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Original Article

Synthesis of Colloidal Silver Nanoparticle (SNP) Using *Vigna Radiata* Seed Exudate: A Novel Green Method

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Abstract

Synthesis of green silver nanoparticle is carried out by many scientists. Most of them use extracts of plant or microb. In the present research unlike others no extract is used. Rather, seed exudates of *Vigna radiata* secreted during germination is used for synthesizing colloidal silver nanoparticle. The siver nanoparticled were characterized using UV-Vis, fourier transform infrared spectroscopy (FTIR) and transmission electron microscopy (TEM). All these characterization suggested formation of silver nanoparticle comparable with chemical synthesis using sodium borohydrate as reducer.

Keywords: Silver Nanoparticle; Vigna Radiata; Seed; Exudate; Green Synthesis.

Introduction

The seed exudes of *Vigna radiata* contain reducing sugar, proteins and polyphenols like vitexin, isovitexin and a C-glucosylflavonoid, during germination [1]. These chemicals are known to have allelopathic effects [1]. Mungbean is affected by its own toxic exudates or by phytotoxins produced when crop residues decompose in the soil [2]. Exudation of amino acids and sugars from imbibing seeds of *Vigna radiata* was reported by Zheng and Kawabata [3]. These exudates were never used properly by farmers, food industry or hourse hold users.

The environmental impact of the waste generated due to synthesis of nanomaterials is well known [4]. Therefore, research is needed for colloidal silver nanoparticle (SNP) synthesis using green precursors to develop environment friendly processes [5]. Green nanoparticles have gained momentum in pharmaceutical and biomedical applications for their low toxicity [6]. Reports have been published on the use of green reducers for synthesis of colloidal silver nanoparticle [4]. Most of the research on green methods are complicated and uses extracts of plant parts [7, 8, 9]. As we all aware of that plant extract mostly contain high amount of different types of chemical compounds including protein, lipid and charbohydrate. Because of their high content of biomoleculaes they are always prone to become microbial growth media. Another drawback of using plant extracts is their variation of the chemical composition with respect to season and maturity of the source. The nanoparticle is produced at the cost of plant material and therefore the industrial level production might lead to over exploitation of the most suitable plant.

In the view of the above a novel method is designed for synthesizing colloidal silver nanoparticle using exudes of soaked *Vigna radiata* seed. The method did not damage or destroy the seeds, rather, it used up the exudates that comes out of the seed during imbibitions.

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Materials and Methods

Materials

Silver nitrate and $NaBH_4$ (A.R. grade) was obtained from MERCK, India. Seeds of moong bean (*V. radiata*) were purchased from the local market. Double distilled water was prepared in the laboratory.

Protein, Reducing Sugar and Polyphenol Estimation

For the experiment 50 g seeds were surface sterilized as mentioned above and soaked in 200 ml sterile distilled water for 24h. Exudates of 5 ml for protein, 5 ml for reducing sugar and 5 ml for polyphenol estimation were collected at 3rd, 6th, 12th and 24th h of imbibitions. The total volume of the exudates is measured during every collection using a sterile measuring cylinder under sterile condition inside laminar air flow cabinet. These actual volumes were used later for calculating the total amount of protein, reducing sugar and polyphenol exude by seed at a particular time. Protein and reducing sugar estimation was performed using Bradford's and anthrone method respectively. Total polyphenol estimation was done using Cordenunsi *et al.*'s method [28]. In short 500 mL extract was mixed with 2.5 ml 0.2 N Folin's reagent and incubated at room temperature for 5 min followed by addition of 2.0 ml saturated Na_2CO_3 (75 g/L) and incubation for 90 min at 30°C. After incubation, absorption was measured at 765 nm using gallic acid as standard.

Synthesis of Colloidal Silver Nanoparticle Using Sodium Borohydrate

Standard colloidal SNP was prepared following method as described by Phukon et al. [8] using sodium borohydrate as reducer.

