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Influence of Environmentally Synthesized Silver Nanoparticles on Seed Quality Traits in Groundnut

Anitha Rose J. [a++*], Rupesh Kumar Reddy B. [a#], Vasundhara S. [a†] and Narasimha G. [b‡]

[a] *Department of Seed Science and Technology, S.V. Agricultural College, Tirupati, India.*
[b] *Department of Virology, S.V. University, Tirupati, India.*

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Silver nanoparticle (AgNPs) is one of the widely used nanoparticles because of their antimicrobial properties. In this present study, the effect of green synthesized silver nanoparticles and bulk form of silver ($AgNO_3$) at different concentrations (250, 500, 750 and 1000 ppm) on seed quality parameters of groundnut were studied. Silver nanoparticles synthesized using *Azadirachta indica* leaves were characterized by Dynamic light scattering, UV- Vis and FTIR spectrum. Size, zeta potential, UV- Vis absorbance peak and FTIR spectrum of the AgNPs measured 28.6 nm, 24.6 mV, 350 to 490 nm and 3334.53 cm^{-1} respectively. The results showed that AgNPs 1000 ppm was found best in enhancing the seed quality parameters such as germination percentage, root length, shoot length, seedling length, root dry weight, shoot dry weight, seedling dry weight, seed vigour index I& II.

[++] *M.Sc. Scholar;*
[#] *Assistant Professor;*
[†] *Professor and Head;*
[‡] *Associate Professor;*

**Corresponding author: E-mail: jajjaraanitharose@gmail.com;*

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1. INTRODUCTION

Groundnut (*Arachis hypogaea* L.) is a leguminous plant grown in the tropical and subtropical regions. It is the third most important source of vegetable protein and the fourth most important edible oil in the world. The productivity level of groundnut is very low in India, because 80 percent of it is grown under rainfed conditions with low inputs. Groundnut is stored for a period of about 6-9 months before sowing. During this period, seed viability may be lost quickly due to the production of free radicles by lipid peroxidation. In addition to this, the inadequacies of essential plant nutrients particularly micronutrients, significantly influence groundnut growth, yield and metabolic processes. However, surpassing the optimal levels of nutrients required for regular functioning and growth can lead to a decline in overall crop health and growth due to either deficiency or toxicity. Nutrient deficiency arises when the supply of an essential nutrient is significantly insufficient, resulting in diminished plant growth or quality [1]. The present technologies available to prolong the vigour and viability of groundnut seeds are not satisfactorily alleviating the practical problem. Therefore, a viable alternative seed treatment approach is required to effectively manage seed deterioration in groundnut [2].

Recently, nano science and nano technology is one of the most important areas of modern science and technology. Nanoparticles are used widely in seed science for improving germination, vigour and to induce stress tolerance. Particle size may affect agronomic as well as physiological parameters of plants. Decreased particle size leads to a greater quantity of particles per unit weight of a substance, thereby enhancing the specific surface area of a fertilizer as well [3]. The nanoparticles performance depends critically on their size, shape and composition [4]. Extensive research has been conducted in the field of agriculture, harnessing the potential of nanomaterials and nanodevices to tackle diverse challenges.

Green synthesis provides advancement over chemical and physical methods as it is cost effective, environment friendly, easily scaled -up [5]. Throughout storage, silver nanoparticles offer a potential alternative for controlling the growth of insects and pest [6]. Recent studies have reported that a plants response to AgNPs depends on the dosage of nanoparticle. Within the range of concentrations (0, 25, 50,100,200 and 400 ppm), the most effective growth response in *Brassica juncea* seedlings is elicited by the 50 ppm treatment [7]. AgNPs synthesised through the environmentally friendly methods like plant extracts, microbial sources and biotemplates not only demonstrate versatile applicability but also possess a relatively lower toxicity profile. Krishnaraj *et al*. [8]. conducted a study on the impact of biologically synthesized AgNPs on *Bacopa monnieri*, which demonstrated a noteworthy influence on the plants growth characteristics. Similarly, Netala *et al*. [9] described a straightforward and environmentally conscious method for the biosynthesis of AgNPs using aqueous callus extract of *Centella asiatica.*

2. MATERIALS AND METHODS

The experiment was carried out at Department of Seed Science and Technology, S. V. Agricultural College, Tirupati and Department of Virology, S. V. University, Tirupati.

