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Dangi Sandeep
Department of Seed Science and Technology, College of Agriculture, Dharwad, Karnataka, University of Agricultural Sciences, Dharwad, Karnataka, India

Biradarpatil NK
Department of Seed Science and Technology, College of Agriculture, Dharwad, Karnataka, University of Agricultural Sciences, Dharwad, Karnataka, India

Correspondence
Dangi Sandeep
Department of Seed Science and Technology, College of Agriculture, Dharwad, Karnataka, University of Agricultural Sciences, Dharwad, Karnataka, India

Biosynthesis of silver nanoparticles using soybean seed extract

Dangi Sandeep and Biradarpatil NK

Abstract
An eco-friendly green mediated synthesis of inorganic nanoparticle is a fast growing research in the limb of nanotechnology. This study reports the synthesis of silver nanoparticles by using soybean aqueous seed extract which act as both reducing and capping agent without the use of toxic chemicals. Visually, the formation of silver nanoparticles was confirmed by observing the colour changes from light yellow to dark brown colour and an intense peak was observed in the UV- Visual Spectrophotometer at 434 nm. Further, silver nanoparticle was formation was confirmed by Particle Size Analyzer, Atomic Force Microscope and Scanning Electron Microscope. This novel green approach is a rapid, facile and used for large scale production of silver nanoparticle.

Keywords: soybean aqueous seed extract, green synthesis, silver nanoparticles, AFM, SEM and particle size analyzer

Introduction
The field of nanotechnology is one of the most active researches nowadays in modern material science and technology. Nanoparticles are fundamental building blocks of nanotechnology. Metal nanoparticles have a high specific surface area and a high fraction of surface atoms (Arangasamy Leela *et al.*, 2008) [1]. Due to their diverse properties and uses, like magnetic and optical polarizability, electrical conductivity (Chang and Yen, 1995) [2], catalysis, antimicrobial and antibacterial activities (Baker *et al.*, 2005, Shahverdi *et al.*, 2007) [3, 4], DNA sequencing (Cao *et al.*, 2001) [5], and Surface-Enhanced Raman Scattering (SERS) (Matejka *et al.*, 1992) [6] they are gaining the interest of scientist for their novel methods of synthesis. Silver has long been recognized as one of the nanoparticles having inhibitory effect on microbes present in medical and industrial process. Silver is an effective antimicrobial agent, exhibits low toxicity and has diverse *in vitro* and *in vivo* applications (Mani *et al.*, 2012) [7].
Green synthesis of nanoparticles is an eco-friendly approach which might pave the way for researchers across the globe to explore the potential of different herbs in order to synthesize nanoparticles. This kind of a synthesis includes usage of plant or microbes as a source. Among this, plants are considered as a best suited option as they can be made available easily for large scale biosynthesis of nanoparticles. Nanoparticles synthesized by plants are more stable and faster. As the nanoparticles are synthesized from plants, these nanoparticles seem to incorporate the plant peptides as capping agents during synthesis, which stabilizes the nanoparticles as well as enhances their antagonistic properties. A variety of techniques including physical and chemical methods have been developed to synthesize silver nanoparticles. The physical methods are highly expensive and chemical methods are harmful to the environment. Therefore, there is a growing need to develop eco-friendly nanoparticles that do not use toxic chemicals in the synthesis. Synthesis of silver nanoparticles from soybean seed and leaves have already been reported. As the nanoparticles are synthesized from plants, these nanoparticles seem to incorporate the plant peptides as capping agents during synthesis, which stabilizes the nanoparticles as well as enhances their antagonistic properties. Silver nanoparticles (AgNPs) have become the focus of intensive research owing to their wide range of applications in physical and biological sciences including agricultural and allied fields (Sharma *et al.*, 2014) [8]. Silver nanoparticles exhibit improved properties depending upon their size, morphology and distribution (Prasad and Ponneri, 2014) [9]. Plants are the reservoir of various phytochemicals and their extracts have been used for the synthesis of metal nanoparticles. Keeping this in view a present investigation was taken on green synthesis of silver nanoparticles using soybean seed extract.
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Materials and Methods

The experiment was conducted during 2016-17, at the Nanotechnology Laboratory, University of Agricultural Sciences, Dharwad. Green synthesis of silver nanoparticles, silver nitrate ($AgNO_3$) was used as a precursor and soybean (DSb-21) aqueous seed extract as both reducing and capping agent.

