Extracellular Synthesis of Silver Nanoparticles by Callus of *Medicago sativa*

H. S. Hegazy[1], G. H. Rabie[1], Lamis D. Shaaban[1], Diana S. Raie[2]

[1] Botany Dept., Faculty of Science, Zagazig University, Egypt
[2] Process Design and Development Dept., Egyptian Petroleum Research Institute, Egypt
rabiegam@hotmail.com, hegazyshegazy@gmail.com, lamis_shaaban@yahoo.com, raiediana@yahoo.com

Abstract: Plants supply technology with a green style for making nano-metals. The present article is an experimental trail for synthesis of silver nanoparticles by secretions from *Medicago sativa* callus. Hypocotyls-derived cultured tissues are initiated and then sub-cultured on MS medium supplemented with 3 % sucrose, 2 mg/l 2,4D and 1 mg/l BA. Silver nitrate is used as a bulk material. A whole-cell callus is exposed to aqueous silver nitrate. At room temperature, poly-shaped and poly-sized silver nanoparticles are fabricated. Also, we investigate the effect of pH on the biosynthesis process. At pH 10, there are significant effects on shape and size. The stabilizing agent could be belonging to polyphenol.
[H. S. Hegazy, G. H. Rabie, Lamis D. Shaaban, Diana S. Raie. **Extracellular Synthesis of Silver Nanoparticles by Callus of *Medicago sativa*.** *Life Sci. J* 2014;11(10):1211-1214]. (ISSN:1097-8135). http://www.lifesciencesite.com. 180

Key words: *Medicago sativa*, Callus, Biosynthesis, Nanoparticles, Metal Stress, Silver

1. Introduction:

Using plants for synthesis of nano-silver has achieved an advanced position among its bio-counterparts. From one hand, silver nanoparticles have many biotechnological applications due to their anti-microbial behavior **(Caballero-Díaz *et al.*, 2013)**. From the other hand, phyto-making of nano-metals is a green method; as it is rapid, economic, and safe, and a single step style **(Mude *et al.*, 2009; Satyavani *et al.*, 2011)**. However, it is still facing several problems to be used in industry. One of the main challenges is the hazard of food shortage. Consequently, botanists are trying to find solutions to overcome this risk.

Applying tissue culture techniques in biotechnology has superior advantages over being from plant origins. It allows upstream and downstream processing. It is an incessant source for plant biomass. So, it overcomes the difficulties faced by dealing with perennial and seldom plants. Also, it avoids the cost of harvesting and competition of nutrition. There are many examples for using cultured tissues derived from different explants, such as shoot-tips **(Demissie and Lele, 2013)** and leaf-bits **(Mude et al., 2009)**, leaf **(Asmathunisha et al., 2013)**, rhizomes **(Malabadi et al., 2012 c)**, stem **(Satyavani et al., 2011)**, and seeds **(Lukman et al., 2011)**, for nano-production. Although the variety of plants used for nano-silver fabrication, most of bio-mass used are in the form of cell free extracts, that motivates us to explore a new area of using callus as a whole-cell for silver nanoparticles synthesis **(Malabadi et al., 2012 a; Demissie and Lele 2013)**.

Due to its simplicity of in vitro culturing and richness of anti-oxidant metabolites **(Lukman et al., 2011)**, in the current work, we test the ability of cultured tissue from *Medicago sativa* to biosynthesize silver nanoparticles. In addition, we test the effect of pH, using ammonium hydroxide **(Gorup et al., 2011)** and nitric acid **(Lukman et al., 2011)** for preparing alkaline and acidic conditions, on nano-silver shape and size.

2. Materials and Methods

Surface sterilization and Callus induction:

Seeds of *Medicago sativa* have been kindly provided by Forage Crops Research Section, Field Crops Research Institute of the Agricultural Research Centre, Giza, Egypt. Surfaces sterilized seeds are transferred to sterilized Petri dishes containing moist Whatman No. 3 filter paper and incubated in the dark at 25 ºC for germination. After 7 days, we cut hypocotyls of 3-5 mm and plant them on the callus induction medium. Cultured explants are incubated in growth chamber at constant temperature of 25 ±2 ºC. Under similar conditions of callus initiation from hypocotyls, they are maintained three sub-cultures (each sub-culture after three weeks). Callus is initiated and sub-cultured on MS medium, supplemented with 3 % sucrose, 2 mg/l 2,4 D and 1 mg/l BA. Before 0.8 % agar addition, pH of the medium is adjusted at 5.8 ± 0.2. The semi-solidified medium is autoclaved for 20 min at 121°C for sterilization **(El-Hattab et al., 2005)**.

