

## ***Tagetes erecta* mediated phytosynthesis of silver nanoparticles: an eco-friendly approach**

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**Abstract.** Dhuldhaj UP, Deshmukh SD, Gade AK, Yashpal M, Rai MK. 2012. *Tagetes erecta* mediated phytosynthesis of silver nanoparticles: an eco-friendly approach. *Nusantara Bioscience* 4: 109-112. Nanotechnology is a multidisciplinary field having applications in various fields like medicine, pharmacy, engineering, and biotechnology. An important step in nanotechnology is to develop simple and eco-friendly method for the nanomaterial synthesis. Here we describe simple and eco-friendly method for synthesis of silver nanoparticles by extract of *Tagetes erecta* plant leaves. The phytosynthesis (synthesis by plant) of silver nanoparticles was detected by color change from light-green to dark-brown. Synthesis of silver nanoparticles was confirmed by UV-Vis spectrophotometry, further characterization includes nanoparticle tracking analysis system (NTA) (LM20) and transmission electron microscopy (TEM). TEM analysis confirms the synthesis of the polydispersed spherical silver nanoparticles of 20-50 nm, with an average size of 30 nm.

**Keywords:** *Tagetes erecta*, eco-friendly, nanoparticles, phytosynthesis, TEM.

**Abstrak.** Dhuldhaj UP, Deshmukh SD, Gade AK, Yashpal M, Rai MK. 2012. *Fotosintesis nanopartikel perak yang diperantarai oleh Tagetes erecta: pendekatan ramah lingkungan.* *Nusantara Bioscience* 4: 109-112. Nanoteknologi merupakan bidang multidisiplin yang dapat diaplikasikan pada berbagai bidang seperti kedokteran, farmasi, teknik, dan bioteknologi. Sebuah tahap penting pada nanoteknologi adalah mengembangkan metode yang sederhana dan ramah lingkungan untuk menyintesis nanomaterial. Dalam penelitian ini, digambarkan metode sederhana dan ramah lingkungan untuk sintesis nanopartikel perak oleh ekstrak daun tanaman kenikir *Tagetes erecta*. Fotosintesis (sintesis oleh tanaman) dari nanopartikel perak terdeteksi oleh perubahan warna dari hijau-terang ke gelap-coklat. Sintesis nanopartikel perak dikonfirmasi dengan spektrofotometer UV-Vis, lebih lanjut karakterisasi meliputi sistem analisis pelacakan nanopartikel (NTA) (LM20) dan mikroskop elektron transmisi (TEM). Analisis TEM menegaskan terjadinya sintesis bola nanopartikel perak dari 20-50 nm yang multidispersi, dengan ukuran rata-rata 30 nm.

**Kata kunci:** *Tagetes erecta*, ramah lingkungan, nanopartikel, fotosintesis, TEM.

### INTRODUCTION

Nanotechnology is the branch of science dealing with the synthesis of nanomaterials and nanoparticles of size 1-100 nm and their technological applications in various fields (Tenover 2006). The combination of the nanotechnology and biology presents the new branch of nanotechnology called Nanobiotechnology. It deals with the application of biotechnological principle for the development of devices, and systems at nano level (Hasselov et al. 2008; Kholoud et al. 2010).

There are different methods for nanoparticles synthesis as chemical, physical and a recently developed biological method. Biological method is cost-effective and eco-friendly than chemical and physical methods (Rai et al. 2008; Raveendran et al. 2003). In biological method, fungi, bacteria, and plants are used for the synthesis of nanoparticles. Biosynthesis of inorganic materials, especially metal nanoparticles using microorganisms (Mandal et al. 2006, Gade et al. 2010) and plants (Gardea-Torresdey et al. 2003) were carried out. Various plants and

fungi having the potential of metal nanoparticle synthesis, proving that biological synthesis of metal nanoparticles is simple and cost-effective (Rai et al. 2008). Among the biological agent *Fusarium* sp. (Ingle et al. 2008, 2009; Bawaskar et al. 2010), *Aspergillus niger* (Gade et al. 2008), was proved to be a novel agent for synthesis of silver nanoparticles.

