

Original Research Article

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Effect of Biosynthesized Silver Nanoparticles using *Achyranthes aspera* Roots on Seed Quality Parameters of Groundnut

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ABSTRACT

Nanotechnology is considered as an emerging technology due to the possibility to advance well-established products and to create new products with totally new characteristics. Biosynthesized silver nanoparticles (Ag NPs) using *A. aspera* roots and standard Ag NPs were characterized by zetasizer, UV-Visible spectrophotometer and scanning electron microscope (SEM). Efficacy of biosynthesized and standard Ag NPs was attributed on quality parameters of Groundnut seed. The average particle size of Ag NPs was 50.37 nm (Standard) and 23.21 nm (Biosynthesized). The characteristic absorbance peak was observed at 407.40 and 420.80 nm for standard and biosynthesized Ag NPs, respectively. SEM images revealed that, both the standard and biosynthesized Ag NPs were spherical in shape. Ag NPs at 150 ppm was found best in enhancing the seed quality parameters such as germination per cent, speed of germination, root length, shoot length, etc. The studies also revealed that, the effect of biosynthesized Ag NPs was on par with the standard Ag NPs in enhancing the groundnut seed quality. Hence, biosynthesized Ag NPs could be used as a new potential alternative for seed dormancy breaking in groundnut.

Keywords

Biosynthesis,
Uttarani,
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Introduction

Nanotechnology is a broad interdisciplinary area of research, development and industrial activity which has grown very rapidly all over the world for the past decade. It is considered as an emerging technology due to the

possibility to advance well-established products and to create new products with totally new characteristics and functions in a wide range of applications. Nanoscience studies the phenomena, properties and responses of materials at atomic, molecular and macromolecular scales in general at sizes

between 0.1 and 100 nm (Bhushan, 2004). Nanomaterials often show unique and considerably changed physical, chemical and biological properties compared to their macro scaled materials (Sharma *et al.*, 2009).

Their performance depends critically on their size, shape and composition (Sathyavathi *et al.*, 2010).

An array of physical, chemical and microbial methods has been used for synthesis of noble metal nanoparticles of particular shape and size (Balagurunathan *et al.*, 2011).

Green synthesis provides advancement over chemical and physical methods as it is cost effective, environment friendly, easily scaled-up and further there is no need to use toxic chemicals, high pressure and energy.

Groundnut (*Arachis hypogaea*) is a species in the legume or "bean" family. Groundnut seed is usually stored for a period of 9 to 12 months before sowing.

It is stored as unshelled pods and as kernels for different purposes. Both forms are vulnerable to attack by a *plethora* of storage pest after harvest.

However, seed viability is getting lost quickly due to the production of free radicals by lipid peroxidation during storage.

The present technologies available to prolong the vigour and viability of groundnut seeds are not satisfactorily alleviating the practical problem. So an alternative simple and practicable seed treatment to control seed deterioration of groundnut is needed (Shyland Natarajan, 2014).

Silver nanoparticles may be an alternative to control growth of insects and pests during storage (Al-Othman *et al.*, 2014).

Materials and Methods

The experiments were carried out at Centre for Nanotechnology, Department of Processing and Food Engineering and at Seed unit, UAS, Raichur.

Materials

The *Achyranthes aspera* (locally called as *Uttarani*) roots were collected from University of Agricultural Sciences, Raichur. Groundnut seeds were collected from APMC, Raichur.

Standard silver nanoparticles were procured from Sisco Research Laboratories Pvt. Ltd., Mumbai, India.

Biosynthesis of silver nanoparticles using *Achyranthes aspera* roots

Initially, To prepare plant extract, 5 g of dried root powder and 100 ml of distilled water was heated together at 60 °C for 30 min in water bath and filtered through Whatman filter paper No. 1.

The filtrate was stored at 4°C for further experiments (Kalidasan and Yogamoorti, 2014). The root extract of *A. aspera* (10 ml) was diluted with distilled water (90 ml). Further, 1.5 mM AgNO₃ (100 ml) solution was prepared and stored.