2.1 Materials

Fresh leaves of *Azadiractha indica* (Neem), were collected at S. V. Agricultural College campus, Tirupati, Andhra Pradesh, India. The pods of groundnut variety Dheeraj (TCGS 1073) were procured from the Regional Agricultural Research Station, Tirupati.

2.2 Synthesis of Silver Nanoparticles

The neem leaves collected were washed thoroughly with regular tap water, followed by double distilled water in order to eliminate dirt on the surface of leaves. Subsequently, the leaves were air dried to avoid excessive weight during weighing. A quantity of five grams of neem leaves was precisely weighed using an electronic balance. These leaves were then finely chopped and macerated with distilled water using a motor and pestle. The resultant leaf extract was filtered through muslin cloth and subsequently diluted with distilled water to achieve a total volume of 1000 ml. For the synthesis of silver nanoparticles (AgNPs), 0.1 mM (6 mg) $AgNO_3$ is added to the leaf extract and then allowed to incubated at room temperature. After some time, colour of the leaf extract was changed from green to dark brown (Fig.1). The transformation in colour indicated the successful formation of silver nanoparticles, in accordance with the findings of Vaishnavi *et al*., 2020.

2.3 Characterization of Silver Nanoparticles

The nanoparticles particle size and distribution were verified utilizing techniques such as Dynamics Light Scattering (DLS) and Zeta Potential (ZP). DLS, employing a multiple scattering laser diffraction method, was utilized to ascertain the particle size distribution of the nanoparticles. Additionally, Zeta potential analysis was conducted to investigate the nanoparticles negative potential values, crucial for nanoparticle reduction and stability. The reduction of nanoparticle ions was observed through UV-Visible spectrophotometry (UV-1800). The presence of functional groups like amino and carboxyl groups within the nanosuspension was identified using Fourier Transform Infrared Spectroscopy (FTIR).

2.4 Seed Treatment

Groundnut (Dheeraj) seeds collected from the Regional Agricultural Research Station (RARS), Tirupati were soaked with the biosynthesized silver nanoparticles and $AgNO_3$ suspensions at various concentrations (250, 500, 750 and 1000 ppm) for three hours and then shade dried for one hour. Seeds were kept for germination using between paper method. Four replicates of 100 seeds from each treatment were taken at random and placed at uniform spacing in between two wetted germination paper towels. The paper towels were rolled and placed in plastic trays in upright position and the trays were placed in a germinator maintained at 25 ± 2 °C temperature and 95 ± 5 % relative humidity (RH) for 10 days. After 10 days of the test period, observations were recorded on germination (%), root length (cm), shoot length (cm), seedling length (cm), root dry weight (g), shoot dry weight (g), seedling dry weight (g) and seed vigour index I & II were calculated.

2.5 Germination Percentage

At the end of germination test, number of healthy seedlings within each replication was recorded and the germination percentage (%) was calculated and presented as a percentage using the formula provided below:

Germination (%) = Number of normal seedlings / Total number of seed sown x 100

2.6 Root Length, Shoot Length and Seedling Length (cm)

At the time of germination count, ten healthy seedlings were randomly selected for the assessment of root length, shoot length and seedling length. The measurement of root length was taken from the point of attachment of seed to the tip of primary root, while the shoot length was measured from the point of attachment of seed to the tip of the leaf. The resultant mean values were expressed in centimetres. The sum of root and shoot length of ten seedlings was computed and their mean was expressed as seedling length in centimetres.