Use of plant extracts for the nanoparticles synthesis is rapid, eco-friendly and simple alternative to bacterial and fungal system as leaf extract is easily prepared and there is no need of isolation, culture, and maintenance of bacterial and fungal culture. Plant extracts have more potential for reducing metal ions than microbes (Rai et al. 2008). The successful examples of synthesis of nanoparticles either intracellularly or extracellularly include geranium leaf-extract (Shivshankar et al. 2003, 2004, 2005) sun-dried leaves of *Cinnamomum* (Huang et al. 2007), *Azadirachta indica* (neem) (Shankar et al. 2004), *Aloe vera* (Chandran et al. 2006), *Capsicum annum* (Li et al. 2007), *Carica papaya* (Mude et al. 2009), *Opuntia ficus-indica* (Gade et al. 2010b), and *Murraya koenigii* (Bonde et al. 2012).

The present study focuses on the development of the simple, novel and eco-friendly method for phytosynthesis of silver nanoparticles from *Tagetes erecta*.

## MATERIALS AND METHODS

### Synthesis of silver nanoparticles

Leaves of the *Tagetes erecta* were collected from the garden of S.G.B. Amravati University, Amravati, Maharashtra, India. After several washes with tap water, leaves were surface sterilized with  $\text{HgCl}_2$  (0.1%) for 1-2 min to remove microbial contamination, if any. These leaves were cut into small pieces and crushed with mortar and pestle by adding little amount of double sterilized distilled water. The extract obtained was filtered with Whatman filter paper No. 42, the volume was adjusted to 100 ml by double sterilized distilled water. The extract was challenged with the silver nitrate (1mM) solution, and incubated at room temperature. Only leaf extract without silver nitrate (1 mM) treatment was used as the control and the experiments were carried out in triplicate.

### Detection and characterization of silver nanoparticles

#### Visual observation

Synthesis of silver nanoparticles was detected by change of color from light-green to dark-brown of the filtrate challenged with silver nitrate.

#### By UV-visible spectrophotometer

The synthesis was confirmed with the help of UV-Vis spectrophotometer (Perkin-Elmer, Lambda 25) by scanning the absorbance spectra in the range of 200-800 nm wavelengths.

#### Nanoparticle tracking and analysis system (NTA) (LM 20)

The nanoparticles tracking analysis was carried out by using the liquid sample of silver nanoparticles prepared by diluting with the nuclease-free water and 0.5 ml of diluted sample was injected onto the sample chamber and observed through LM 20. Nanoparticles present within the laser beam path were observed by optical instrument (LM-20, NanoSight Pvt. Ltd., UK) having CCD camera and size of the nanoparticles was measured on the basis of Brownian motion of the particles.

#### TEM analysis

The synthesized silver nanoparticles were also characterized by TEM (Philips, CM 12), on conventional carbon-coated copper grids (400 meshes, Plano GmbH, Germany). For TEM analysis, 5  $\mu\text{L}$  of silver nanoparticles sample was taken, and three images of each sample were selected for the clarification of the composition.

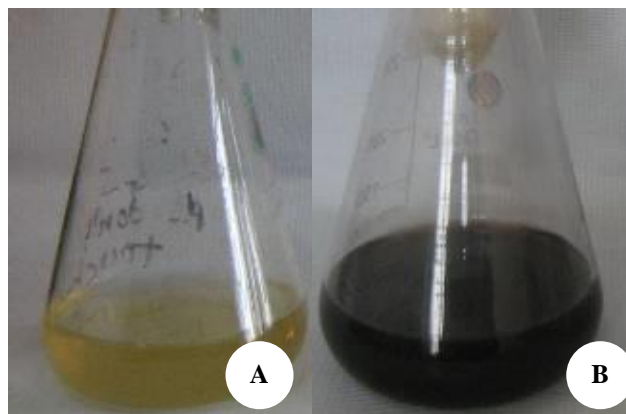
## RESULTS AND DISCUSSIONS

The indication of the silver nanoparticles synthesis was marked by the rapid change in the color of plant extract from light-green to dark-brown after treatment with silver