Synthesis of Colloidal Silver Nanoparticle using V. Radiata Seed Exudate

Two methods were followed for synthesis of silver nanoparticle using *Vigna radiata* seeds. In the first method surface sterilized *Vigna radiata* seeds (5, 10, 20, 30 and 40 numbers with average weight 61.0 ± 1.1 mg/seed) were soaked in 20 ml $AgNO_3$ (0.001M) solution under sterile condition. Hour wise observations were made using UV-Vis spectrophotometer (Multiscan Go 151001063C of Thermo Scientific, USA). In the second method we would like to confirm further that seeds are secreting exudates to the solution and they are responsible for silver nanoparticle synthesis. To proof the same we soaked 10 seeds (weight and surface sterilization as specified above) in 20 ml sterile double distilled water. Seeds were allowed to secret exudates to the soaked distilled water for 24 h. Ten (10) ml of $AgNO_3$ (0.001M) solution was added drop wise to the decanted seed less exudates solution. Incubated in dark at 25°C and UV-V is spectrophotometric reading were recorded at 5th and 24th hour.

Characterization

FTIR analysis of the colloidal SNP formed was performed after centrifuging, drying and mixing the pellet with KBr (FTIR grade). The SNP/KBr pellet was analyzed using a Nicolet FTIR machine. Transmission electron microscopic (TEM) examination of silver nanoparticle was done using model no. JEM-100CX II (JEOL Japan).

Results and Discussions

The protein, reducing sugar and polyphenol estimation of the seed exudate is presented in the Fig. 1. As presented in the figure the seed exudate contains lowest crude protein, highest reducing sugar and intermediate amount of total polyphenols. Slope of the lines presented in the figure suggest that rate of exudation of protein and reducing sugar increases at 6 h and then remains constant from 6-12 hrs. The same for polyphenol remains constant up to 12 h and increases slightly after that.

It was observed that the colour of $AgNO_3$ solution with *V. radiata* seeds started turning to golden yellow at 10 h when compared with colourless controls. These observations when analyzed using UV-Vis spectrophotometre (200-800 nm). The $AgNO_3$ solution with *V. radiata* seeds showed the presence of a hump near 441 nm suggesting formation of colloidal SNP (Figure 2a). The same finding when further observed using TEM silver nanoparticles formation is confirmed (Figure 5c). As observed in Figure2a, the absorption (A.U.) of 5 seeds in $AgNO_3$ is half than that of SNP ($NaBH_4$) at 10th h after incubation. These suggest that *V. radiata* seeds exudates are either weak reducer or less amount of exudate secretion than required to reduce all $AgNO_3$ in the solution. The hour wise SNP formation by the exudates of 5 seeds from 0 to 10th h was presented in Figure 2b. The additional peaks observed near 250 nm and 350 nm might be due to seeds exudate of *V. radiata* and did not correspond to SNP (Figure 2a, 5 seeds in distilled water). It has been observed that deep black opaque precipation (SNP) was formed in solution inoculated with 20, 30 and 40 seeds. Black SNP is often seen in colloidal SNP synthesis due to formation of $AgNO_3$ [9]. Since most of the SNP got precipitated due to formation of $AgNO_3$ therefore no peaks corresponding to SNP can be observed in Figure 3A, neither they are following seed number corresponding optical density for SNP. Secretion of K^+ ions by *V. radiata* is reported by Promila and Kumar [10]. Therefore, it might be suggested that at high numbers of seeds (20 and above seeds in 20 ml solution) might secret enough amount

