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Antibacterial activity of Silver Nanoparticles Synthesized from *Fusarium semitectum* and Green Extracts

Sarvamangala R. Patil

Department of Biotechnology, Vishweshwariah College of Applied Sciences,
Gulbarga-585103, Karnataka, India

Abstract: ***Biological synthesis of silver nanomaterials is an important aspect of nanotechnology today. The present study deals with the synthesis of silver nanoparticles employing Fusarium semitectum and leaf extracts of papaya, neem and parthenium. The formation of nanoparticles was monitored by UV-Vis spectral analysis. The synthesized silver nanoparticles were tested for antibacterial activity against selected pathogenic bacterial strains like Pseudomonas aeruginosa, Salmonella typhi, Staphylococcus aureus and Streptococcus pyogenes. The antibacterial activity of silver nanoparticles from F. semitectum and parthenium extract was highest against S. pyogenes followed by S. aureus, P. aeruginosa and S. typhi when compared to the reference drug. The antibacterial activity against Gram positive bacteria was comparatively better than against Gram negative bacteria. Application of silver nanoparticles in sewage treatment showed a significant decrease in the bacterial load. This effect was found to be concentration and time dependent.***

Keywords: Silver nanoparticles, *Fusarium semitectum*, Green extracts, and Antibacterial activity

1. Introduction

Nanoparticles are being used as fundamental building blocks of nanotechnology. Nanoparticles are regarded as highly reactive species because of large surface area. The most effectively studied nanoparticles today are those made from noble metals, in particular Ag, Pt, Au, Pd.[1]. Among metal nanoparticles, silver nanoparticles play a significant role in the field of biology and medicine. Silver was known only as a metal till recently and it is only when the nano era came into existence that people started to believe that silver could even be produced at the nanoscale. The silver nanoparticles have been tested in various fields of biological science viz. drug delivery, wound treatment, binding of HIV gp120 protein[2], in water treatment and as an antibacterial compound[3][4].

The silver nanoparticles have been synthesized using a variety of methods [5][6], Among various methods, biological method is considered as an eco-friendly. With the increasing demand for green synthesis processes, the field of nanoparticle synthesis employed either biological microorganisms or plant extracts as a simple and viable alternative to chemical procedures and physical methods[1][7]. It is to be noted that those organisms which contain the "Silver resistance machinery" can synthesize silver nanoparticles provided that the concentration of the silver ions does not cross the "threshold limit". Various microorganisms used for the production of silver nanoparticles include *Pseudomonas stutzeri, Klebsiella pneumoniae, Escherishia coli, Enterobacter cloaca*[8][9]. Extracts from bio-organisms may act both as reducing and capping agents in silver nanoparticles synthesis. The reduction of Ag+ ions by combinations of biomolecules found in these extracts such as enzymes/proteins, amino acids, polysaccharides and vitamins is environmentally benign, yet chemically complex. But, the mechanism which is widely accepted for the synthesis of silver nanoparticles is the presence of enzyme "Nitrate reductase".

The fungi are the most suitable organism's forbiosynthesis of nanoparticles as their metabolic activity lead to precipitation of nanoparticles in external environment. A method for the synthesis of nanoparticles of gold [10] and silver[11] intracellularly in Verticillium fungal cells has been reported. The intracellular synthesis of nanoparticles may accomplish a better control over the size and shape distribution of the product, harvesting and recovery are more cumbersome and expensive. The extra cellular synthesis by comparison is more adoptable. Some researchers found that the silver nanoparticles can be synthesized extracellularly by using fungi *Colletotrichum* sp.[12], *Aspergillus fumigatus*[13], *Fusarium oxysporu* [14] and *Fusarium semitectum*[15], Plant mediated biological synthesis of nanoparticles is gaining importance due to its simplicity and ecofriendliness. Biosynthesis of silver nanoparticles by plants such as Alfalfa, Aloevara, Carica papaya, Eucalyptus, Tamarind, Euphorbia etc have been reported.