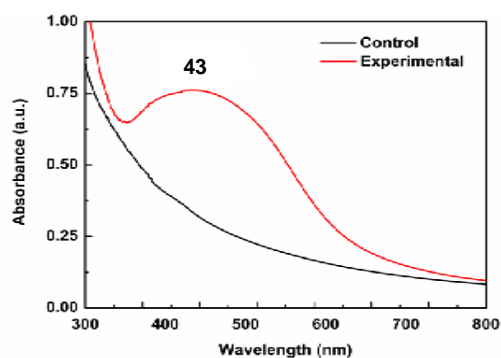
nitrate (1 mM  $\text{AgNO}_3$ ) at room temperature. The color change was due to the reduction of silver ions to silver nanoparticles, i.e.  $\text{Ag}^+$  to  $\text{Ag}^0$  and appearance of brown color indicates Silver nanoparticle synthesis (Figure 1). The conformation of the silver nanoparticle synthesis was done by UV-visible spectrophotometer analysis, which shows the absorbance peak at 438 nm (Figure 2). These observations are similar to those reported by many researchers (Ingle et al. 2008; Raheman et al. 2011).

The dispersion characteristics, i.e. average size and particle size distribution were measured with NTA by NanoSight LM-20. NTA allows individual nanoparticles in a suspension to be microscopically visualized, and based on the Brownian motion the size distribution and average size of the particle were obtained. It shows that the average size of nanoparticles is 40 nm most repeated nanoparticles was of 37 nm, the concentration of the nanoparticles was  $1.47 \times 10^8$  particles/ $\text{mL}^{-1}$ . The particle size distribution histogram and 3-D plot of particle size distribution were shown in Figure 3 and 4, respectively. Particle size and intensity distribution of nanoparticles were shown in Figure 4 which was found to be similar to the results obtained by Montes-Burgos et al. (2010) and Raheman et al. (2011).

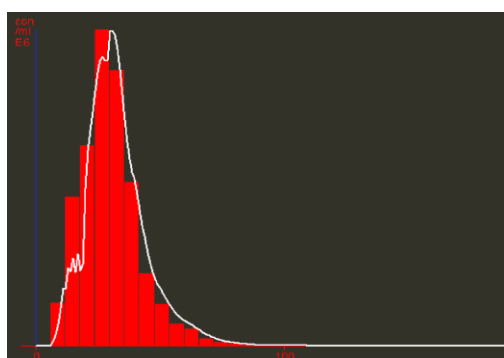
Finally, the synthesis of spherical and polydisperse silver nanoparticles by the leaf extract was confirmed by TEM analysis and it was found that the particles are in the range of 20-50 nm having 30 nm as the average diameter (Figure 5). Even the size predicted by TEM analysis was smaller than predicted by NTA analysis. Generally, NTA analysis resulted in larger particle sizes measurements compared to TEM, which is in agreement with previous work (Farkas et al. 2010, 2011). The differences can be mainly explained by the bias from each specific method (Hasselov et al. 2008). For example, TEM is a number based method has a bias towards the smaller particle sizes compared to NTA which is a number based method but fails to probe the weakly scattering particles (below 10-20 nm) and is consequently mainly measuring the NP aggregates which may explain some of the differences in size measurements.



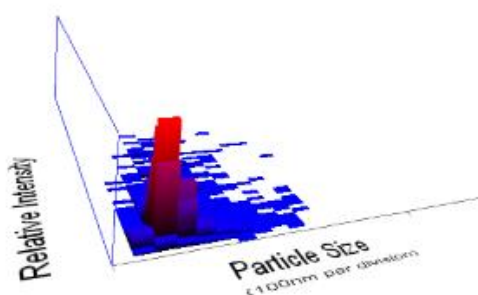
**Figure 1.** Synthesis of silver nanoparticles from leaf extract of *Tagetes erecta* (A) Leaf extract before treatment with  $\text{AgNO}_3$  (control) and (B) Leaf extract after treatment with  $\text{AgNO}_3$  (experimental)