of K^+ and other ions leading to aggregation of SNP and therefore obtained as blue precipitate as referred by Caro et al. [9]. The aggregation is further confirmed by TEM analysis as shown in Figure 5b as compared to Figure 5a (SNP synthesized using $NaBH_4$). Whereas, in the present experiment it has been observed that 10 seeds in 20 ml of $AgNO_3$ (0.001M) did not provide any blue precipitate of SNP. On comparing the 10 seeds treatment with 5, at 10^{th} h it can be seed from Figure 2a and Figure 3a that SNP formation is less in 10 seeds, which might be due to secretion of K^+ and other ions and their interference with other reducing exudates of seeds. Therefore, it has beed concluded that 5 seeds in 20 ml $AgNO_3$ (0.001M) solution is optimum to provide SNP. On allowing the treatments (5 and 10 seeds) to stand upto 24^{th} hour we found that in case of the later SNP absorption becomes double against stady absorption of the former (Figure 3b). This suggest that 10 seeds treatment might be slow in SNP synthesis at 10^{th} h but at 24^{th} h they can provide SNP comparable to $NaBH_4$ based synthesis (Figure 3b and Figure 2a).

Looking into the potency of 10 seeds at 24^{th} h for the second method, exudate is collected (as specified in materials and methods section) and allowed for reaction with 10 ml (0.001M) $AgNO_3$ solution. The spectrophotometric observation of the reaction was made at 5^{th} and 24^{th} h and found to be producing SNP (at 24^{th} h) equivalent to $NaBH_4$ (Figure 4a and Figure 2a). The formation of SNP was confirmed using TEM analysis of the sample (Figure 5d). This results confirm that major SNP is synthesized by the seed exudate and not by a process of internalization of $AgNO_3$ and then secretion of SNP [7].

To confirm the utilization of $AgNO_3$ by different numbers of seeds (5, 10, 20, 30 and 40) and $NaBH_4$, Fourier transform infrared spectroscopy (FTIR) analysis was performed (Fig. 4b). The pattern of the FTIR analysis confirms the complete utilization of $AgNO_3$ by 40 seeds [11]. As seen in the figure the signature of the $AgNO_3$ decreases with increase in the number of *V. radiata* seeds in a constant volume of 20 ml. For 40 seeds the FTIR patter is almost similar like $NaBH_4$ reduced silver nanoparticle.

TEM examination of SNP prepared using $NaBH_4$, black SNP precipitate (20 seeds in 20 ml $AgNO_3$ of 0.001M), 5 seeds in 20 ml $AgNO_3$ solution and SNP synthesis using seed less exudates (second method) are presented in Fig. 5 a, b, c and d, respectively. It can be seen that all SNP are mixture of spherical, elongated, triangular, tetragonal and hexagonal in shapes. The triangular SNP is not observed in $NaBH_4$ based method (Fig. 5a). The size of the SNP varies from several 7 nm small SNP, few 14 nm medium SNP to 52 nm large SNP in $NaBH_4$ method (Fig. 5a).

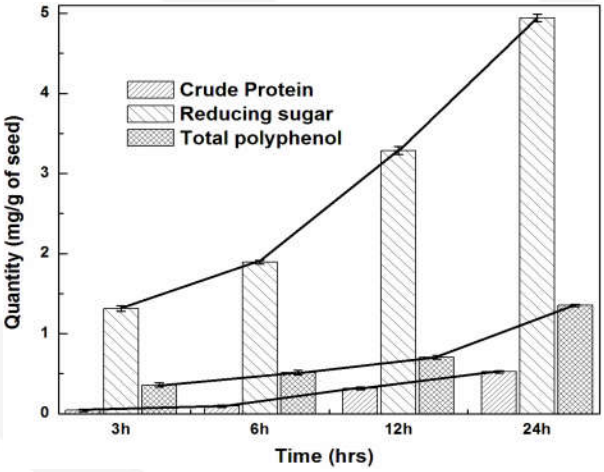

Fig. 1: Crude protein, reducing sugar and polyphenols content of the seed exudate/g of seed with respect to four different soaking time