Soybean seed extraction method

Genetically pure and fresh seeds of soybean var. DSb-21 were collected from Seed Unit, UAS Dharwad. These seeds were dried for 10 days at room temperature**.** Dried 60 g seeds were weighed and washed thoroughly with distilled water for 3-4 times. Later washed seeds were placed in clean 500 ml beaker containing 250 ml distilled water. It is boiled for 30 mins on induction coil. After seed leachate was cooled for 10 mins and filtered with Whatman paper No.1. Filtered extract was stored in refrigerator for further use.

Preperation of $AgNO_3$ stock solution

Precursor was used in the synthesis of silver nanoparticles was silver nitrate ($AgNO_3$) with concentration of 1 mM. The silver nitrate ($AgNO_3$) was purchased from Himedia Laboratories, Mumbai, India and it was used as a precursor in the silver nanoparticle synthesis process.169.86 g of $AgNO_3$ in 1 liter of distilled water gives us 1mM of $AgNO_3$ solution.

Synthesis of silver nanoparticles

30 ml of culture tube was taken in which 10 ml of $AgNO_3$ is added then 1 ml of soybean seed extract was added. Culture tube containing 10 ml of $AgNO_3$ and 1 ml of soybean seed extract was exposed to sunlight for three hours. While exposing solution slightly start turning to brown colour after three hours the whole solution turns to dark brown colour which preliminarily confirms the synthesis of silver nanoparticles.

The experiment was conducted during 2016-17, at the Nanotechnology Laboratory, University of Agricultural Sciences, Dharwad. Green synthesis of silver nanoparticles, silver nitrate ($AgNO_3$) was used as a precursor and soybean (DSb-21) seed extract as both reducing and capping agent. Preparation of soybean seed extract by boiling of 60 g of dried seeds in 250 ml of water for 30 min. Extract was filtered by Whatman paper No.1 for removal seed debris and stored at 4^{o}C for further experiment. Green synthesis of silver nanoparticles by exposing of reaction mixture (10 ml of 1 mM $AgNO_3$ and 1 ml of soybean seed extract) to bright sunlight for three hours. Colour changes was observed from colourless to dark brown it indicate preliminary confirmation of nanoparticles formation (Photo-2), further confirmation by UV-Visible Spectrophotometer, Atomic Force Microscope (AFM) and Scanning Electronic Microscope (SEM).

