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Biosynthesis of Silver Nanoparticles for Mosquito Control

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ABSTRACT

Biological synthesis of nanoparticles has received increased attention due to a growing need to develop environmentally benign technologies in material synthesis. In present work silver nanoparticles (AgNps) were synthesized intracellular by using *Pseudomonas aeruginosa sp.* The silver nanoparticles were characterized by Transmission Electron Microscopy (TEM), UV-Visible Spectroscopy (UV). Larvicidal activities of silver nanoparticles were analyzed as per the standard procedures. The *Pseudomonas aeruginosa* synthesized AgNPs were found effective against the larvae and pupae of *Cx. quinquefasciatus*. The larvaes of *Cx. quinquefasciatus* were found highly susceptible to the synthesized AgNPs at the same test concentrations. The mortality could be observed after different hours of exposure. The present suggested that the bacterial mediated AgNPs can be use to kill larva, pupa of filarial vector and could be significantly reduce parasite transmission and therefore lead to reduced filarial risk.

Keywords: Biosynthesis, *Pseudomonas aeruginosa*, silver nanoparticles, Larvicidal activities

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INTRODUCTION

Silver nanoparticles are emerging as one of the fastest growing materials due to their unique physical, chemical and biological properties; small size and high specific surface area. Biological synthesis of nanoparticles has received increased attention due to a growing need to develop environmentally benign technologies in material synthesis. Several plant species have been utilized in this regard (Mondal *et al.*, 2014)[1].

According to WHO (WHO, 2005)[2] there were about 219 million cases of malaria in 2010 (with an uncertainty range of 154 million to 289 million) and an estimated 660,000 deaths (with an uncertainty range of 490,000 to 836,000). Malaria mortality rate has fallen by more than 25% globally since 2000 and by 33% in the WHO African region. Most deaths occur among children living in Africa, where malaria claims the life of a child every minute. Country-level burden estimates available for 2010 show that an estimated 80% of malaria deaths occur in just 14 countries and about 80% of cases occur in 17countries. Together, the Democratic Republic of the Congo and Nigeria account for over 40% of the total estimated malaria deaths globally. Owolade *et al.*, (2008)[3] reportedly put forth the idea of nanoparticles helping in development of new pesticides, insecticides and insect repellants. In the coming years, it would surely be an important and ecofriendly tool for larval control. *Bacillus thuringienesis ssp*. Israelensis and *Bacillus sphaericus* are two insecticidal organisms that are used for the control of larvae in many countries, due to their excellent larvicidal properties (Wirth *et al.*, 2010)[4]. The larvicidal activity of cobalt nanoparticles synthesized by *Bacillus thuringiensis* against *Anopheles subpictus*and *Aedesaegypti* (Diptera: Culicidae) was studied by Marimuthu *et al.*, (2013)[5].

The use of biologically silver nanoparticles offers numerous benefits of eco-friendliness and compatibility for larvicidal application. The objectives of the present study is to biologically synthesized silver nanoparticles using microorganism and to evaluated their potential for mosquito control

MATERIALS AND METHOD

Bacteria:

Pseudomonas aeureginosa (MTCC 424) was procured from the Microbial Type Culture Collection (MTCC), IMTECH, Chandigarh, India.

Synthesis of AgNps:

The *Pseudomonas aeruginosa* (MTCC 424) was grown in 250-mL Erlenmeyer flasks containing 100 ml Nutrient broth at 37° C and 150 rpm for 24 hours. After incubation, biomass was separated by centrifugation and washed with sterile distilled water to remove the traces of media components, and, challenged with AgNO3 solution (1 mM). Incubate the

solution for 48 hr and after that centrifuged the solution and separate out the biomass and supernatant solution.

Characterization of Ag-NPs:

After 48 hours of incubation of the above mixture, the preliminary detection of Ag-NPs was carried out by visual observation of color change of the cell filtrate. These samples were later subjected to optical measurements, which were carried out by using a UV-Vis spectrophotometer (Shimadzu 1650 PC) and scanning the spectra between 430 nm at the resolution of 1 nm. A Transmission electron microscopy (TEM) was used to record the micrograph images of synthesized Ag-NPs. The silver nanoparticles synthesized showed sharp adsorption peak at 430 nm and which is the characteristic property of surface plasmon resonance of the silver nanoparticles.