2.7 Seed Vigour Index I

Seed vigour index I was computed by adopting the following formula as given by Abdul-Baki and Anderson [10] and was expressed in whole number.

Seed vigour index = Germination (%) x Mean seedling length (cm)

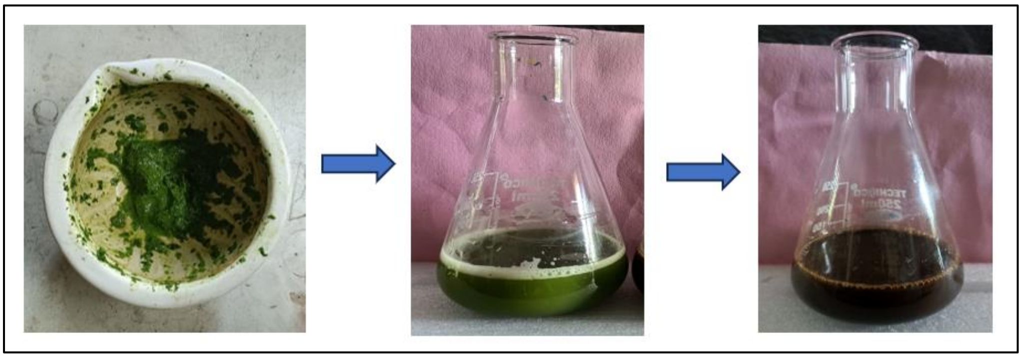

Fig.1 Green synthesis of silver nanoparticles

2.7.1 Root dry weight, shoot dry weight, seedling dry weight (g)

During the germination count, ten healthy seedlings were selected randomly from the germinated seeds. These selected seedlings were placed in brown paper bags and subjected to a hot air oven at 70± 2^{0}C for 24 hr. Following the cooling period, the roots and shoots were meticulously separated from seedlings and their dry weights were measured using an electronic weighing balance. The sum of root dry weight and shoot dry weight of ten seedlings was calculated and their mean was expressed as seedling dry weight. The average weight was calculated and was expressed in grams per seedling.

2.8 Seed Vigour Index II

The seed vigour index II was calculated by using the formula given by Abdul-Baki and Anderson [10] and was expressed in whole number.

Seed vigour index II **=** Germination (%) x Mean seedling dry weight (g)

3. RESULTS AND DISCUSSION

During the synthesis process, the transition of colour from green to dark brown indicates the successful transformation of silver nanoparticles. This alteration in colour is attributed to the surface plasmon vibrations specific to silver nanoparticles. Comparable observations were made by Rahman *et al.* [11] and Vaishnavi *et al.* [12]. where extracts from leaves of *Ficus religiosa, Pterocarpus santalinus* led to the transformation of the filtrate from green to dark brown colour. The presence of bioactive compounds like polyphenols, terpenoids, flavonoids, carbohydrates, vitamins and trace elements within the plant extract has been found to play a main role in reduction of silver nanoparticles [13].

3.1 Characterization of Silver Nanoparticles

The particles size distribution and zeta potential of the nanoparticles were assessed using this Dynamic light scattering, a technique that gauges particle size by analyzing the rate of fluctuations in the intensity of laser light scattered by particles as they undergo diffusion within a solvent. The zeta potential value of the AgNPs was 24.6 mV (Fig. 2) which shows that the nanoparticles are highly stable. The particle size distribution values of AgNPs was 28.6 nm (Fig. 3) which indicates that the particles are dispersed evenly. The UV-Vis spectrum shows the peak at 420 nm (Fig. 4) and FTIR spectrum of AgNPs shows a peak at 3334.53 cm^{-1} (Fig. 5) which corresponds to N-H stretching of secondary amine.

