Ecotoxicology and Environmental Safety 120 (2015) 400–408

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv

# Synthesis, characterization and biocompatibility of silver nanoparticles synthesized from *Nigella sativa* leaf extract in comparison with chemical silver nanoparticles

Rayhaneh Amooaghaie [a,*], Mohammad Reza Saeri [b], Morteza Azizi [c]

[a] Department of Biology, Shahrekord University, Shahrekord, Iran
[b] Department of Material Engineering, Shahrekord University, Shahrekord, Iran
[c] Department of Material Engineering, Shahrekord University, Shahrekord, Iran

ARTICLE INFO

*Article history:*
Received 2 February 2015
Received in revised form
13 June 2015
Accepted 15 June 2015
Available online 26 June 2015

*Keywords:*
Green synthesis
Nano particle
Nigella sativa
Silver
Toxicity

ABSTRACT

Despite the development potential in the field of nanotechnology, there is a concern about possible effects of nanoparticles on the environment and human health. In this study, silver nanoparticles (AgNPs) were synthesized by 'green' and 'chemical' methods. In the wet-chemistry method, sodium borohydrate, sodium citrate and silver nitrate were used as raw materials. Leaf extract of *Nigella sativa* was used as reducing as well as capping agent to reduce silver nitrate in the green synthesis method. In addition, toxic responses of both synthesized AgNPs were monitored on bone-building stem cells of mice as well as seed germination and seedling growth of six different plants (Lolium, wheat, bean and common vetch, lettuce and canola). In both synthesis methods, the colorless reaction mixtures turned brown and UV–visible spectra confirmed the presence of silver nanoparticles. Scanning electron microscope (SEM) observations revealed the predominance of silver nanosized crystallites and fourier transform infra-red spectroscopy (FTIR) indicated the role of different functional groups in the synthetic process. MTT assay showed cell viability of bone-building stem cells of mice was further in the green AgNPs synthesized using black cumin extract than chemical AgNPs. IC50 (inhibitory concentrations) values for seed germination, root and shoot length for 6 plants in green AgNPs exposures were higher than the chemical AgNPs. These results suggest that cytotoxicity and phytotoxicity of the green synthesized AgNPs were significantly less than wet-chemistry synthesized ones. This study indicated an economical, simple and efficient ecofriendly technique using leaves of *N. sativa* for synthesis of AgNPs and confirmed that green AgNPs are safer than chemically-synthesized AgNPs.



## 1. Introduction

Research and development in nanotechnology field has been growing rapidly throughout the world. Silver nanoparticles (AgNPs) are one of the most important and extensively usage of NPs materials. Silver nanomaterials exhibit broad spectrum biocidal activity toward bacteria, fungi, viruses, ad algae. This motivates its use in a large number of biomedical and environmental applications as well as a growing list of consumer products. Due to strong antimicrobial activity, AgNPs are also used in clothing, food industry, sunscreens, as well as in pest and diseases management in agriculture (Abou El-Nour et al., 2010; Yu, 2013; Prabhu and Poulose, 2012; Khot et al., 2012).

One key aspect of nanotechnology concerns the development of rapid and reliable experimental protocols for the synthesis of nanomaterials over a range of chemical compositions, sizes, high monodispersity and in large scale. Nanoparticles can be synthesized using various approaches including chemical and green methods. (U.S. EPA, 2012). The chemical methods of synthesis require short period of time for the synthesis of large quantity of nanoparticles, but problem with most of the chemical and physical methods of nanosilver production is that they are extremely expensive and also involve the use of toxic, hazardous chemicals such as $NaBH_4$, $N_2H_4$, $NH_2OH$, ethanol,…, and often experiences various problems like stability, preparation, control of growth and aggregation of particles and requires capping agents for size stabilization of the nanoparticles. Furthermore, there are evidences that engineered nanosilver can allegedly cause adverse effects on humans, animals and plants as well as the environment (Abou El-Nour et al., 2010; Questera et al., 2013; Prabhu and Poulose, 2012). It is an unavoidable fact that the silver nanoparticles synthesized

* Corresponding author. Fax: +98 38 14424 419.
*E-mail addresses:* Rayhanehamooaghaie@yahoo.com (R. Amooaghaie), Saeri_mohammad@yahoo.com (M.R. Saeri), morteza2105@yahoo.com (M. Azizi).

http://dx.doi.org/10.1016/j.ecoenv.2015.06.025
0147-6513/

have to be handled by humans and must be available at cheaper rates for their effective utilization; thus, there is a need for an environmentally and economically feasible way to synthesize these nanoparticles. The quests for such a method has led to using biological methods for synthesize the silver nanoparticles (Yu, 2013; Prabhu and Poulose; 2012). The green synthesis method possesses the following advantages over traditional chemical methods: (1) green synthesis is simple and usually involves a one-pot reaction; (2) it is amenable to scale up; (3) the toxicity-associated hazardous chemicals are eliminated, increasing the biocompatibility of the resulting product with normal tissues for in vivo applications; and (4) the process is cost-effective. Finally, (5) green biological entities can be used as reducing agents and capping agents, providing AgNPs with enhanced colloidal stability (Park, 2014). In this regard, green synthesis of nanoparticles has received increasing attention due to the growing need to develop environmentally benign technology in nanoparticles synthesis.