Screening of larvicidal activity of silver nanoparticles:

Larvicidal activities of silver nanoparticles were analyzed as per the standard procedures recommended by World Health Organization (WHO, 1996)[6]. The silver nanoparticles solutions were diluted using double distilled water according to desired concentrations. Each test included a set control (silver nitrate and distilled water) with five replicates for each individual concentration. Mortality were assessed every 3 h to determine acute toxicities on fourth instar larvae of *Culex sp*.

Larvicidal activity of nanoparticles test:

Potential larvicidal activity of AgNPs using *Pseudomonas aeruginosa sp.* was visibly observed by changes of the medium color, aliquots were subjected to UV-visible spectroscopy to measure the peak, then the particles were purified by density gradient centrifugation. Releases of silver ions in the solution were measured. The bioreduction of silver nanoparticles was monitored by sampling the reaction mixture at regular intervals and the absorption maxima was scanned by UV-vis spectra, at the wavelength of 200-700 nm in Schimadzu 1601 spectrophotometer operated at a resolution of 1 nm (WHO, 1996)[6]

RESULTS AND DISCUSSION

Biosynthesis of silver nanopartical:

Silver nitrate solution (1 mmol/L) was treated with microbial extracts and incubate for 48 h. The colour of broth changed to dark brown, indicating silver nanoparticles formed (Figure 1).

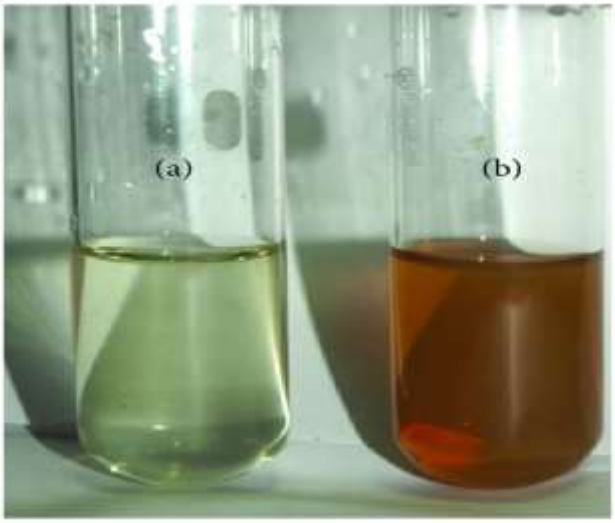

Figure 1: Visual observation of Synthesis of Silver Nanoparticals

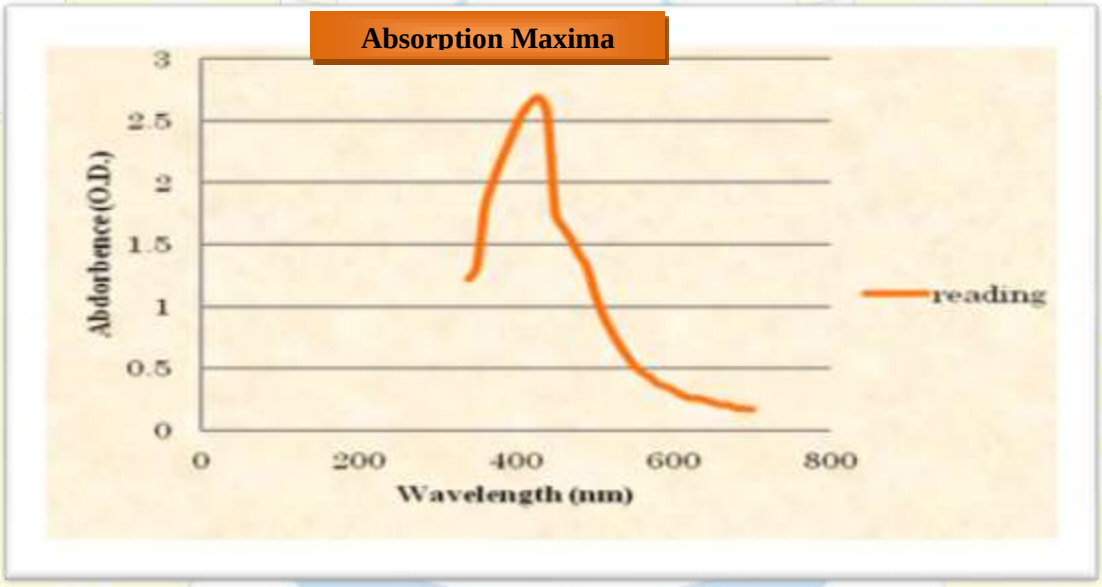

Figure 2: UV – Visible spectrophotometer graph of silver nanoparticles synthesized by *Pseudomonas aeruginosa* showed adsorption peak at 430 nm.