The present study deals with the possibility of exploring biological methods for silver nanoparticle synthesis. The purpose of this study was to analyze the nanoparticle synthesis by *F. semitectum* and plant leaf extracts of papaya, neem and parthenium and determination of antibacterial activity of these nanoparticles against pathogenic bacterial strains such as *Pseudomonas aeruginosa, Salmonella typhi, Staphylococcus aureus* and *Streptococcus pyogenes.*

2. Materials and Methods

2.1 Microorganisms

The standard fungal culture of *Fusarium semitectum* was obtained from Agarkhar Research Institute, Pune. The pathogenic bacterial strains such as *Pseudomonas aeruginosa, Salmonella typhi, Staphylococcus aureus* and *Streptococcus pyogenes* were obtained from Pooja Diagnostic Laboratory, Gulbarga. The fungal culture was maintained on Potato Dextrose agar (pH 5.6) containing (g/L) Potato 200, Dextrose 20 and Agar 15. The pathogenic bacterial cultures used as the test organisms were maintained on Nutrient agar (pH 7.0) containing (g/L) Peptone 5, Beef extract 3, Sodium chloride 5 and Agar 20 at a temperature of 4^0C. Regular sub culturing of the cultures was performed at an interval of every 4 weeks.

2.2 Plant leaves

The fresh leaves of papaya, neem and parthenium were collected from in and around Gulbarga city.

2.3 Sewage Sample

Sewage Sample was collected from City Bus Stand area Gulbarga.

2.4 Biosynthesis of Silver Nanoparticles

Fusarium semitectum was grown aerobically in a liquid medium of pH 5.6 containing (g/L) KH_2PO_4 7, K_2HPO_4 2, $MgSO_4.7H_2O$ 0.1, $(NH_4)_2SO_4$ 1, Yeast Extract 0.6 and Glucose 10. The flasks were incubated on an orbital shaker (ORBITEK-LEBT) at 27^0C agitated at 150 rpm. The biomass was harvested at 72 hrs of growth by filtering through a Whattman filter paper No 1 followed by extensive washing to remove any media components from the biomass. About 20 g (wet weight) was brought in contact with 100 ml of double distilled water in an Erlenmeyer flask and agitated for 72 hrs under the same conditions as mentioned above. After the incubation the cell filtrate was obtained by filtering it through Whattman filter paper No. 1. The resultant filtrate was then mixed with 100 ml of 1mM aqueous silver nitrate solution contained in an Erlenmeyer flask and observed for the color change.

Plant leaf extracts were prepared as per the method of Parashar et. al.[16], Leaves weighing 25 g for each plant were thoroughly washed thrice in distilled water for 15 min, dried and cut into fine pieces. The fragmented leaves were boiled in 500 ml Erlenmeyer flask containing 200 ml of sterile distilled water for 15 min and were filtered using Whattman filter paper No. 1. The resulting fresh leaf extract was added into 1mM aqueous silver nitrate solution and observed for the color change.

2.5 UV-Vis Spectra Analysis

The reaction media of *Fusarium semitectum* and leaf extracts were monitored using UV-Vis Spectrophotometer (Hitachi U-2900) between 200-800 nm at regular time intervals and the absorbance was measured [1].

2.6 Antibacterial activity of silver nanoparticles

Antibacterial activity of the synthesized nanoparticles was determined using agar well diffusion assay method. Approximately 20 ml molten and cooled nutrient agar medium was poured in sterile petridishes. The bacterial test pathogens such as *Pseudomonas aeruginosa, Salmonella typhi, Staphylococcus aureus* and *Streptococcus pyogenes* were grown in nutrient broth for 24 hrs and this culture was used to prepare bacterial lawns. Agar wells of 5 mm were prepared with the help of sterilized stainless steel cork borer. Different concentrations (50, 100 and 150 µl) of silver nanoparticles were added to the wells. Positive control drugs such as gentamycin, chloremphenicol, ciprofloxacin and amoxicillin in the concentration of 1mg/ml were used against *Pseudomonas aeruginosa, Salmonella typhi, Staphylococcus aureus* and *Streptococcus pyogenes* respectively. After diffusion the petriplates were incubated at 37^0C for 24 hrs and zones of inhibition were observed and measured [17].

2.7 Application of silver nanoparticles in sewage treatment

Antibacterial effect of silver nanoparticles was assessed against the bacteria present in sewage and waste water samples by spread plate method. By employing serial dilution technique, the bacteria were isolated from samples before treating with silver nanoparticles and CFU (colony forming units) were recorded. Then 1ml of sample was treated with different concentrations (0.5, 1 and 2 ml) of silver nanoparticles for varying time intervals (3, 4 and 5 hrs) and both the sets were grown on petriplates containing 20 ml of nutrient agar medium. The plates were incubated at 37^0C for 24 hrs and CFU were recorded after 24 hrs.