Prepared diluted plant root extract (100 ml) and AgNO₃ solutions were heated at 60 °C for 30 min in water bath, cooled and kept for further use.

For synthesis of silver nanoparticles, 85 ml of AgNO₃ solution was added to 15 ml of prepared plant root extract. The mixture was heated (45 °C, 1 h) until chemical reaction took place resulting in colour change in the reactants from pale yellow to dark brown.

The appearance of brown colour indicated the formation of silver nanoparticles (Plate 2) (Kalidasan and Yogamoorti, 2014).

Characterization of biosynthesized and standard silver nanoparticles

Characterization of the Standard and biosynthesized Ag NPs obtained using *A. aspera* root extract was performed. Zetasizer (ZETA Sizer, nano383, Malvern, England) was used to measure average particle size (nm) of Ag NPs. (Das *et al.*, 2014).

UV-Visible spectrophotometer (Schimadzu, UV-1800, Kyoto, Japan) to check the absorbance of the Ag NPs was employed (Habibi *et al.*, 2017). The morphological features of biosynthesized Ag NPs were studied by using scanning electron microscope (SEM) (Carl Zeiss Microscopy, EVO 10, Cambridge, UK). Magnification can be adjusted from about 1 to 30,000 times to get clear morphology of silver nanoparticles at the accelerating voltage of 5 to 30 kV with working distance at 10 mm (Joseph *et al.*, 2016).

Priming of groundnut seeds

The standard and biosynthesized silver nanoparticles were dissolved at different concentrations (0, 25, 50, 75, 100, 125 and 150 ppm) in gum arabica and in de-ionized water solution, respectively.

Cleaned groundnut seeds were subjected to priming by soaking in silver nanoparticles solution at 1.00: 0.30 seed to solution ratio for about 4 hours.

The treated seeds were dried under the shade until seeds reached the moisture content of 7 ± 1 % (Khalaki *et al.*, 2016). The seed quality parameters were determined by following the standard procedure described below.

Seed germination test

Seed germination test was carried out by paper towel method as prescribed by International Seed Testing Association (ISTA, 2013). Soaked (30 min) germination papers were used for germination test to keep the seeds moist. Fifty seeds were placed on germination paper in zig-zag manner and rolled from both sides. Likewise, four replications were made for each treatment. The rolled towels with seeds were secured with rubber band and placed in walk in seed germinator (25 ± 2 °C temperature and 90 ± 5 % RH). The number of seeds germinated from each replication were counted daily up to 10 days and remaining seed parameters like root length, shoot length, seedling dry weight and vigour index I were taken on 10th day.

Percent germination and Speed of germination

The number of seeds germinated in each treatment was counted then, germination percentage and Speed of germination was calculated using the following formulas (ISTA, 2013).

$$\text{Seed germination(\%)} = \frac{\text{Number of normal seedings}}{\text{Total number of seeds}} \times 100$$

$$\text{Speed of germination} = \frac{X_1}{Y_1} + \frac{X_2 - X_1}{Y_2} + \dots + \frac{X_n - X_{n-1}}{Y_n}$$

Where,

X_n = No. of seeds germinated on nth day

Y_n = No. of days from sowing on nth day

Root length and shoot length

The root length was measured from the tip of primary root to the base of hypocotyls with the

help of the scale and the mean root length was expressed in centimetres (ISTA, 1993). The shoot length was measured from the base of primary leaf to the base of hypocotyls and the mean shoot length was expressed in centimetres (ISTA, 1993).

Seedling dry weight

Ten randomly selected seedlings were taken in butter paper and dried in hot air oven at 70 °C for 24 h.

Then, the seedlings were removed and allowed to cool in desiccators for 30 min before weighing in an electronic balance.

The average weight was calculated and expressed in milligrams (Almutairi and Alharbi, 2015)

Vigour index I

Vigour index I was worked out by multiplying the per cent germination (%) and mean seedling length (cm) as follows (Abdul-Baki and Anderson, 1973).

Seedling vigour index I = Per cent germination (%) × Mean seedling length (cm).

