticles of *Allium cepa* against *Ae.aegypti*

Time Duration	LC50 value(ppm)	95% Confidential limit		Chi-square χ^2	S.E	Slope
		LCL	UCL			
24 hours	3.379	1.730	6.602	33.57	0.855	2.701
48 hours	2.494	2.139	2.909	5.876	0.473	3.823
72 hours	1.866	0.940	3.703	31.69	0.833	2.569

Table3: Larvicidal activity of leaf extract of *Allium cepa* against *Cx. Quinquefasciatus*

Time Duration	LC50 value(ppm)	95% Confidential limit		Chi-square χ^2	S.E	Slope
		LCL	UCL			
24 hours	24.55	18.64	32.32	9.919	0.880	2.121
48 hours	20.23	15.08	27.15	9.441	0.871	2.135
72 hours	16.86	15.10	18.83	6.034	0.557	2.067

LCL – Lower Confidential Limit UCL – Upper Confidential Limit

Table 4: Larvicidal activity of leaf extract synthesized silver nanoparticles of *Allium cepa* against Cx. *Quinquefasciatus*

Time Duration	LC50 value(ppm)	95% Confidential limit		Chi-square χ^2	S.E	Slope
		LCL	UCL			
24 hours	0.410	0.255	0.658	20.20	0.874	2.494
48 hours	0.256	0.161	0.406	19.88	0.872	2.289
72 hours	0.110	4.613	0.264	9.888	0.882	3.292

LCL – Lower Confidential Limit UCL – Upper Confidential Limit

4. DISCUSSION

Silver has been used for many years for its antimicrobial properties. Alexander the Great used silver vessel to store drinking water (Silver *et al.*, 2006). However, the formulation of silver has changed during antiquity from bulk silver to ionic silver (Kwakye-Awwah *et al.*, 2008). The broad spectrum antimicrobial properties of silver nanoparticles encourage its use in a large number of biomedical and environment applications as well as in growing list cosmetics, clothing, and numerous consumer products (Jain *et al.*, 2011).

Metal nanoparticles can be synthesized by reducing metal ion using some chemicals. In biosynthesis, it is believed that extracts of natural materials act as reducing agent for generation of metal nanoparticles (Monavallil *et al.*, 2010). Previous studies have assured that the reduction of the silver ions into silver nanoparticles during exposure to the sea weed extracts of *Gelidiella* sp could be followed by colour change. AgNPs exhibit dark yellowish brown colour in solution. Absorption spectra of silver nanoparticles formed in reaction media at 72hrs (Bhima *et al.*, 2012). The present study demonstrated the formation of the silver nanoparticles by the reduction of the aqueous silver metal ions during exposure to the extract of *Allium cepa*. The reaction of ions occurred within 1hr at 37^0 C and appearance of reddish brown colour from colourless solution.

Silver nanoparticles synthesized by using *Allium cepa* were formed at 414 nm with polydispersed. UV-Vis spectroscopy is one of the most widely used techniques for structural characterization of silver nanoparticles (Sun *et al.*, 2001). Generally, UV-VIS spectroscopy can be used to examine size and shape of the controlled nano particles in aqueous suspense. The results of the UV-VIS absorption showed increasing colour intensity with increased time intervals and this might be due to the production of the silver nanoparticle (Shrivastava *et al.*, 2010) and the formation of the brownish yellow colour might be due to the excitation of the surface plasmon vibration of the synthesized silver nano particles (Krishnaraj *et al.*, 2010).The broadness of the peak is a good indicator of the size of the nanoparticles. As the particle size increases the peak becomes narrower with a decreased bandwidth (Kong and Jang, 2006). Jain *et al.* (2011) have reported that the absorption spectra of silver nanoparticles were highly symmetric single band absorption with peak at 421nm.

FTIR analysis was carried out to identify the possible interactions between silver and bioactive molecules, which may be responsible for synthesis and stabilization (capping) of silver nanoparticles. The intensed peak 3435^{cm-1}, 2085^{cm-1}, 1639^{cm-1}, 1369^{cm-1}, $1226^{cm-1}$and 532^{cm-1} indicated the presence of hydroxyl (OH) group, benzene ring, carboxylic (C=O) group, alkyl halide group respectively. The results of the FTIR used to identify the possible bio molecules responsible for the stabilization of the synthesised silver nanoparticles. The prominent peaks of the FTIR results are showing the correspond values to the amide group (N-H stretching- 3435), alkane group (CH-2085) alkene (CC- 1639, 1369, 1226 and 532) and ether groups (COC-1 031.73). The observed peaks are considered such as flavonoids, triterpenoids and polyphenols (Asmathunisha *et al.*, 2010). Hence, the terpenoids are proved to have good potential activity to convert the aldehyde groups to carboxylic acids in the metal ions. Further, amide groups are also responsible for the presence of the enzymes and these enzymes are responsible for the reduction synthesis and stabilization of the metal ions, further, polyphenols are also proved to have potential reducing agent in the synthesis of

the silver nanoparticles (Mukunthan *et al.,* 2011).