Questera et al. (2013) have recently reviewed several biological systems including a wide number of organisms, including bacteria, yeast, fungi, algae, and plants. Several products such as: total extracts, carbohydrates and polyphenolic compounds are able to fabricating various types of metal nanoparticles like silver, gold, palladium, and others (Park et al., 2014). One of the highly prevalent and widely studied among green methods is the synthesis of metal nanoparticles using plant extracts that has been found to be cost, effective and environmental friendly (Jha et al., 2009). Although green synthesis of AgNPs by various plants has been reported (Ashok et al., 2010; Ahamed et al., 2011; Padma et al., 2012; Sathyavathi et al., 2010), the potential of plants as biological materials for the synthesis of nanoparticles is yet to be fully explored. In order to better describe the bioreduction of metal ions and the biosynthesis of the metal/semiconducting nanoparticles with different shapes, investigation of the synthesis process with various plants is important.

On the other hand, the more widespread our use of silver nanomaterials enhances the potential for human and ecosystem exposure. The silver nanoparticles may be released to the environment from discharges at the point of production, from erosion of engineered materials in household products (e.g., antibacterial coatings and silver-impregnated water filters), and from washing or disposal of silver-containing products. Despite its widespread use, there are an escalating controversy surrounds their use. Consensus remains elusive on subjects as essential as how it behaves in the human body, animals and plants and the environment, and the extent to which its use may contribute to bacterial resistance (Nowack and Bucheli, 2007; Seltenrich, 2013). However, if the amount of nano-scaled silver entering environment becomes higher than the tolerable levels for vital organisms, critical environmental infrastructure might be impacted. Many reports have demonstrated that chemical AgNPs are highly toxic to bacteria, fish, clams, rats, algae and plants (Ma, 2010.; Seitz et al., 2015; Nowack and Bucheli, 2007; Seltenrich, 2013; von Moos and Slaveykova, 2014; Yu et al., 2013). Therefore, concerted efforts have been invested in the development of non-toxic nanoparticles using green methods for utility in a wide spectrum of applications.

Given that the increase in production of commercial AgNPs may have potential negative impacts on ecosystems, our knowledge regarding the toxicity of AgNPs must be broadened to predict the environmental and human health risks associated with these materials. Thus, this study was designed for a simple, cost-effective and environmentally benign synthesis of AgNPs at ambient conditions using *Nigella sativa* leaf extract as a reducing and stabilizing agent. In addition, although there are some the information on toxicity of chemically-synthesized AgNPs, the information on biological response of human, animal and plant cells to green synthesized AgNPs is very limited. Before developing the technology further, it was equally important to evaluate toxicity of such phyto-synthesized NPs in view of their possible biological contamination. Therefore, in this research, toxicity of AgNPs synthesized by *N. sativa* leaves extracts were compared with the ones which were synthesized by an ordinary chemical reduction on vitality of bone-building stem cells of mice and seed germination and seedling growth of six different plants (canola, lettuce, Lolium, wheat, bean and common vetch).

## 2. Materials and method

### 2.1. Synthesis of silver nanoparticles using green method

In brief, *N. sativa* leaf biomass is prepared by sieving crushed shadow dried leaves using a sieve with 200 μm mesh openings. *N. sativa* powder was thoroughly washed in distilled water and cut into fine pieces. 1 g of this powder was homogenized completely in 40 ml double distilled water and incubated with constant stirring (100 rpm) at 80 °C for 20 min. The resultant mixture then filtered using Whatman filter papers No.1 to remove debris. This extract was used for generating silver nanoparticles. The extract was stored at 4 °C for future uses. For green synthesis 2 ml above cumin extract was added to 70 ml solution of 5 mM silver nitrate ($AgNO_3$-Merck) drop-wise in a 250-mL Erlenmeyer. Solution incubated with constant stirring (200 rpm) at 64 °C for 4 h in dark. This was followed by observation of color change to yellowish brown indicating formation of silver nanoparticles and the biological synthesis of silver nanoparticles was checked using UV spectrophotometer.

### 2.2. Synthesis of silver nanoparticles using chemical method

For the chemical synthesis of AgNPs, firstly, 2 ml sodium borohydrate (2 mm in methanol) was added to 45.0 ml of distilled water and the solution was mixed to 25 ml aqueous solution of $AgNO_3$ (5 mM) drop-wise with constant stirring at 64 °C and the color change was observed. reduction of Ag to nanoparticles was completed in 30 min by drop-wise adding obtained suspension to 20 ml aqueous solution of three sodium citrate (2 mm) as a capping agent with constant stirring (200 rpm) at 50–60 °C

### 2.3. Characterization of synthesized-AgNPs

The optical absorption of the green synthesized AgNPs were measured using a double beam UV–vis spectrum (Ultra spec pro 4000 spectrophotometer) having a resolution of 1 nm, in the wavelength range 300–800 nm at room temperature.

Each of the colloidal solution containing AgNPs were centrifuged at 12,000 rpm for 15 min, and the pellets was discarded and the supernatants were again centrifuged at 8000 rpm for 15 min. This time, the supernatants were discarded and the final pellets were dissolved in 0.1 mL of deionized water. The pellet was mixed carefully and dried by freeze dryers (Zirbus technology D-37539-Germany). Size and morphology of nanoparticles were determined using laser particle size analyzer (HORIBA LB-550-Japan). The cover slip itself was used during scanning electron microscopy (SEM) analysis. The samples were then gold coated using a coater (JEOL, Akishima-shi, Japan, and Model No. JFC-1600) and applied field emission scanning electron microscopy (FESEM, TESCAN, Czech) method. The IR spectra of native and treated leaf extract's samples were recorded using Magna IR-550 FTIR spectrophotometer, using transmittance mode operating at a scan rate of 2 $cm^{-1}$(JASCO, Tokyo, Japan) in range of 400–4000 $cm^{-1}$.