Results and Discussion

During synthesis, addition of root extract of *A. aspera* into the aqueous solution of silver nitrate led to the change in the colour of the solution from pale yellow to dark brown within reaction duration.

This might be due to the reduction of Ag⁺ ions, indicating the formation of Ag NPs. Excitation of the surface plasmon resulted strong light scattering by the electric field at a wavelength resonance which made the pale yellow solution turned to dark brown colour.

This might be due to the presence of bioactive compounds such as polyphenols, terpenoids, flavonoids, carbohydrates, vitamins and trace elements present in the plant extract played an important role in reduction of silver nanoparticles (Sivakumari *et al.*, 2018).

Characterization of silver nanoparticles

Zetasizer

The characterization of standard and biosynthesized silver nanoparticles was done in terms of average particle diameter from the intensity distribution analysis by using zetasizer. The size distribution histogram of zetasizer indicated that, the size of standard and biosynthesized silver nanoparticles was 50.37 and 23.21 nm, respectively (Fig. 1). The variation in particle size was probably due to change in climatic conditions during biosynthesis (Zainala *et al.*, 2013).

These results are in good agreement with (Kalidasan and Yogamoorti, 2014) who reported that, the size of biosynthesized Ag NPs using *A. aspera* root extract was 105 nm. Earlier it was reported that, an average particle size of biosynthesized silver nanoparticles were 19.60 and 25.50 nm using *Pongamea pinnata* seed and *Achyranthes aspera* leaf extract, respectively (Beg *et al.*, 2016, Bobbu *et al.*, 2016).

UV-Visible spectrophotometer

The reduction of Ag NPs in the aqueous solution of the silver complex during the reaction with the root extract of *A. aspera* was confirmed by the UV-Visible spectra. From Fig. 2, it is observed that, the surface plasmon resonance band was located at wavelength of 407.40 and 420.80 nm for standard and biosynthesized Ag NPs, respectively. This observed intense band was attributed due to the excitation of free electrons in the

nanoparticles which indicated the presence of silver nanoparticles. Characteristic absorption peak at 413 nm for biosynthesized Ag NPs using *A. aspera* was reported earlier (Kalidasan and Yogamoorti, 2014).

It was also reported that, SPR band located at wavelength 450 nm for biosynthesized silver nanoparticles using *Achyranthes aspera* (Sivakumari *et al.*, 2018).

Scanning Electron Microscope (SEM)

The clear magnified (8.07 KX) SEM image at the accelerating voltage of 10.00 kV with working distance of 9.50 mm, showed that, uniformly distributed standard and biosynthesized silver nanoparticles were in spherical shape (Fig. 3).

This might be due to the availability of different quantity and nature of capping agents present in the leaf extract (Srirangam and Rao 2017).

The present results are in good agreement with the findings of Sivakumari *et al.*, 2018, Allafchian *et al.*, 2016 and Premasudha *et al.*, 2015 for biosynthesized Ag NPs (spherical shape) using *A. aspera*, *Phlomis* leaf extract and *Eclipta alba* leaf extract as reducing agent, respectively.

Effect of standard and biosynthesized silver nanoparticles on seed quality

parameters of groundnut seeds

Groundnut seeds when treated with standard and biosynthesized Ag NPs in different concentrations significantly outperformed compared to control in terms of per cent germination, speed of germination, shoot length, root length, seedling dry weight and vigour index. Significant differences were also observed between the doses.

Percent germination and speed of germination

Per cent germination and speed of germination of the groundnut seeds increased with increasing the concentration of standard and biosynthesized Ag NPs as compared to control (Table 1).

It is noticed that among all the treatments, 150 ppm recorded the maximum germination (91.75 %) and speed of germination (26.49). In all treatments, germination percentage and speed of germination of standard Ag NPs was on par with the biosynthesized Ag NPs. The reason for rapid germination could be due to the penetration of nanoparticles into the seed coat facilitating the influx of water inside the seed and activated the enzymes in early phase, thereby enhancing the speed of germination (Sridhar, 2012). Almutairi and Alharbi, 2015 found that, Ag NPs at 2000 ppm had increased germination speed (1.59 seeds/ day) for watermelon over the control (0.85 seeds/day).