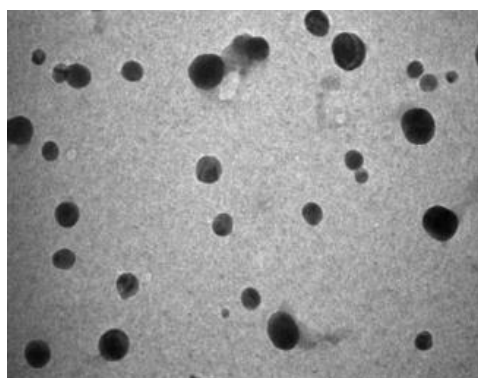
**Figure 2.** UV-visible spectra of leaf extract (showing no peak) and silver nanoparticles showing peak at 438 nm



**Figure 3.** NTA (Nanosight-LM 20) nanoparticle size distribution histogram showing the average size of 37 nm



**Figure 4.** NTA (Nanosight-LM 20) 3-D plot of nanoparticle size distribution



**Figure 5.** TEM micrograph showing synthesis of polydispersed and spherical silver nanoparticle having average size of 30 nm (scale bar 200 nm)

For the development of green synthesis, Raveendran et al. (2003) suggested three main factors in nanoparticle synthesis should be considered, i.e. solvent choice, the use of an environmentally benign reducing agent, and the use of a non-toxic material for nanoparticle stabilization. In the present study, water is used as an environmentally benign solvent, replacing toxic organic solvents from chemical methods and biomolecules from leaf extract of *Tagetes erecta* was used as both reducing and stabilizing agents for green synthesis.

## CONCLUSION

The leaf extract of *Tagetes erecta* has potential of silver nanoparticles synthesis. These synthesized silver nanoparticles are found to be stable. The green synthesis method is not only cost effective but also eco-friendly, simple and efficient than the others, and has appreciable control over size and shape of the nanoparticles.

## REFERENCES

- Bawaskar M, Gaikwad S, Ingle A, Rathod D, Gade A, Duran N, Marcato PD, Rai M, 2010. A New Report on Mycosynthesis of Silver Nanoparticles by *Fusarium culmorum*. *Curr. Nanosci* 6: 376-380.
- Bonde SR, Rathod DP, Ingle AP, Ade RB, Gade AK, Rai MK. 2012. *Murraya koenigii* Mediated Synthesis of Silver Nanoparticles and Its Activity against Three Human Pathogenic Bacteria. *Nanosci Methods* 1: 25-36.
- Chandran SP, Chaudhary M, Pasricha R, Ahmad A, Sastry M. 2006. Synthesis of gold nanotriangles and silver nanotriangles using Aloe vera plant extract. *Biotech Prog* 22: 577-579
- Farkas J, Christian P, Gallego-Urrea JA, Roos N, Hasselov M, Tollefsen KE, Thomas KV. 2010. Effects of silver and gold nanoparticles on rainbow trout (*Oncorhynchus mykiss*) hepatocytes. *Aquat Toxicol* 96: 44-52.
- Farkas J, Christian P, Gallego-Urrea JA, Roos N, Hasselov M, Tollefsen KE, Thomas, KV. 2011. Uptake and effects of manufactured silver nanoparticles in rainbow trout (*Oncorhynchus mykiss*) gill cells. *Aquat Toxicol* 101: 117-125.
- Gade AK, Ingle AP, Whiteley C, Rai MK. 2010a. Mycogenic metal nanoparticles: progress and applications. *BiotechnolLett* 32 (5): 593-600.
- Gade AK, Bonde P, Ingle AP, Marcato PD, Duran N, Rai MK. 2008. Exploitation of *Aspergillus niger* for synthesis of silver nanoparticles, *J Biobased MaterBioener*2: 243-247.
- Gade AK, Gaikwad SC, Tiwari V, Yadav A, Ingle AP, Rai MK. 2010b. Biofabrication of silver nanoparticles by *Opuntia ficus-indica*: *In vitro* antibacterial activity and study of the mechanism involved in the synthesis. *Curr Nanosci* 6: 370-375.
- Gardea-Torresdey J, Gomez E, Jose-Yacamán M, Parsons J, Peralta-Videa J, Tioani H. 2003. Alfalfa sprouts: A natural source for the synthesis of silver nanoparticles. *Langmuir* 19: 1357-61.
- Hasselov M, Readman JW, Ranville JF, Tiede K. 2008. Nanoparticle analysis and characterization methodologies in environmental risk assessment of engineered nanoparticles. *Ecotoxicology* 17: 344-361.
- Huang J, Li Q, Sun D, Lu Y, Su Y, Yang X, Wang H, Wang Y, Shao W, He N, Hong J, Chen C. 2007. Biosynthesis of silver and gold nanoparticles by novel sundried *Cinnamomum camphora* leaf. *Nanotech*18: 11-20.
- Ingle A, Gade A, Pierrat S, Sonnichsen C, Rai M. 2008. Mycosynthesis of silver nanoparticles using the fungus *Fusarium acuminatum* and its activity against some human pathogenic bacteria. *Curr Nanosci* 4: 141-44.
- Ingle I, Gade A, Bawaskar M, Rai M. 2009. *Fusarium solani*: a novel biological agent for the extracellular synthesis of silver nanoparticles. *J Nanopart Res*11: 2079-85.