Biosynthesis of silver nanoparticles:

A known mass of fresh callus of *Medicago sativa* are soaked in non-aerated silver nitrate (0.1 M) at room temperature without light exposure **(Gardea-Torresdey et al., 1999)**. The bulk silver is pH adjusted (pH at 2, 5, 7, 9, 10, and 11) in non-pH adjusted bulk material **(Gorup et al., 2011; Lukman et al., 2011)**. All

reactions are proceeding for 24 h in dark and at room temperature.

Characterization of silver nanoparticles:

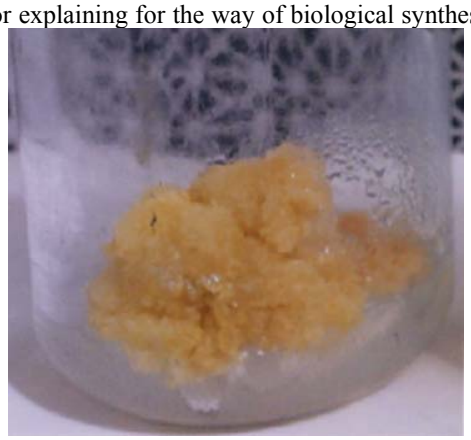
The appearance of a yellowish color in solution is an initial observation of nano-silver formation. The mixtures are subjected for optical measurements using UV-Vis spectrophotometer through examining the spectra between 250 to 800 nm. Reaction solutions are sampled for TEM for morphological analysis. Silver nanoparticle is separated by centrifugation **(Malabadi et al., 2012b)**. The precipitate is washed by deionized water and finally dried to be processed for FTIR analysis **(Satyavani et al., 2011; Demissie and Lele, 2013)**.

Statistical Analysis

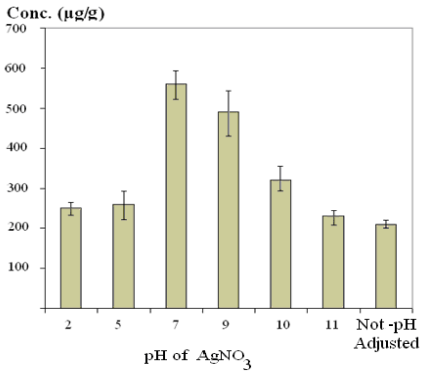
Statistical analysis is done by SPSS 11.0. Samples are analyzed in triplicates. The difference in either shapes or sizes of nano-silver bio-synthesized by callus biomass either with or without pH adjustment of aqueous silver nitrate is calculated by one way ANOVA test.