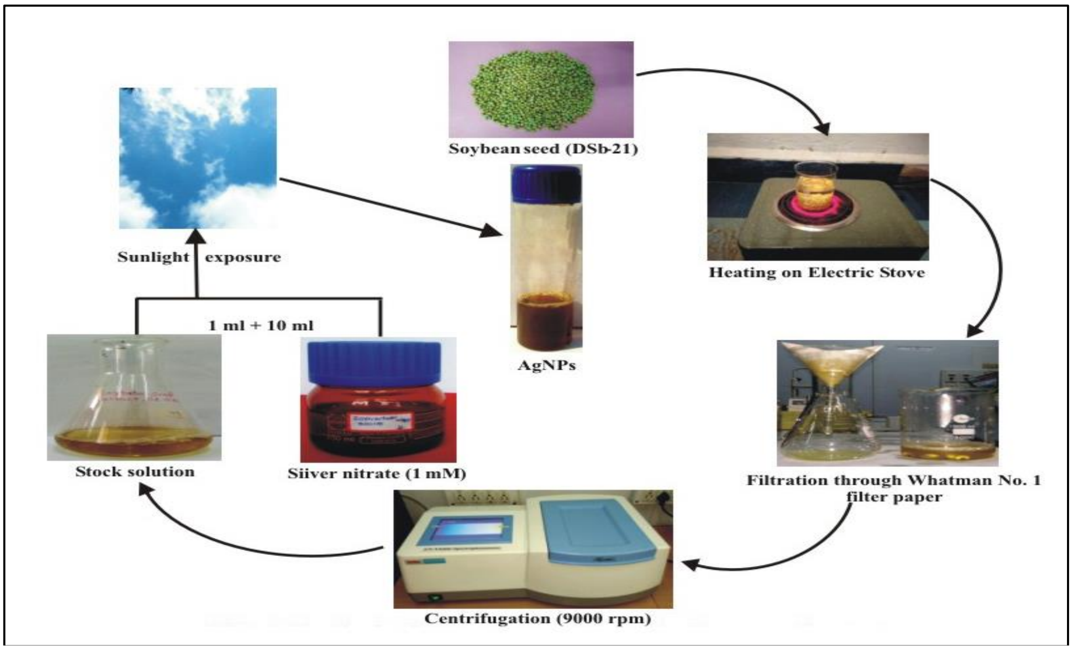

Photo 1: Flow chart of synthesis of Silver nanoparticles using soybean seed extract

Characterization of nanoparticles

The synthesised nanoparticles were characterized using UV-Vis Spectroscopy (Spectrum, SP-UV500DB/VDB) over a range of 200-700 nm. Green synthesized silver nanoparticles particle shape, sizes and distribution characterized by AFM (Nanosurf AG, Swiss, Flex AFM, with 24bit C3000) (Fig. 3) and SEM (Scanning Electron Microscope) analysis.

UV-visual spectroscopy

UV-visible spectroscopy was used to detect and confirm the presence of various ions in a given sample based on their optical absorbance peaks. As the size of the nanoparticles decreases, the band gap increases and thus the optical absorbance increases as compared to that of the bulk particles and therefore their colour changes.

Atomic force microscope (AFM)

AFM was carried out to study the size distribution and the shape of the nanoparticles based on the phenomena of transmittance of the electron beam through an ultra thin specimen. The transmitted electrons carried an image of the specimen which was further focused to analyze the shape and the size of the specimen.

Particle size analyzer (PSA)

In most applications theoretical calculations predict the relative effects of particle size, particle composition, composition of the surrounding medium and wavelength of light. In order to find out the particles size distribution the AgNPs powder was dispersed in water by horn type ultrasonic processor (Vibronics, VPLP1). The data on particle size distribution were extracted in Zetasizer Ver. 6.20 (Mal1052893, Malvern Instruments).

Scanning electron microscope (SEM)

SEM was carried out to study the size distribution and the shape of the nanoparticles based on the phenomena of transmittance of the electron beam through an ultra thin specimen. The transmitted electrons carried an image of the specimen which was further focused to analyze the shape and the size of the specimen.

Results and Discussion

Silver ion reduction was observed when 1 mM silver nitrate solution was mixed with 30 per cent aqueous extract of soybean seed (variety DSb-21) and kept under bright sunlight for three hours. Initial stages of reduction showed change in colour from almost colourless to dark brown, which clearly indicated the formation of silver nanoparticles in the reaction mixture as shown (Fig.1). Colour change could be observed within ten minutes from colourless to faint yellow, indicating the formation of silver nanoparticles. The colour intensity increased with the increased time of exposure to sunlight. As time elapsed the yellow coloured solution eventually became dark brown by three hours, which may be due to the increased concentration of nanoparticles as well as reduction in particle size. There was no significant change beyond three hours indicating the completion of the reduction reaction within three hours.

It is well known that silver nanoparticles exhibit a yellowish-brown colour in aqueous solution due to excitation of surface plasmon vibrations in silver nanoparticles (Jae and Beom, 2009) [10]. Reduction of silver ions to silver nanoparticles could be followed by a colour change and UV-Vis spectroscopy. The technique outlined above has proven to be very useful for the analysis of nanoparticles (Henglein, 1993; Sastry *et al.*, 1997; Sastry *et al.*, 1998) [11-13]. Therefore, the progress in conversion reaction of silver ions to silver nanoparticles was followed by a colour change and spectroscopic techniques. Figure 1 shows the photographs of sample solutions containing silver nitrate (left beaker) and silver nitrate in the presence of optimized amounts of soybean seed extract solutions after completion of the reaction (right beaker). The appearance of a dark brown colour confirms the existence of silver nanoparticles in the solution (Photo-2).