In present investigation, third instar larvae of *Ae. aegypti* and *Cx. Quinque-fasciatus* were treated with biosynthesized silver nanoparticles and the percent mortality was assessed against various concentrations ranging from 1- 5ppm. The LC_{50} value of synthesized silver nanoparticles was 3.379 and 0.410ppm. The mechanism which causes the death of the larvae could be explained due to the nanoparticles can enter through oral cavity as well as body membrane of the Mosquito larvae. These AgNPs in the intracellular space can bind to sulphur containing proteins or to phosphorus containing compounds like DNA, leading to the denaturation of some organelles and enzymes, subsequently, the decrease in membrane permeability and disturbance in proton motive force causes loss of cellular function and finally cell death. Moreover in the present case, it is evident from the GC-MS spectra that the active compounds Para-coumaric acid ,Para-hydroxybenzoic acid, Propionaldehyde, Protocatechuic acid, Raffinose and Sinapic acid which are having high larvicidal activity are also contributing towards the high mortality rates with less concentration of AgNPs. Sakulku *et al.* (2009) have reported the low release rate of nanoemulsion with large droplets size that resulted in prolonged mosquito repellent activity compared to the nanoemulsion with small droplets.

In the present study acetone extract of *Allium cepa* was tested for its efficiency against third instar larvae of *Ae. aegypti* and *Cx. quinquefasciatus*. The larvae were subjected to different concentrations (20-100ppm) of the leaf extract and 79.596 and 24.551 ppm was recorded as LC50 value respectively for *Ae. aegypti* and *Cx. quinquefasciatus*. Similarly the effect of marine cleome *Lobophora variegate* was highly potential and showed LD50 value of 70.38 and 95.52 ug ML-1 on the second instar larvae of *Ae. aegypti* was studied by Manilal *et al.*, (2011). Aqueous (Physiological saline) extract of seed kernel from soap nut *Sapindus emarginatus* (Sapindaceae) was found to exhibit, a strong anti-mosquito activity as evident from its ability to inflict 100% mortality of all the developmental stage of *Ae. aegypti* (Koodalingam *et al.*, 2009).

In the present study, the biosynthesised silver nanoparticles from leaf extract of *Allium cepa* showed potential larvicidal activity against *Ae. aegypti* and *Cx. quinquefasciatus* larvae. Hence, the larvicidal activity of the silver nanoparticles might be due to the denaturation of the sulfur-containing proteins or phosphorous containing compound like DNA that, leads to the denaturation of organelles and enzymes (Choi *et al.,* 2008) and thus reduces the cellular membrane permeability and reduction in ATP synthesis which finally causes the loss of the cellular function and cell death (*Sap-Iam et al.,* 2010).

The size and shape of nanoparticles plays an important role in many of the pharmaceutical, industrial and biological applications. The formed silver nanoparticles are characterized by UV-Vis and FTIR analysis; these silver nanoparticles are highly stable and had significant mosquito larvicidal activity against the third instar larvae of *Ae. aegypti* and *Cx. quinquefasciatus*.

5. CONCLUSION

Today, environmental safety is considered to be of paramount importance. An insecticide does not need to cause high mortality on target organisms in order to be acceptable but should be eco-friedly in nature. Phytochemicals may serve as these are relatively safe, inexpensive and readily available in many parts of the world. Several plants are used in traditional medicines for the mosquito larvicidal activities in many parts of the world. According to Bowers *et al* (1995), the screening of locally available medicinal plants for mosquito control would generate local employment, reduce dependence on expensive and imported products, and stimulate local efforts to enhance the public health system. The ethno-pharmacological approaches used in the search of new bioactive toxins from plants appear to be predictive compared to the random screening approach. The recently developed new isolation techniques and chemical characterization through different types of spectroscopy and chromatography together with new pharmacological testing have led to an interest in plants as the source of new larvicidal compounds. Synergestic

approaches such as application of mosquito predators with botanical blends and microbial pesticides will provide a better effect in reducing the vector population and the magnitude of epidemiology. The presented green synthesis shows that the environmentally benign and renewable source of *Allium cepa* can be used as an effective reducing agent for the synthesis of AgNPs. This biological reduction of metal would be boon for the development of clean, nontoxic, and environmentally acceptable green approach to produce metal nanoparticles, involving organisms even ranging to higher plants. The formed Ag NPs are highly stable and have significant larvicidal activity.

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