3. Results

The development of reliable process for the synthesis of silver nanomaterials is an important aspect of nanotechnology today. In the present work, the extra cellular biosynthesis of silver nanoparticles was carried out by using culture supernatant of *F. semitectum*. The Erlenmeyer flasks with the fungal filtrate has a pale yellow color before the addition of Ag+ ions which changed to a brownish color on completion of the reaction with silver ions for 24 hrs (plate 1). Similar results were displayed from the aqueous leaf extracts of three plants papaya, neem and parthenium (plate 2 & 3). Of the three plants studied the intensity of color development was significantly higher in parthenium and hence it was considered for further studies. The appearance of brownish color in solution containing the biomass/leaf extract is a clear indication of the formation of silver nanoparticles in the reaction mixture.

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Figure 1: Silver Nanoparticles synthesis from *Fusarium semitectum*

a **b**

Figure 2: Silver Nanoparticles synthesis from leaf extracts a. Neem b. Papaya

3.1 UV-Vis Spectra Analysis

The UV-Vis spectra (Fig. 1) recorded for the aqueous silver nitrate-*F. semitectum* reaction medium as a function of time indicated that the silver surface plasmon band occurs at around 425 nm and steadily increased in intensity. Figure 2 shows the UV-Vis spectra of parthenium leaf extract at various time intervals. The absorbance was found to increase with increase in time up to 72 hrs and thereafter it declined. The technique outlined above has been very useful for the analysis of nanoparticles. As illustrated in the figure the strong surface plasmon resonance was centered approximately at 450nm.

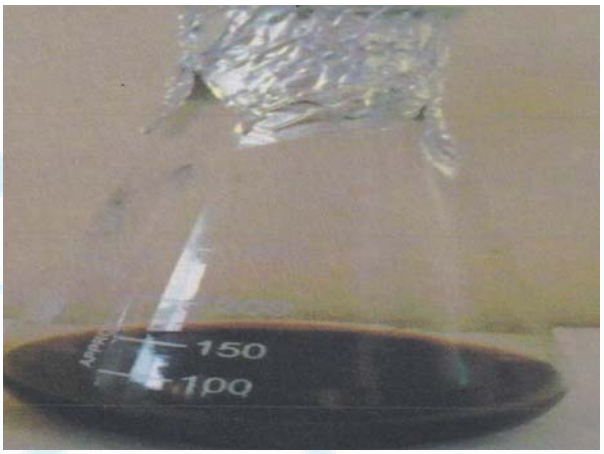

Figure 3: Silver Nanoparticles synthesis from leaf extracts of Parthenium

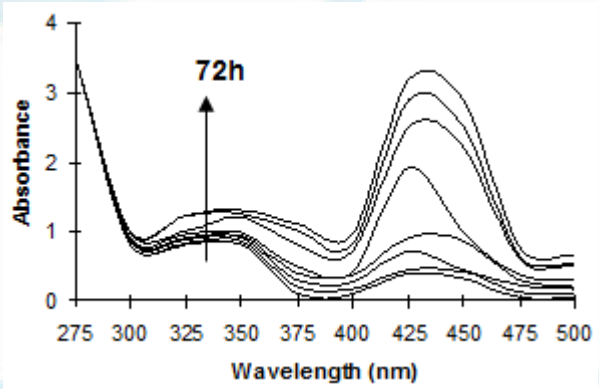

Figure 4: UV-Vis spectral analysis of silver nanoparticles from *F. semitectum*

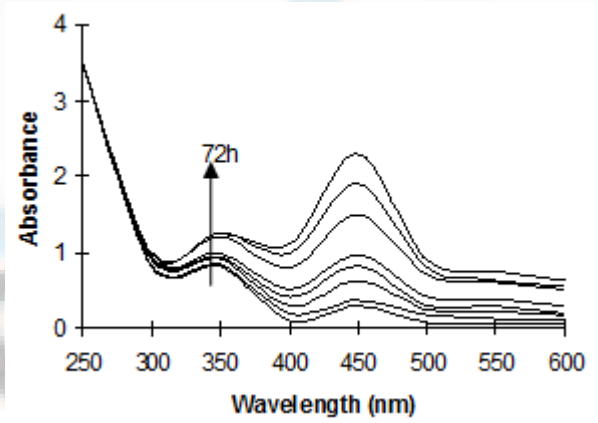

Figure 5: UV-Vis spectral analysis of silver nanoparticles from parthenium leaf extract