Photo 2: Reaction mixture (10 ml of $AgNO_3$ and 1ml of soybean seed extract) before (left) and after (right) exposing to sunlight

The colour change of reaction mixture was due to the presence of protein and enzymes in seed extract. It helps to reduction of silver nitrate to silver nanoparticles (Awwad *et al.*, 2013) [14] and for further confirmation by UV-Visible spectrophotometer, peak was observed at 434 nm (Fig. 2). This is because of a phenomenon called Surface Plasmon Resonance (SPR) exhibited by silver nanoparticles.

UV-Vis spectroscopy

The silver nanoparticles were characterized by UV-Vis spectroscopy, one of the most widely used techniques for structural characterization of silver nanoparticles (Sun *et al*., 2001) [15]. The absorption spectrum (Figure-1) of the yellowish-brown silver nanoparticle solution prepared with the proposed method showed a surface plasmon absorption band with a maximum of 434.8 nm, indicating the presence of Ag nanoparticles. UV-spectra reading of the reaction mixture (silver nitrate and soybean seed extract) as function of time is given in figure-2.

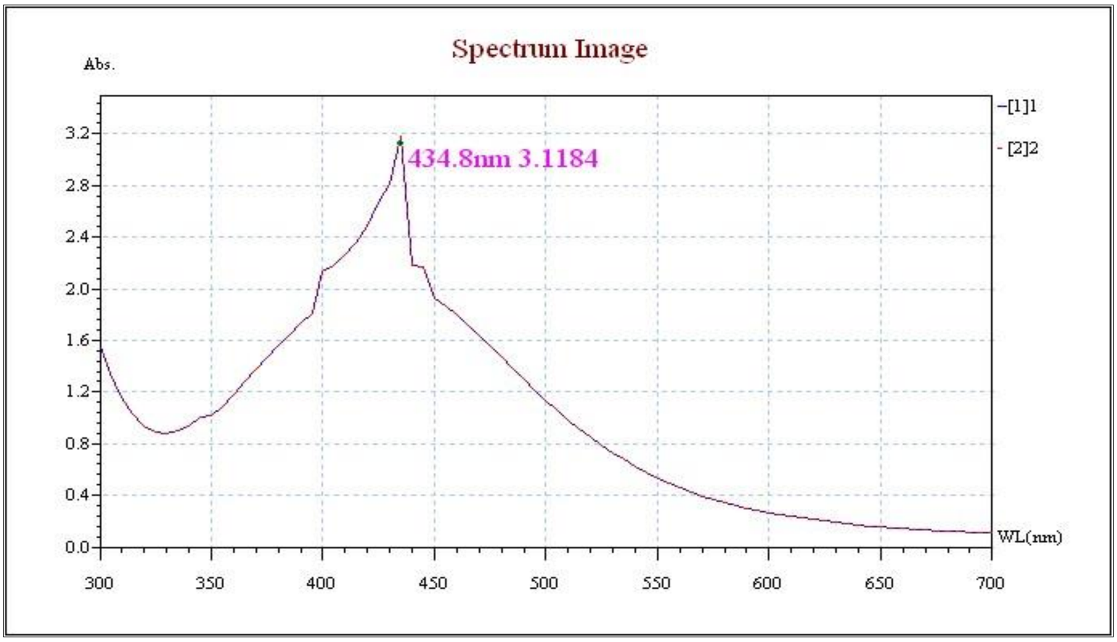

Fig 1: UV-Vis absorption spectrum of AgNPs

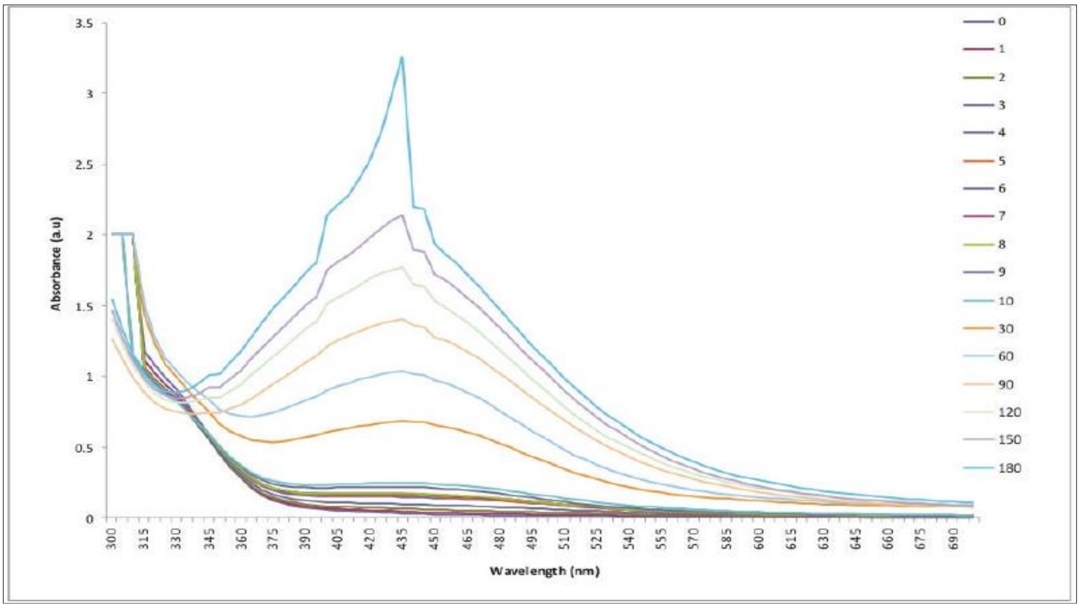

Fig 2: UV-spectra reading of the reaction mixture (silver nitrate and soybean seed extract) as function of time

Atomic Force Microscope (AFM)

The synthesized silver were characterized by atomic force microscope with relation to size, shape the data showed 2D and 3D images of nanoparticles. The AFM results of soybean based AgNPs recorded particle size ranging between 60-80 nm and they were spherical in shape as shown in photograph-3.

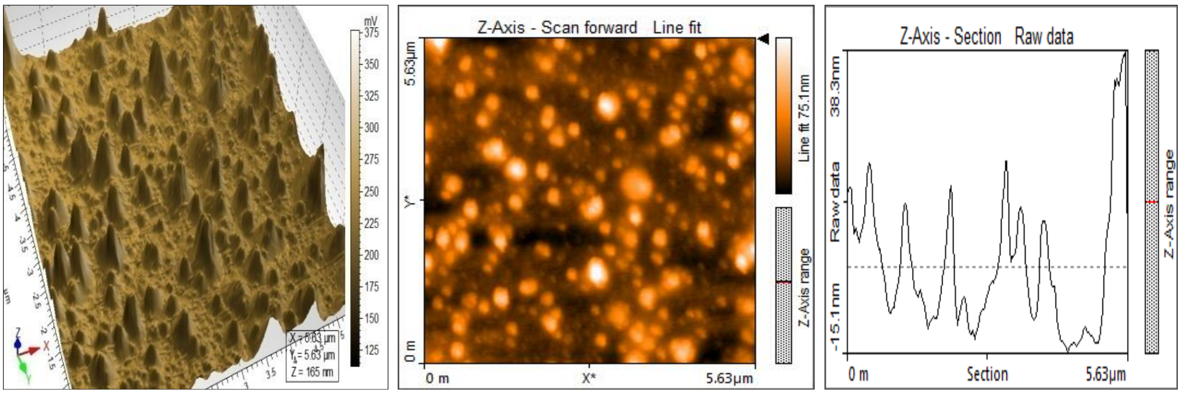

Photo 3. 2, 3: dimension and graphical images of Ag NPs synthesized from soybean seed extracts and characterized by atomic force microscope

Particle Size Analyzer by diffuse light scattering method

Particle size refers to the details of particles size (d.nm) and zeta potential range based on the count rates. The particle size analysis showed a size of nanoparticles d. mean volume of 60.18 nm and with the specific sizes ascertained as 12.91 nm and 12.89 nm as shown in figure 3.