Root length and shoot length

Nanoparticle treated germinated seeds exhibited maximum root and shoot length than control (Table 2).

Standard and biosynthesized Ag NPs treated seeds at 150 ppm induced maximum root length *i.e.*, 22.55 and 22.10 cm, respectively compared to control (15.81 cm). In all the treatments, biosynthesized Ag NPs showed on par results with standard Ag NPs (Table 2). Also, standard and biosynthesized Ag NPs proved best by giving maximum shoot length (6.15 and 5.82 cm, respectively) at the same dosage. A positive effect of Ag NPs on seedling growth of *V. radiata* was observed due to the enhanced uptake of water and nutrients by the treated seeds (Koizumi *et al.*, 2008).

These results were in good agreement with Pandey *et al.*, 2014 who observed the maximum root length (6.50 cm) due to the application of Ag NPs (100 ppm) on *Brassica*

Juncea, The application of Ag NPs (1000 ppm) on onion seeds showed increased shoot length (7.50 cm) over the control (5.40 cm) (Anandaraj and Natarajan, 2017).

Plate.1 *Achyranthes aspera* root powder



Plate.2 Biosynthesized silver nanoparticles



Fig.1 Average particle size of a) standard and b) biosynthesized silver nanoparticles

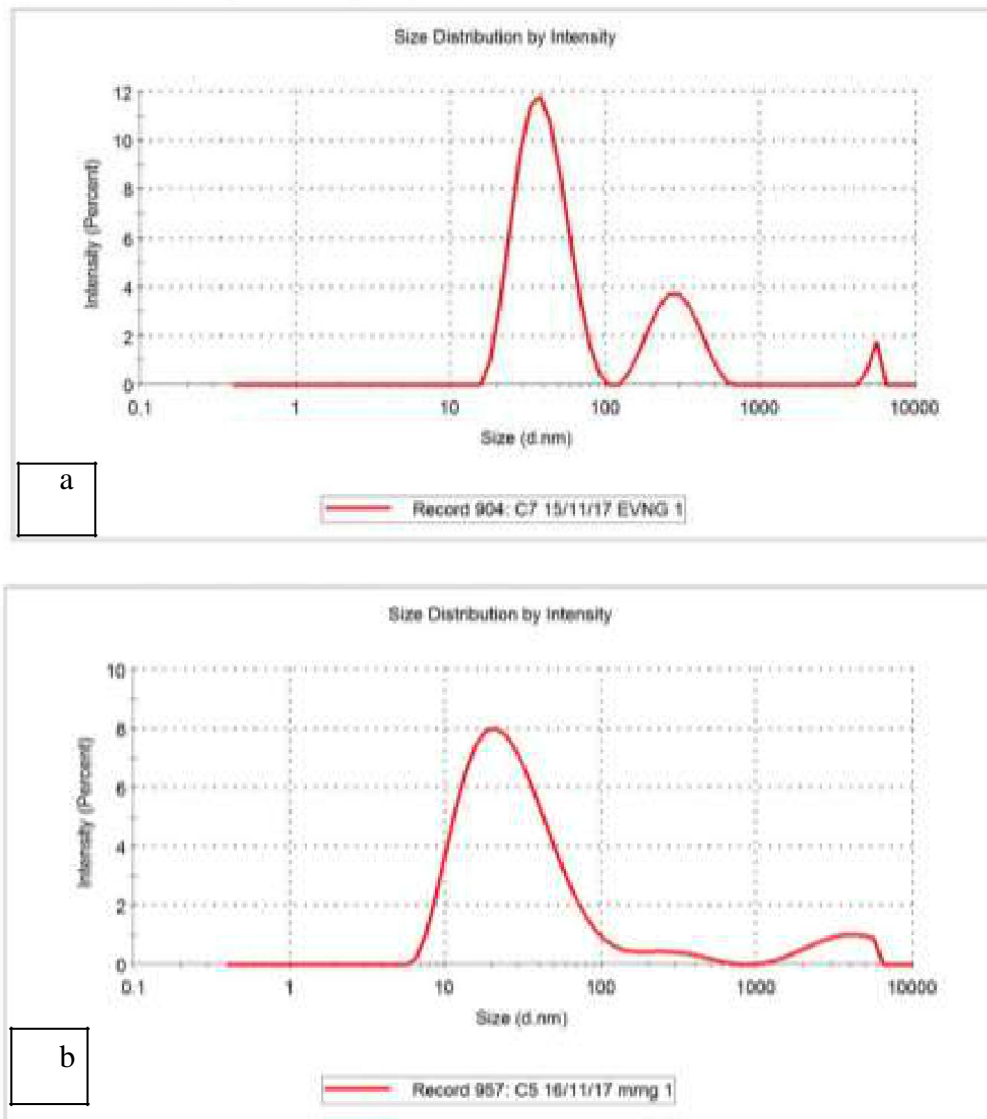


Fig.2 UV-Visible spectrum of a) standard and b) biosynthesized silver nanoparticles