3. Results and Discussion

In the current study, we present cultured tissue from *M. sativa* as a nano-maker. Callus is initiated and sub-cultured on MS medium supplemented with 2 mg/l of 2,4 D and 1 mg/l BA **(Fig. 1.A)**. Exposing fresh weight of cultured tissue to the aqueous silver nitrate puts callus in a metal stress condition, as a result bio-secretions and/or bio-sorption are expected. The elemental analysis of callus approves the later probability of silver bio-sorption **(Fig. 1.B)**. Also, the gradual changes in silver substrate into yellow with respect to pH support the former possibility of extracellular exudates and increase the chance of nano-silver formation **(Fig. 2)**. The yellowish color is feature for silver nanoparticles as a result of excitation of surface plasmon resonance **(Lukman et al., 2011)**.Bottom-up style is the most preponderant route used for explaining for the way of biological synthesis of nano-colloids. It depends on the ability of reducer to induce nucleation. This is followed by controlled growth of nuclei, and stabilizing the nano-clusters **(Lukman et al., 2011)**. According the previous hypothesis, bio-mass used to fabricate nanoparticles is supposed to have reducers and stabilizers. Moreover, we find, in case of pH adjusted bulk substrate, the darkest yellowish color occurs at pH 10. Samples are subjected to UV-Vis spectrophotometer. Notable, except at pH 2, all samples are yellowish and absorb in visible region. These spectra verify the probability of nano-silver production **(Fig. 3)** and then confirmed by TEM images **(Fig. 4)**. We find multiple shapes including spherical, disk and irregular shapes. In non-pH adjusted case, size ranges from 2 to 50 nm. At pH 10, size ranges from 35 to 40 nm. In addition, the mean of size of nanoparticles produced at both cases are significantly different. They confirm the guess arises from broad UV-visible spectra of being poly-sized and poly-shaped. This variation in size and shape may be due to insufficient concentration of reducers and/or stabilizers as a result of incomplete metabolic pathways of de-differentiated callus cells **(Lukman et al., 2011)**. Reducing agents supposed to be antioxidants secreted in response to metal stress. Biomolecules stabilize nanoparticles are expected using FTIR spectrum. **Fig.5** represents probable stabilizer biomolecules. Bands at 1550, 1635, 2200, and 3221 cm^{-1}which are corresponding to amide linkages between the amino acid residues in proteins, carbonyl groups (C=O) from polyphenols, side chain vibrations consisting of C-H stretching of aliphatic group, polyphenolic OH group **(Asmathunisha et al., 2013; Demissie and Lele 2013)**. Also, it may be due to presence of different reducers with different concentration. However, the significant effect of pH on bio-synthesized nano-silver is a result of the steric effect **(Lukman et al., 2011)**.

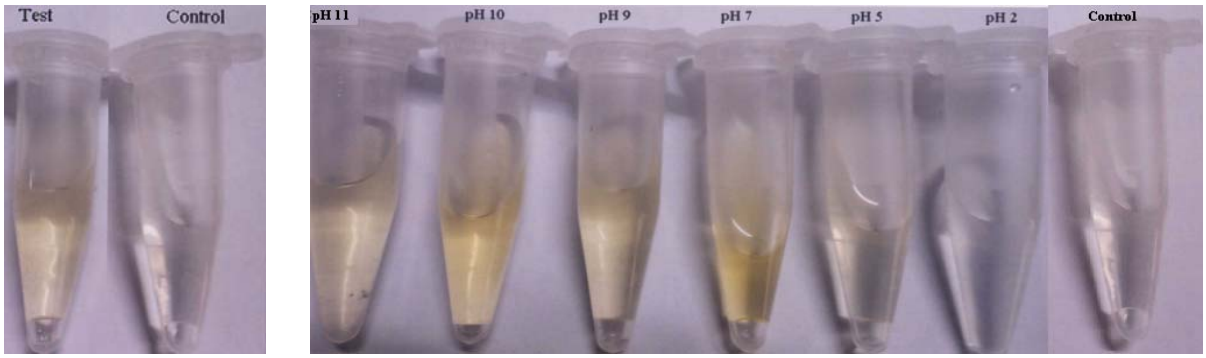


A



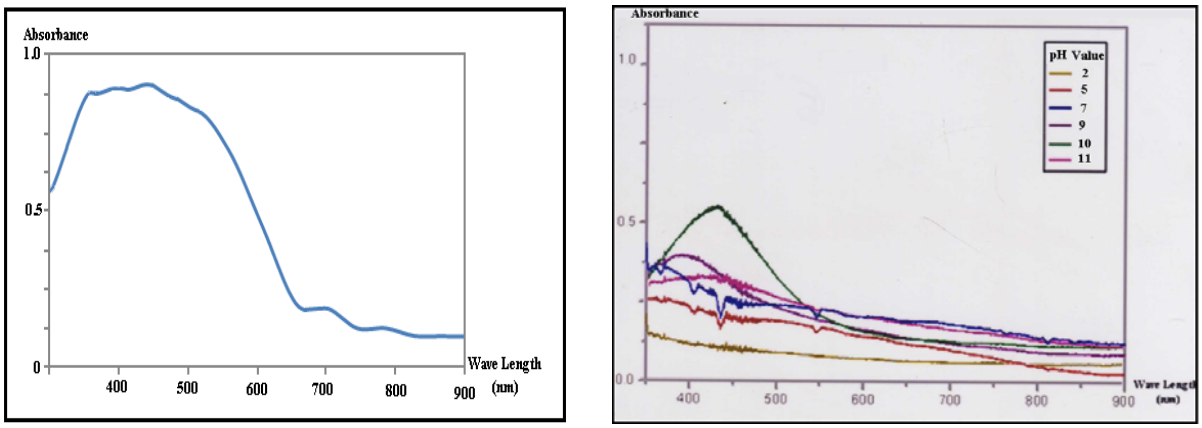
B

Fig. 1 A. Optical image of callus derived from hypocotyls of *Medicago sativa* B. Quantitative elemental analysis of silver absorbed by callus biomass either with or without pH adjustment of aqueous silver nitrate