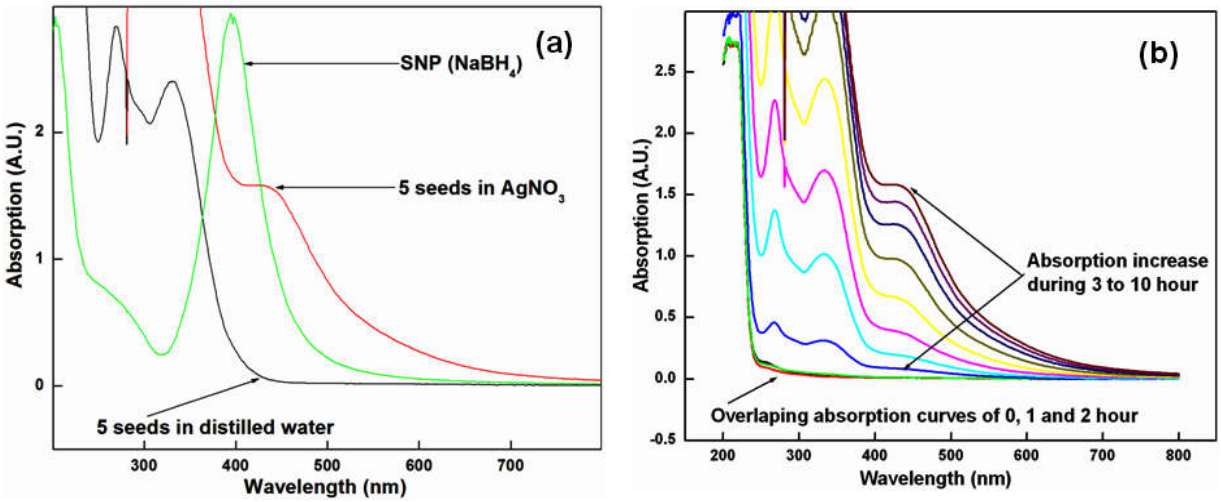

Fig. 2: UV-Vis absorption pattern of SNP synthesized by 5 seeds soaked in 20 ml 0.001M $AgNO_3$ solution **(a)** at 10^{th} h with positive and negative control; **(b)** from 0 to 10^{th} h

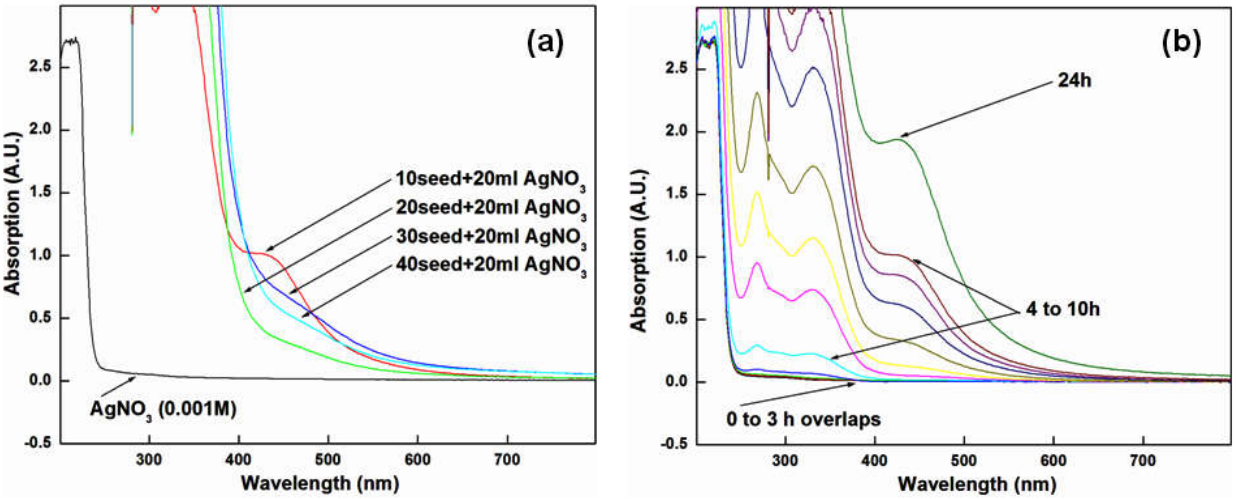

Fig. 3: UV-Vis absorption pattern of SNP synthesized by 10, 20, 30 and 40 seeds soaked in 0.001M $AgNO_3$ solution **(a)** at 10^{th} h along with a blank; **(b)** for 10 seeds only from 0 to 24^{th} h