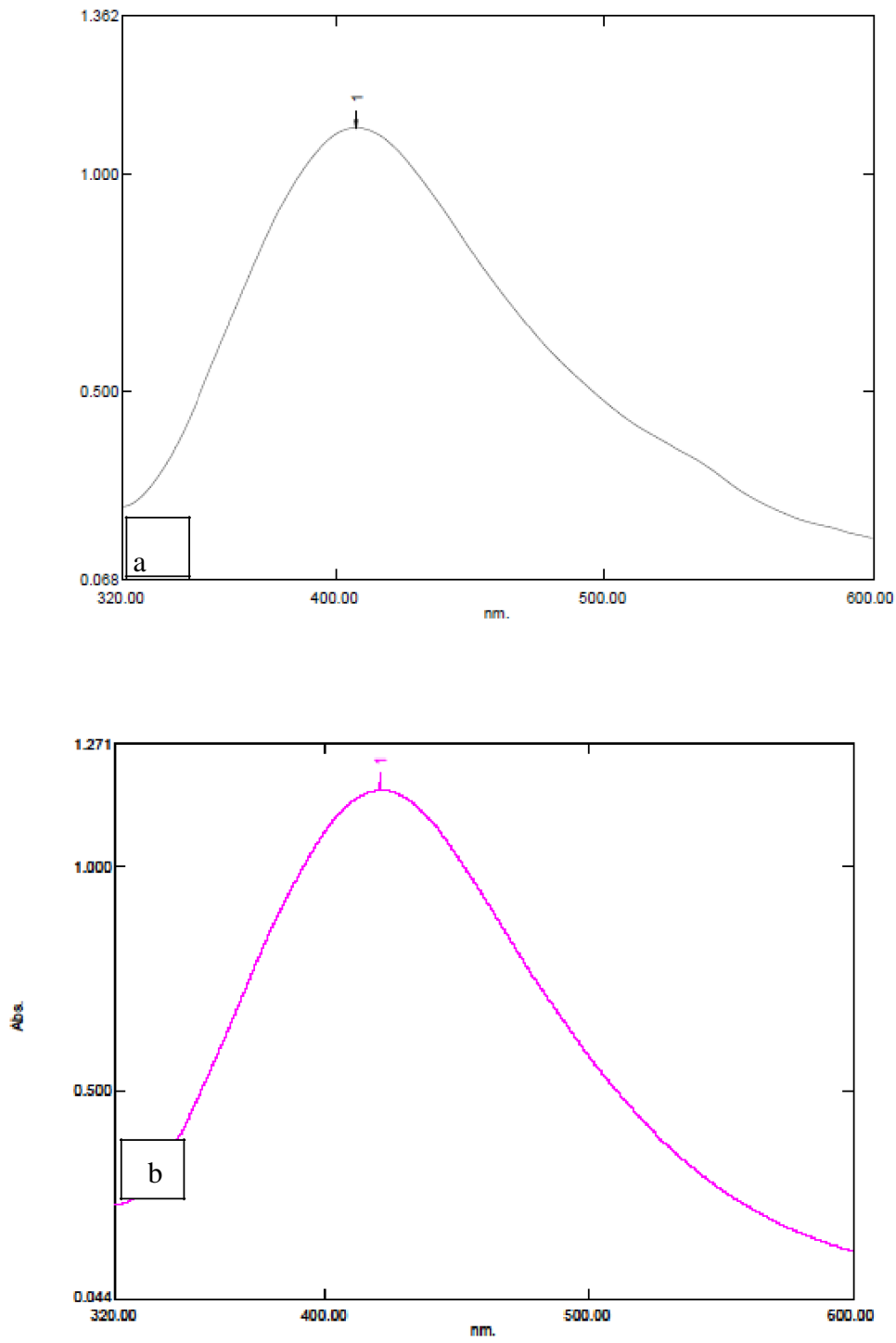


Fig.3 SEM image of a) standard and b) biosynthesized silver