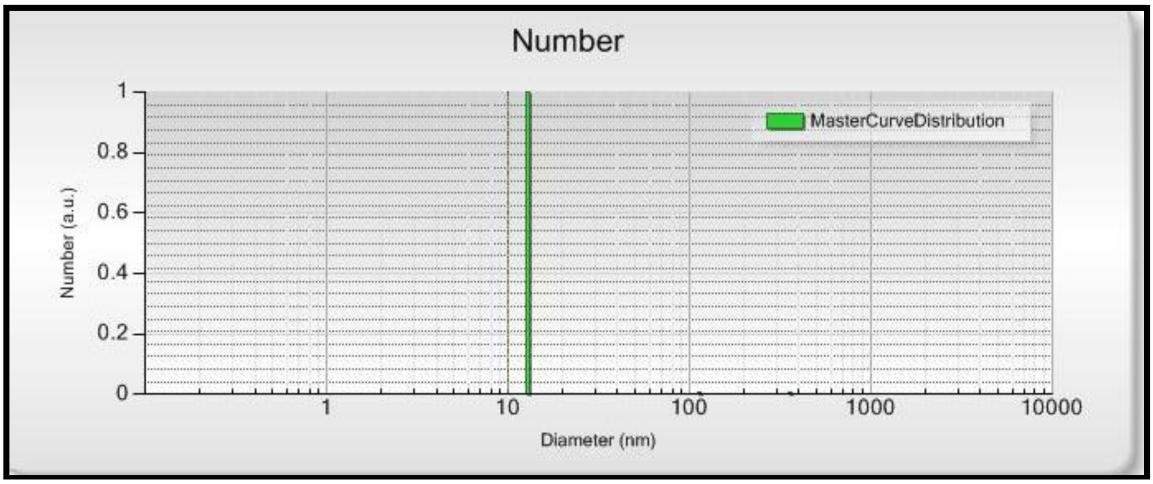

Fig 3: Particle size analysis of AgNP's

Scanning Electron Microscope (SEM)

SEM analysis of the silver nitrate solution (Control) and reduced form of silver nitrate solution were clearly distinguishable owing to their size difference. The SEM image shows high density AgNps synthesized by soybean seed extracts further confirming the presence of AgNPs. The shape of the AgNPs was spherical and cubic; the sizes of AgNPs ranges between were 12.50 nm and 120.50 nm as confirmed by SEM image (Photo-4).

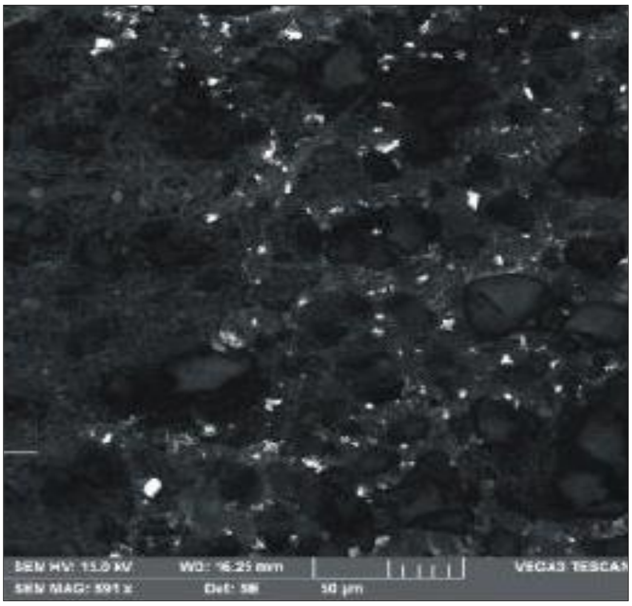

Photo 4: SEM image of nanoparticles

Conclusion

Green synthesis of silver nanoparticles from soybean seed extract was rapid, eco-friendly and cost effective due to easily available seed source and also act as both reducing and capping agent. Green synthesized silver nanoparticles was characterized by UV-Vis (430-435 nm), AFM (60-80 nm). Particle Size Analyzer and SEM (Shape: spherical and cubic).

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