Characterization of silver nanoparticles:

After UV – Visible spectrophotometer, further characterization was carried out by scanning electron microscopy, transmission electron microscopy and X – ray diffraction pattern generated by transmission electron microscope. On analysis, Spherical silver nanoparticles were observed and the size of silver nanoparticles synthesized by *Pseudomonas aeruginosa* 38–42 nm (Figure 3). The silver nanoparticles were found to be capped by sodium citrate. On X – ray diffraction pattern generated by transmission electron microscope, it was confirmed that the silver nanoparticles are crystalline in nature.

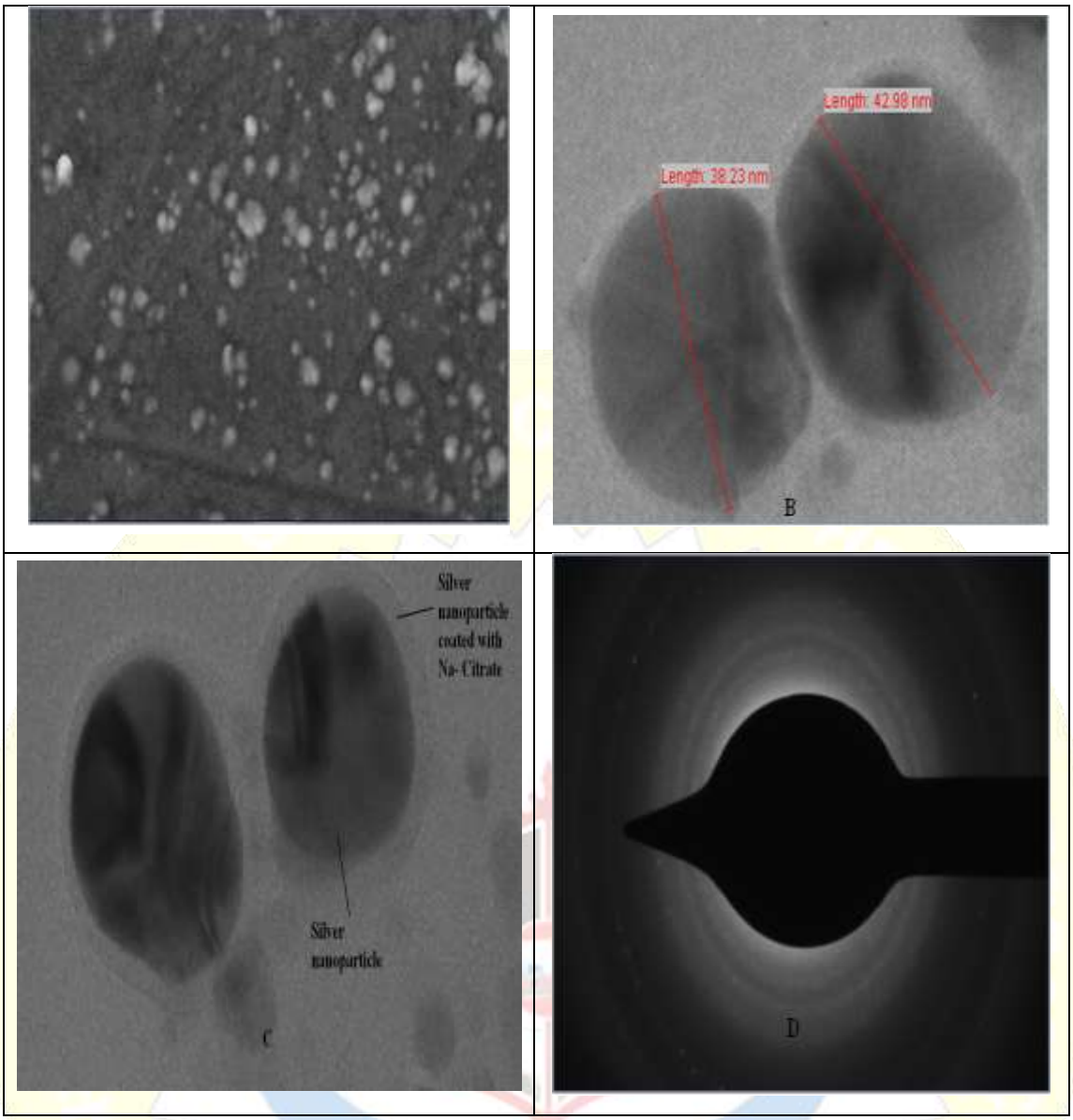

Figure 3: Showing SEM, TEM and XRD images of silver nanoparticles synthesized by *Pseudomonas aeruginosa* (A: SEM images, B and C: TEM images, D: XRD image).