nanoparticles

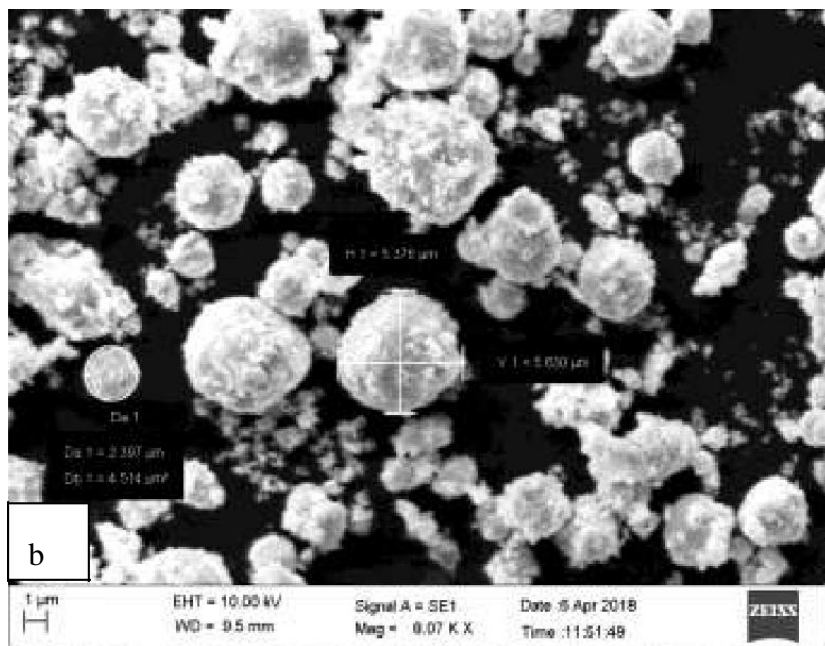
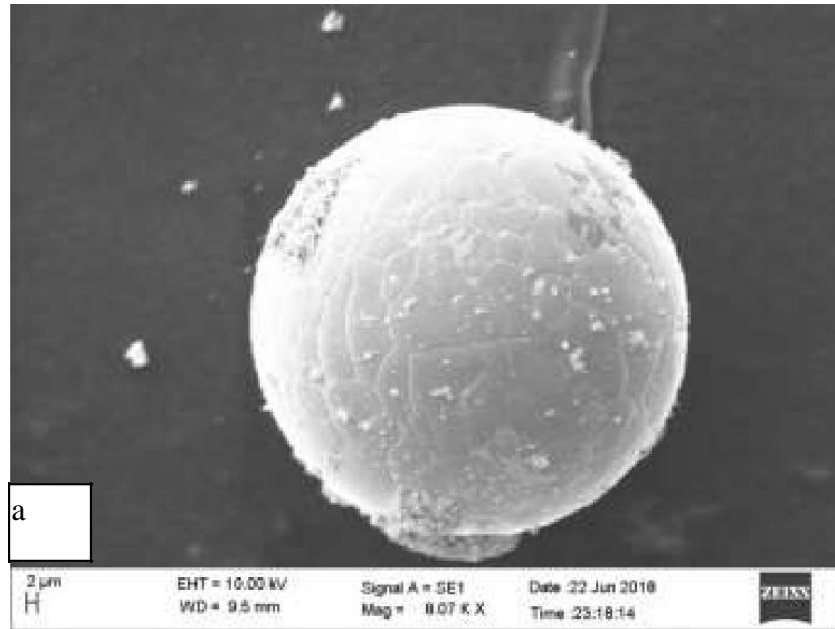