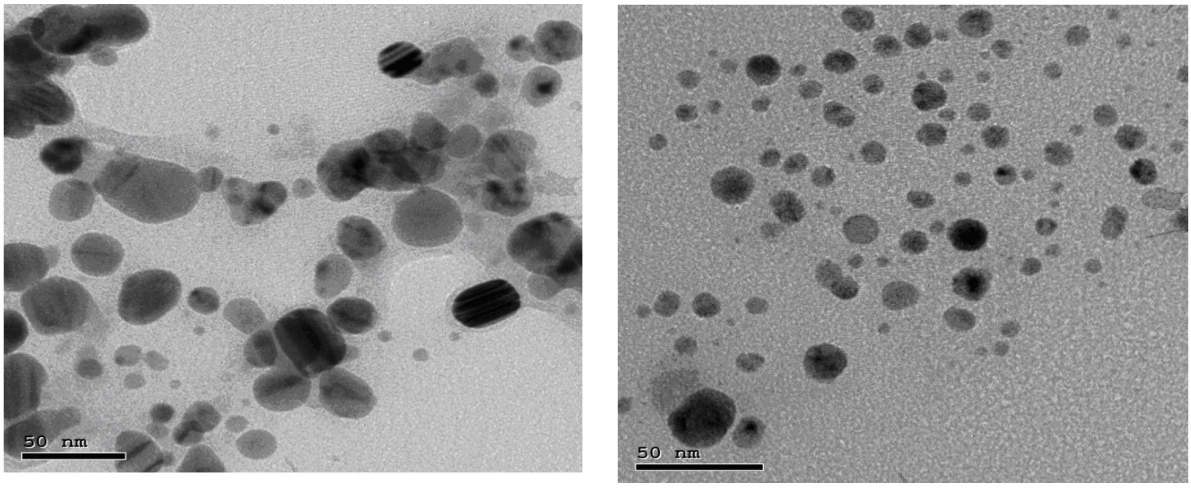
A B

Fig. 2 Yellowish color of nano-silver biosynthesized by callus A. without pH adjustment. B. at different pH



A B

Fig. 3 UV-visible spectra of nano-silver biosynthesized by callus A. without pH adjustment. B. at different pH



A B

Fig. 4 TEM images of nano-silver biosynthesized by callus A. without pH adjustment B. at pH 10

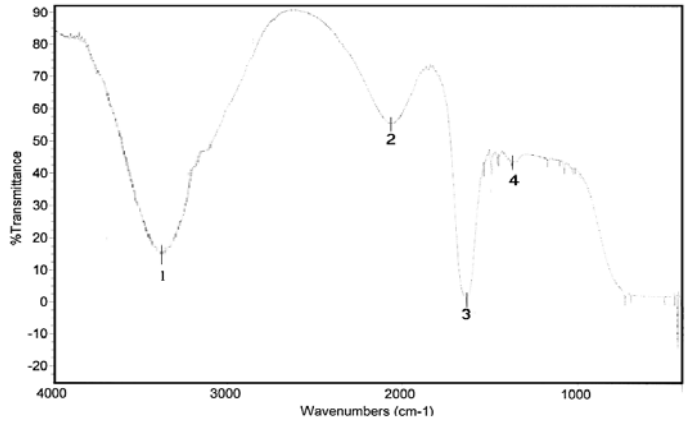


Fig. 5 FTIR spectrum of nano-silver biosynthesized by callus exudates

Conclusion

Medicago sativa callus as a whole cell bio-mass can provide reducers and stabilizers for silver nanoparticles synthesis. At room temperature, nano-silvers are fabricated with variable shapes and sizes. Also, the steric effect resulting from pH can control shapes of nanoparticles and has a significant effect on size. The stabilizer could be belonging to polyphenols. The reducing agent is supposed to be a member of antioxidants.