UV-Vis Spectrophotometer and TEM Analysis of Synthesized AgNPs By mixing the fungal liquid component of *Pseudomonas aeruginosa* with the aqueous solution of Ag ions, the colour of fungal liquid changed from white to dark brown colour after 72 h of incubation. The change in colour is a signal for the formation of AgNPs. Figure 1(a) shows the UV-vis spectra of AgNPs synthesized by using the *Pseudomonas aeruginosa* recorded from the reaction medium before and after immersion of AgNO3 (2) after 72 h. Absorption spectra of AgNPs formed in the reaction medium has a broad absorption band centered at ca. 480 nm. The presence of broad resonance indicated an aggregated structure of the AgNPs in the solution.

Mosquito Larvicidal activity of Silver nanoparticles

Tables 1 represents the range of mortality in the mosquito larvae due to biologically synthesized silver nanoparticles. On the basis of these observations, the dosage mortality

lines were drawn Uniform range of mortality with increase in percent concentrations of silver nanoparticles was observed and it showed lowest LC50 value of 21%.

Table 1: Larvicidal activity of silver nanoparticles

% concentration	Corrected % mortality
0.05	14.28
0.1	28.57
0.2	42.85
0.3	52.38
0.4	71.32
0.5	89.0

The *Pseudomonas aeruginosa* synthesized AgNPs were found effective against the larvae and pupae of *Cx. quinquefasciatus*. The larvaes of *Cx. quinquefasciatus* were found highly susceptible to the synthesized AgNPs than the pupae at the same test concentrations. The mortality could be observed after different hours of exposure. The mortality was scored after 1 h. The early three instars of *Cx. quinquefasciatus* were found more susceptible to the synthesized AgNPs and shown the 100% mortality after 1 h of exposure. While, the fourth instar larvae were less susceptible to the synthesized AgNPs. Duran *et al.,* (2011)[7] discussed involvement of the enzyme NADPH-dependent nitrate reductase in production of AgNPs, while Vigneshwaran *et al.,* (2006)[8] showed the role of reducing sugars in AgNPs production, AgNPs synthesis were also reported from combination of reducing agents and terpenoids polyols, eugenol, quinines and Phyllanthin (Jha *et al*., 2009; Kasthuri *et al.,* 2009; Singh *et al.,* 2010)[9-11]. The plant latex used in the present study also showed the presence of proteins and secondary metabolites (terpenoids, tannins, alkaloids and others), so we may preliminarily conclude there is an interaction of enzymatic and non-enzymatic compounds in AgNPs formation.

Shaalan *et al.,* (2005)[12] reported that varying results obtained in lethal concentration values can be due to differences in the levels of toxicity among the insecticidal components of different plants, and the effect of plant extracts can vary significantly depending on plant species, plant part, age of the plant part, extraction solvent, seasonal variation, and mosquito species

In prokaryotic systems, AgNPs have multiple targets for biocidal effects by causing structural damage generation of reactive oxygen species, interfering with DNA replication, and reacting with the thiol enzyme group (Liau *et al.,* 1997; Feng *et al.,* 2000)[14,15]. Patil *et al*. (2012)[13] also pointed out the antagonistic effect of AgNPs on enzymes and proteins regardless of the Gram characteristics in bacteria. The mechanism of larvicidal action of silver nanoparticles requires more detailed study.

CONCLUSION:

Despite advances in medical science, mosquitoes in almost all tropical and subtropical countries are responsible for the transmission of pathogens causing some of the most life threatening and debilitating diseases, like malaria, yellow fever, dengue fever, chikunguniya, filariasis, encephalitis, *etc.* Repeated use of synthetic insecticides for mosquito control disrupts natural biological systems. The use of biological control agents is therefore essential. Microbes have been reported to reduce metal ions and stabilize nanoparticles with a wide size range. In the present investigation, the application of bacterial mediated AgNPs to kill larva, pupa of filarial vector could significantly reduce parasite transmission and therefore lead to reduced filarial risk.

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