Table.1 Effect of Ag NPs on per cent germination and speed of germination of groundnut seeds

Concentration (ppm)	Per cent germination		Speed of germination	
	Standard Ag NPs	Biosynthesised Ag NPs	Standard Ag NPs	Biosynthesised Ag NPs
0 (control)	77.28		21.09	
25	81.14	80	23.32	23.25
50	83.75	82.87	23.62	23.35
75	85.15	84.12	24.63	24.39
100	88.16	86.42	24.97	24.82
125	91.02	89.37	26.34	26.28
150	91.75	90.81	26.49	26.37
+SE (m)	0.97		0.46	
C. D at 1 %	3.75		1.77	

Table.2 Effect of silver nanoparticles on root length and shoot length of groundnut Seeds

Concentration (ppm)	Root length (cm)		Shoot length	
	Standard Ag NPs	Biosynthesised Ag NPs	Standard Ag NPs	Biosynthesised Ag NPs
0 (control)	15.82		3.88	
25	18.12	17.90	5.15	4.87
50	19.42	18.37	5.37	5.07
75	20.32	18.67	5.71	5.31
100	21.25	19.32	5.72	5.50
125	21.87	20.71	5.97	5.65
150	22.55	22.10	6.15	5.82
+SE (m)	0.64		0.29	
C. D at 1 %	2.45		1.12	

Table.3 Effect of Ag NPs on seedling dry weight and vigour index I of groundnut seeds

Concentration (ppm)	Seedling dry weight (mg)		Vigour index I	
	Standard Ag NPs	Biosynthesised Ag NPs	Standard Ag NPs	Biosynthesised Ag NPs
0 (control)	1497		1520	
25	1792	1761	1886	1821
50	1817	1790	2076	1944
75	1864	1848	2217	2018
100	1917	1880	2378	2145
125	2025	1996	2535	2356
150	2126	2052	2633	2536
+SE (m)	13.13		63.94	
C. D at 1 %	50.31		244.89	

Seedling dry weight and Vigour index I

The priming of silver nanoparticles on the groundnut seeds at different concentrations resulted in a significant increase in seedling dry weight. The seedling dry weight increased by 29.58 and 27.04 % @ 150 ppm for standard (2126 mg) and biosynthesized (2052 mg) Ag NPs, respectively against control (1497).

The application of nanoparticles on seed enhanced the level of organic compounds such as protein, chlorophyll and phenols in the primed seeds. Therefore primed seeds showed increased seedling dry weight which might be due to better seedling length (Syriyaprabha *et al.*, 2012). Similar results were also observed by Khalaki *et al.*, 2016 through the application of silver nanoparticles on *Thymus Kotschyanus*.

From the data (Table 3), it is observed that, the higher vigour index I (2633) about 42.27 % was recorded with standard Ag NPs at 150

ppm over the control, which was statistically on par with biosynthesized Ag NPs (2536) at 150 ppm. The results are in good agreement with those published by Shyla and Natarajan, 2014 who reported that, the application of Ag NPs on groundnut seeds increased vigour index by about 40.35 % against control.

The current study revealed that the application of silver nanoparticles by priming method had significantly enhanced the seed quality of groundnut seeds at 150 ppm, in terms of seed germination, speed of germination, root length, shoot length, seedling dry weight and vigour index I. The seed quality parameters were concentration dependent.

Since the effect of treatment T₄ @ 125 and T₅ @ 150 ppm were on par in both the case of standard and biosynthesized Ag NPs, lower dosage (125 ppm) was suggested for the seed treatment.

The effect of biosynthesized Ag NPs was on par with the standard Ag NPs in all the treatments. Hence, the biosynthesized Ag NPs

could be used as a new alternative potential for seed dormancy breaking in groundnut.

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