3.2 Antibacterial activity of silver nanoparticles

In the present study, the silver nanoparticles synthesized from *F. semitectum* were evaluated for antibacterial activity against Gram positive and Gram negative bacteria of high pathogenic nature. The results (Table 1) showed that the silver nanoparticles from *F. semitectum* at the concentration of 150 µl have shown maximum zone of inhibition compared to 50 µl and 100 µl. The zone of inhibition was 50 % in case of *P. aeruginosa* and *S. aureus* when compared to positive

control drug. The highest effect (92.8%) was recorded in case of *S. pyogenes* when compared to standard antibiotic. The bacterium *S. typhi* was found to be more resistant to silver nanoparticles as the zone of inhibition was only 31.0%. Table 1 also depicts the results of antibacterial activity of silver nanoparticles from leaf extracts of parthenium. The activity is comparable to that of the silver nanoparticles synthesized from *F. semitectum.*

Table 1: Antibacterial Effect of Silver Nanoparticles

Bacteria	*Reference drug (100 µl)*	*Silver nanoparticles*					
		50 µl		*100 µl*		*150 µl*	
		a	*b*	*a*	*b*	*a*	*b*
P. aeruginosa	22	08	05	10	08	11	10
S. typhi	29	06	05	07	06	09	08
S. aureus	28	09	08	11	10	14	13
S. pyogenes	14	09	09	11	11	13	12

Values indicate the zone of inhibition in mm.
*a - *F. semitectum* *b- Parthenium leaf extract

3.3 Silver nanoparticles in sewage treatment

Silver nanoparticles have been used as antimicrobial compounds for coliform found in waste water (Jain and Pradeep, 2005). In the present study, the CFU of bacteria before treating with silver nanoparticles were 312 x 10^5 /ml in sewage water. The CFU of bacteria was reduced to 17 x 10^5 /ml and 22 x 10^5 /ml in case of silver nanoparticles from *F. semitectum* and leaf extracts of parthenium respectively at a concentration of 2ml and a time interval of 5hrs (Table 2). This effect is found to be concentration and time dependent.

4. Discussion

Synthesis of silver nanoparticles has attracted considerable attention owing to their diverse properties like catalysis, magnetic and optical polaraizabiltiy and antimicrobial activity. The use of environmentally benign materials like plant leaf extracts, bacteria and fungi for the synthesis of silver nanoparticles offers numerous benefits of ecofriendliness and compatibility for pharmaceutical and biochemical applications as the methods do not use toxic chemicals.

Table 2: Silver nanoparticles in sewage treatment

Sl. No.	*Time interval (h)*	*Silver Nanoparticles*					
		0.5 ml		*1.0 ml*		*2.0 ml*	
		a	*b*	*a*	*b*	*a*	*b*
1.	3	250	283	99	112	47	54
2	4	140	154	39	47	21	29
3	5	95	116	30	41	17	22

Values indicate number of CFU x 10^5 /ml
*a - *F. semitectum* *b- Parthenium leaf extract

The present study deals with the production and characterization of silver nanoparticles from *F. semitectum* and plant leaf extracts. The study focused on the antibacterial activity of the synthesized nanoparticles and their application in sewage treatment.