Acknowledgement

We are acknowledging the Academy of Scientific Research and Technology (ASRT) for funding our current work through Scientists foe Egypt: Next Generation (SNG) program.

References:

Asmathunisha, N. and K. Kathiresan (2013). Rapid biosynthesis of antimicrobial silver and gold nanoparticles by in vitro callus and leaf extracts derived from *Lycopersicon esculentum* mill. *Int. J. Pharm. Bio Sci.,* 4(1): 334-344.

Caballero-Díaz, E.;C. Pfeiffer;L. Kastl;P. Rivera-Gil;B. Simonet;M. Valcárcel;J. Jiménez-Lamana; F. Laborda, and W. J. Parak (2013). The Toxicity of Silver Nanoparticles Depends on Their Uptake by Cells and Thus on Their Surface Chemistry. *Part. Part. Syst. Charact.*, 30: 1079–1085.

Demissie, A. and S. Lele (2013). Phytosynthesis and characterization of silver nanoparticles using callus of jatropha curcas: A biotechnological approach. *Int. J. Nanosci.,* 12(2): 1-7.

El-Hattab, A.; W.M. Shanawy; M.A. Koronfel and W.M. El-Nabawy (2005). Effect of media and varieties on callus formation and plant regeneration of alfalfa (*Medicago sativa* l.). *Egypt. J. Appl. Sci.* 20: 652-670.

Gardea-Torresdey, J. L.; K. J. Tiemann; G. Gamez; K. Dokken'; S. Tehuacanero; and M. Jose-Yacaman (1999). Gold nanoparticles obtained by bio-precipitation from gold (III) solutions. *J. of Nanopart. Res.*,*1*(3), 397-404.

Gorup, L. F.; E. Longo; E. R. Leite and E. R. Camargo (2011). Moderating effect of ammonia on particle growth and stability of quasi-monodisperse silver nanoparticles synthesized by the Turkevich method. *J. of Colloid Interface Sci.*, 360(2): 355-358.

Lukman, A.I.; B. Gong; C.E. Marjo, U. Roessner and A.T. Harris (2011). Facile synthesis, stabilization, and anti-bacterial performance of discrete ag nanoparticles using *Medicago sativa* seed exudates. *J. coll. Interface. Sci.,* 353: 433-444.

Malabadi, R.; N. Meti; G. Mulgund; K. Nataraja and S. Kumar (2012 b). Synthesis of silver nanoparticles from in vitro derived plants and callus cultures of *Costus speciosus* (koen.); assessment of antibacterial activity. *Res. Plant Biol.,* 2: 32-42.

Malabadi, R.B.; R.K. Chalannavar; N.T. Meti; G.S. Mulgund; K. Nataraja and S.V. Kumar (2012 c). Synthesis of antimicrobial silver nanoparticles by callus cultures and in vitro derived plants of *Catharanthus roseus. Res. Pharm.* 2(6): 18-31.

Malabadi, R.B.; S.L. Naik; N.T. Meti; G.S. Mulgund; K. Nataraja and S.V. Kumar (2012 a). Silver nanoparticles synthesized by in vitro derived plants and callus cultures of *Clitoria ternatea*; evaluation of antimicrobial activity. *Res. Biotechnol*., 3(5): 26-38.

Mude, N.; A. Ingle; A. Gade and M. Rai (2009). Synthesis of silver nanoparticles using callus extract of carica papaya-a first report. *J. Plant Biochem. Biotechnol.,* 18**:** 83-86.

Nabikhan, A.; K. Kandasamy; A. Raj and N.M. Alikunhi (2010). Synthesis of antimicrobial silver nanoparticles by callus and leaf extracts from saltmarsh plant, *Sesuvium portulacastrum* l. *Coll. Surface. B: Biointerface.,* 79: 488-493.

Satyavani, K.; T. Ramanathan and S. Gurudeeban. (2011). Green synthesis of silver nanoparticles by using stem derived callus extract of bitter apple (*Citrullus colocynthis*). *Dig. J. Nanomat. & Biostruct.,* 6(3): 1019-1024.