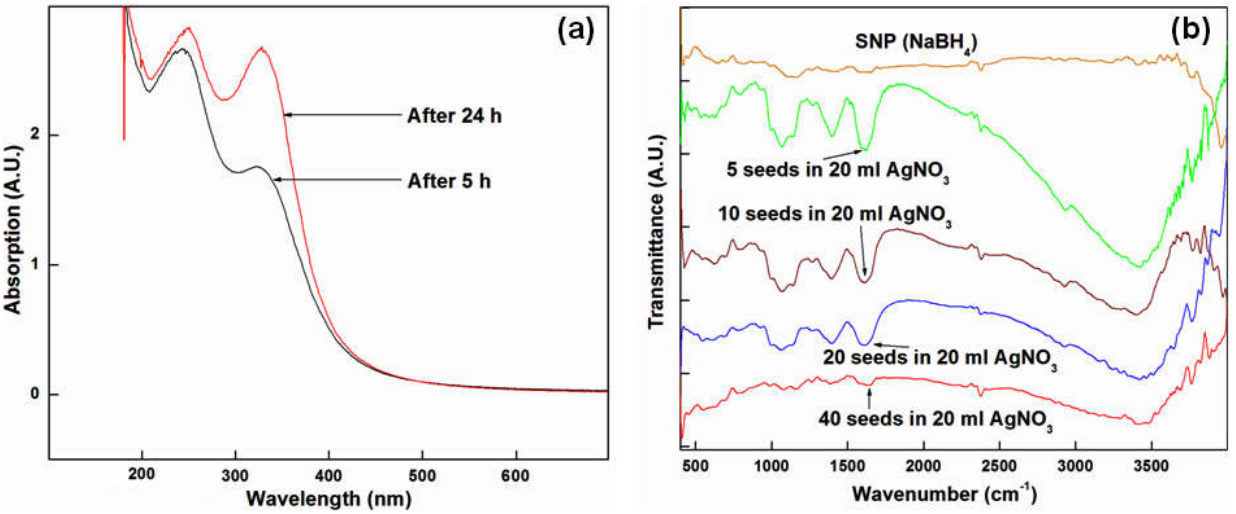

Fig. 4: (a) UV-Vis absorption pattern of SNP synthesized using seed free exudate after 5th and 24th h; **(b)** FTIR spectroscopic patterns of SNP synthesized using different number of seeds (5-40) and $NaBH_4$

The same in case of blue precipitate solution of SNP are 11 nm small, 20 nm medium and 75 nm big SNP (Figure 5b). It should be noted that small sized SNP (around 11 nm) are less in blue SNP solution, large (around 75 nm) and medium (around 20 nm) sized SNP are more in Figure 5b. Further, the aggregation of SNP as suggested from visual observation can be seen in the Figure 5b. The aggregation seen in the Figure 5b is mostly among large SNP with large and small. Most of the medium and small SNP do not aggregate. In case of the SNP produced by 5 seeds the small (around 3 nm) and medium (28 nm) SNP are of equal proportions and few large (around 42 nm) has been seen (Figure 5c). The SNP synthesized using second method (seed free exudate) some aggregation seen towards lower left side (Figure 5d). The small (around 10 nm), medium (around 20 nm) and large (around 41 nm) are seen in Figure 5d. Two hexagonal particles were seen (marked with arrow) in seed free exudate based method (Figure 5d) which were not present in other (Figure 5a, b, c).