The fungal filtrate of *F. semitectum* and leaf extracts of three different plants like papaya, neem and parthenium were screened for silver nanoparticle production. The formation of silver nanoparticles was known by the color change of the reaction medium from pale yellow to brownish. The change in color is known to arise owing to the surface plasmon resonance of metal nanoparticles [7]. Among the three extracts the intensity of color development was the highest in parthenium extract. The formation of nanoparticles was confirmed by UV-Vis spectral studies in the range of 200-800nm. In the spectrophotometric analysis the reaction mixture of *F. semitectum* and parthenium extract exhibited a strong absorption with an absorbance peak at approximately 425nm and 450nm respectively. Arangaswamy and Munusamy[1] reported the synthesis of silver nanoparticles using six different plants i.e., *Oryza sativa, Zea mays, Sorghum bicolor, Saccharam officinalis, Basella alba* and *Helianthus anus*. Parashar et. al.,[16] reported the characterization of silver nanoparticles from parthenium leaf extract showing an absorbance peak between 400-500nm. Our results are in good agreement with those of the authors. It is also observed from the spectra (Fig 1 and 2) that the absorption steadily increased in intensity as function of time of reaction. Shiv Shankar et. al.,[18] suggested that the shoulder at 370nm corresponds to the transverse plasmon vibrations in silver nanoparticles whereas the peak at 450nm due to excitation of longitudinal plasmon vibrations.

In recent years the resistance to commercially available antimicrobial agents by pathogenic bacteria has been increasing at an alarming rate and has become a serious problem. There is an increasing need to search for new antimicrobial agents from natural and inorganic substances. Among the inorganic antimicrobial agents silver has been employed most widely. The antibacterial activity of silver nanoparticles has been broadly studied, the silver nanoparticles act on broad range of target sites both extracellularly as well as intracellularly. They take advantages of the oligodynamic effect that silver has on microbes, where by silver ions bind to reactive groups in bacterial cells, resulting in their precipitation and inactivation. The present study revealed that the antibacterial

activity of silver nanoparticles from *F. semitectum* and parthenium extract was highest against *S. pyogenes* i.e., 92.8% and 85.7% respectively when compared to the reference drug followed by *S. aureus, P. aeruginosa* and *S. typhi*. Saxena et. al.,[19] reported the antibacterial activity of silver nanoparticles from onion extract against *E.coli* and *S. typhimurium*. Dharmendra et. al.,[20] have studied the antibacterial effect of silver nanoparticles synthesized by a physical, top-down approach technique against *E. coli* and *B. subtlis*. They found that the effect is time, dose and strain dependent. They also reported that the silver nanoparticles cause antibacterial effect by rupturing cell membrane. In the present study too, the antibacterial activity increased with increased concentration of silver nanoparticles. Silver nanoparticles are reported to have antibacterial effect against *Klebsiella pneumoniae* and *S. aureus* [4][5] when used alone and effective against *E. coli, B. subtilis* 1021, *P. syringae pv. syringae* 2440, *Xanthomonas compestris pv. vesicatoria, Azotobacter chrococcum* SL 206 and *Rhizobium tropici* when used in combination with silica[21]. To enhance the antibacterial effect of silver nanoparticles, they are also combined with antibiotics [22] or Ultrasonic irradiation [20]. The present study revealed that the antibacterial activity against Gram positive bacteria was comparatively better than against Gram negative bacteria.

Research is underway to use nanotechnology in water purification for safe drinking. Nanoparticles especially silver are expected to play a crucial role in water purification since they have been used against coliforms found in sewage. In the present study, the treatment of the sewage sample with the nanoparticles significantly decreased the bacterial load. This decrease was a function of incubation time and the concentration of nanoparticles. Dharmendra et. al.,[23] reported on the combined antibacterial effect of silver nanoparticles and ultrasonic irradiation on *E. coli* cells isolated from waste water and observed a reduction in the number of colonies to very few after 35 minutes of treatment of silver nanoparticles.

5. Conclusions

The present work concludes that biological synthesis of nanoparticles employing *F. semitectum* and leaf extracts of plants is a reliable method and produces stable silver nanoparticles with a promising potential as an antibacterial agent. The antibacterial attributes of nanoparticles is highly beneficial to combat the problem of alarming drug resistance in pathogenic bacteria. Such nanoparticles can be conveniently applied for the treatment of sewage too. Further advances are needed in order to turn the concept of nanoparticle technology into realistic practical application.

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Author Profile

Dr. Sarvamangala R. Patil has received her B.Sc., M.Sc., M.Phil and PhD degrees in Microbiology from Gulbarga University, Gulbarga in 1993, 1996, 1997, and 2004 respectively. Presently she is working as Head, Dept. of Biotechnology, Principal of Vishweshwariah College of Applied Sciences Gulbarga, and Director of Biotechnology Finishing School (Supported by Dept. of IT, BT and Science & Technology, Govt. of Karnataka).