- Kholoud MM, El-Nour A, Eftaiha A, Abdulrhman AQ, Ammar AA. 2010. Synthesis and applications of silver nanoparticles. Arab JChem 3: 135-140.
- Li S, Qui L, Shen Y, Xie A, Yu X, Zhang L, Zhang Q. 2007. Green synthesis of silver nanoparticles using *Capsicum annum* L. extract. Green Chem 9: 852-858.
- Mandal D, Bolander M, Mukhopadhyay D, Sarkar S, Mukherjee P. 2006. The use of microorganisms for the formation of metal nanoparticles and their application. Appl Microbiol Biotechnol 69: 485-492.
- Montes-Burgos I, Walczyk D, Hole P, Smith J, Lynch I, Dawson K. 2010. Characterisation of nanoparticle size and state prior to nanotoxicological studies. J. NanopartRes12: 47-53.
- Mude N, Ingle A, Gade A, Rai M. 2009. Synthesis of silver nanoparticles using callus extract of *Carica papaya*-A first report. J. Plant Biochem Biotech 18: 83-86.
- Raheman F, Deshmukh S, Ingle A, Gade A, Rai M. 2011. Silver Nanoparticles: Novel Antimicrobial Agent Synthesized from an Endophytic Fungus *Pestalotia* sp. Isolated from Leaves of *Syzygium cumini* (L) Nano Biomed Eng 3 (3): 174-178.
- Rai M, Yadav A, Gade A. 2008. Current trends in phytosynthesis of metal nanoparticles. Crit. Rev Biotechnol 28(4):277-284.
- Raveendran P, Fu J, Wallen SL. 2003. Completely "green" synthesis and stabilization of metal nanoparticles, J Amer Chem Soc, 125(46): 13940-13941.
- Shankar SS, Rai A, Ahmad A, Sastry MJ. 2004. Rapid synthesis of Au, Ag, and bimetallic Au core-Ag shell nanoparticles using Neem (*Azadirachta indica*) leaf broth. J Colloid Interface Sci 275: 496-502.
- Shivshankar S, Ahmad A, Sastry M. 2003. Geranium leaf assisted biosynthesis of silver nanoparticles. Biotechnol Prog 19: 1627-1631.
- Shivshankar S, Rai A, Ahmad A, Sastry M. 2004. Rapid synthesis of Au, Ag, and bimetallic Au core-Ag shell nanoparticles using Neem (*Azadirachta indica*) leaf broth. J. Colloid Interface Sci 275: 496-502.
- Shivshankar S, Rai A, Ahmad A, Sastry M. 2005. Controlling the optical properties of lemongrass extract synthesized gold nanotriangles and potential application in infrared-absorbing optical coatings. Chem Mater 17: 566-572.
- Tenover FC. 2006. Mechanisms of antimicrobial resistance in bacteria. AmJ Medicine119: 3-10.