The results established that colloidal SNP synthesis can be carried out using *Vigna radiata* seed exudes. Reports regarding synthesis of SNP using seed exudate during soaking is rare. Most of the time the justification for green synthesis is to increase biocompatibility, economic viability and use of renewable resources but rarely it is bio waste. The present research is only a preliminary investigation with one dicot species and huge amount of work like purification of the seed exudate, characterization of compounds and their individual potential for synthesis of SNP have to be studied in future. With increasing research on SNP synthesis from green precursor along with rapid industrial applications, will soon become cause of exploitation of certain

species in near future. Under, these circumstance more research on bio waste based method for SNP synthesis can prevent consumption of biomass for SNP synthesis and these will be available for other purposes like food and energy.

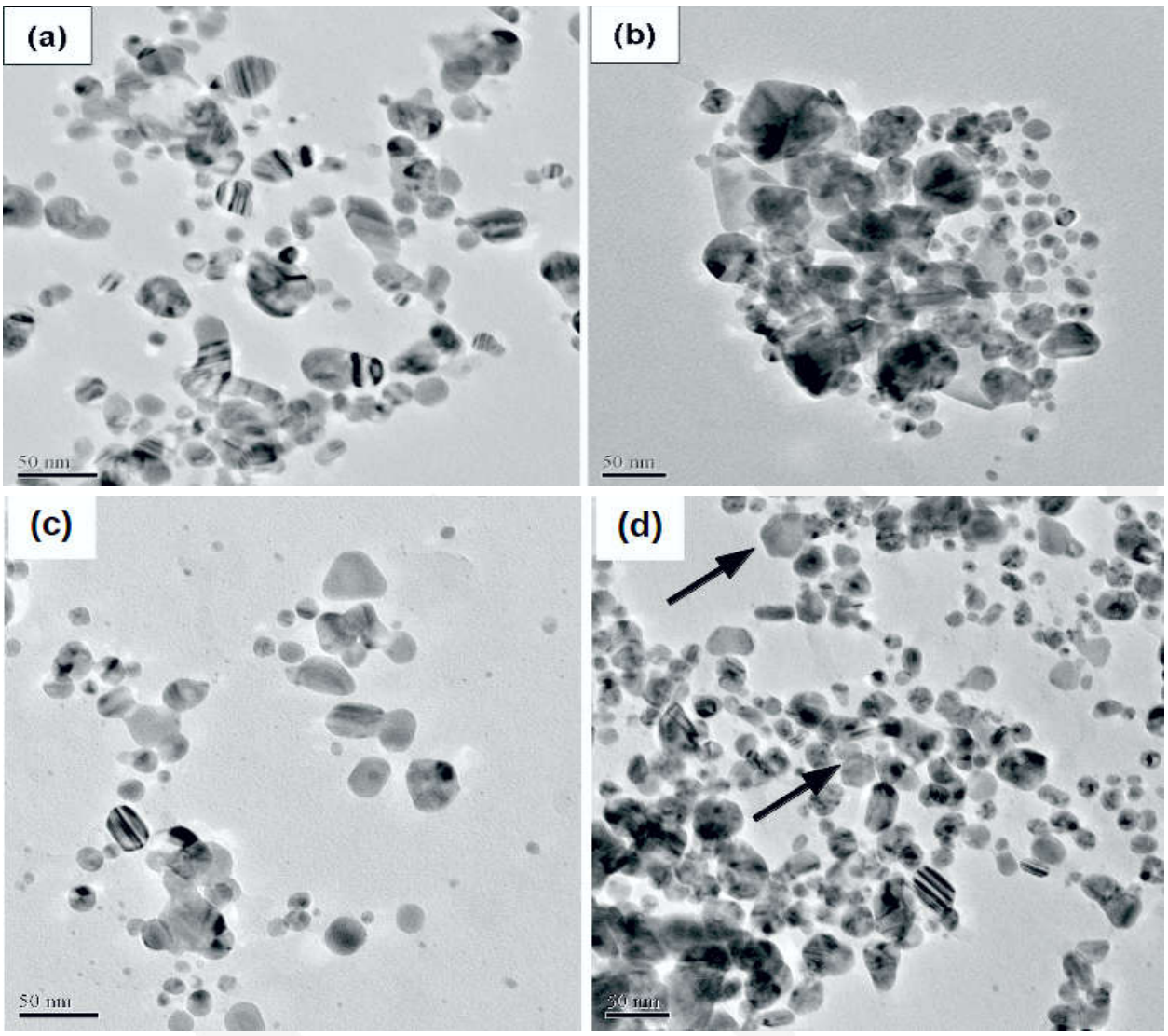

Fig. 5: TEM images of the SNP synthesized using **(a)** $NaBH_4$; **(b)** 20 seeds; **(c)** 5 seeds; **(d)** seed free exudate.

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References

1. C.S. Tang, B. Zhang, Qualitative and quantitative determination of the allelochemical sphere of germinating mung bean, in: Putnam, A.R., Tang, C.S. (Eds.), The science of allelopathy. John Wiley & Sons Inc., New York. 1986; pp. 229-242.
2. S. Lertmongkol, E. Sarobol, C. Premasthira. Allelopathic Effects of Mungbean (Vigna radiata) on Subsequent Crops . Kasetsart J. (Nat. Sci.) 2011; 45: 773-779.
3. S. Zheng, M. Kawabata, Exudation of amino acids and sugars from imbibing seeds of several leguminous crops. Japan. J. Cro. Sci. 2000; 69: 380-384.
4. C. Marambio-Jones, E.M.V. Hoek, A review of the antibacterial effects of silver nanomaterials and potential implications for human health and the environment. J. Nanopart. Res. 2010; 12: 1531–1551.
5. X. Yang et al., Green synthesis of palladium

nanoparticles using broth of Cinnamomum camphora leaf. J. Nanopart. Res. 2010; 12: 1589-1598.

6. A. Ingle, M. Rai, A. Gade, M. Bawaskar, Fusarium solani: a novel biological agent for the extracellular synthesis of silver nanoparticles. J Nanopart. Res. 2009; 11: 2079–2085.
7. J.L. Gardea-Torresdey, E. Gomez, J.R. Peralta-Videa, J.G. Parsons, H. Troiani, M. Jose-Yacaman, Alfalfa Sprouts: A Natural Source for the Synthesis of Silver Nanoparticles. Langmuir. 2003; 19: 1357-1361.
8. V. Dhand, L. Soumya, S. Bharadwaj, S. Chakra, D. Bhatt, B. Sreedhar, Green synthesis of silver nanoparticles using Coffea arabica seed extract and its antibacterial activity. Materials Science and Engineering. 2016; C58: 36-43.
9. S. Patra, S. Mukherjee, A. K. Barui, A. Ganguly, B. Sreedhar, C. R. Patra, Green synthesis, characterization of gold and silver nanoparticles and their potential application for cancer therapeutics. Materials Science and Engineering. 2015; C53: 298-309.
10. P. Phukon, J.P. Saikia, B.K. Konwar, Enhancing the stability of colloidal silver nanoparticles using polyhydroxyalkanoates (PHA) from Bacillus circulans (MTCC 8167) isolated from crude oil contaminated soil. Coll. & Surf. 2011; B86: 314-318.
11. C. Caro, P.M. Castillo, R. Klippstein, D. Pozo, A.P. Zaderenko. Silver Nanoparticles: Sensing and Imaging Applications, in: D. P. Perez (Eds.), Silver Nanoparticles. InTech, Croatia, 2010; pp. 201-223.
12. K. Promila, S. Kumar. *Vigna radiata* seed germination under salinity. Biola. Plantm. 2000; 43: 423-426.
13. K. Gogoi, J.P. Saikia, B.K. Konwar, Immobilizing silver nanoparticles (SNP) on Musa balbisiana cellulose, Coll. & Surf. 2013; B102: 136-138.