3.2 Effect of Silver Nanoparticles on Groundnut Seed Quality Parameters

3.2.1 Germination percentage

The germination percentage of groundnut seeds exhibited an increment with rising concentrations of AgNPs in comparison to both control and $AgNO_3$ treated seeds (Table 1). It is noted that among all the treatments, AgNPs 1000 ppm recorded the maximum germination percentage (72.66 %) which is followed by AgNPs 750 ppm (72.00 %). The results were in agreement with Mahakham *et al.* [14] that phytosynthesized silver nanoparticles significantly improved the germination performance in aged seeds of rice. Smitha *et al.* [5] noticed that the biosynthesized silver nanoparticles was best in enhancing the seed germination in groundnut. The rapid germination observed may be attributed to the penetration of nanoparticles into the seedcoat, which facilitates the influx of water into the seed and activates early-phase enzymes, thereby enhancing the speed of germination [15] %. As for the bulk concentrations $AgNO_3$ 250 ppm (70.00 %) showed maximum germination percentage and the increasing concentration of the $AgNO_3$ resulted in the decrease in germination percentage. The results are in agreement with Sarabi *et al.* [16] that at lower concentrations minimum germination percentage was recorded but with the increase in concentration the seeds of *Brassica napus* did not germinate. Fayez *et al*. [17] findings indicated that $AgNO_3$ reduced plant growth parameters by uptaking silver from $AgNO_3$, potentially leading to the inhibition of grain germination in barley seedlings due to enhanced silver accumulation in the seeds.

3.2.2 Root length, shoot length and seedling length

The results demonstrated that AgNPs had a promotory effect on root, shoot and seedling length of groundnut (Table 1). AgNPs treated groundnut seeds at 1000 ppm induced maximum root length (16.80 cm) compared to all treatments. The maximum shoot length (11.98

cm) and seedling length (28.78 cm) was observed at the same 1000 ppm concentration. AgNPs @750 ppm showed on par results with 1000 ppm. The results are in consistent with Smitha *et al*. [5] that the application of AgNPs by priming method had significantly enhanced the root length in groundnut. Sharma *et al*. [7] showed the similar findings that there is a sharp increase in root length in the silver nanoparticle treated *Brassica juncea* seedlings. Anandaraj and Natarajan [18] reported that the application of AgNPs (1000 ppm) on onion seed showed increased shoot length over control. Vuong *et al*. [19] revealed that the response of peanut root length and shoot length increases with the increasing concentrations of AgNPs which is in consistent with the results. The previous reports suggested that AgNPs have positive effects on seedling growth, yield and metabolism in different species [20]. Similar reports of increase in seedling length when fenugreek seeds treated with AgNPs were observed by Hojjat and Hojjat [21]. Whereas an inhibitory effect of bulk $AgNO_3$ on root length, shoot length and seedling length was observed with the increase in concentration (Table 1). The results are in consistent with Yasur *et al*. (2013) while working on castor. Ghasemifar *et al*. [22] demonstrated that the presence of elevated levels of silver, attributed to both $AgNO_3$ and AgNPs resulted in a detrimental impact on growth primarily through inducing significant lipid peroxidation. Szablińska-Piernik *et al*. [23] explained that higher concentrations of $AgNO_3$ had toxic effects on growing pea seedlings.

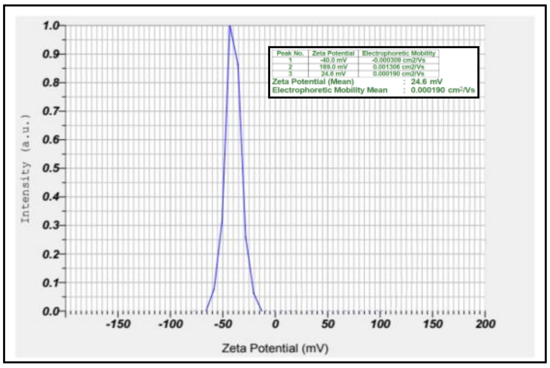

Fig. 2. Zeta potential of AgNPs

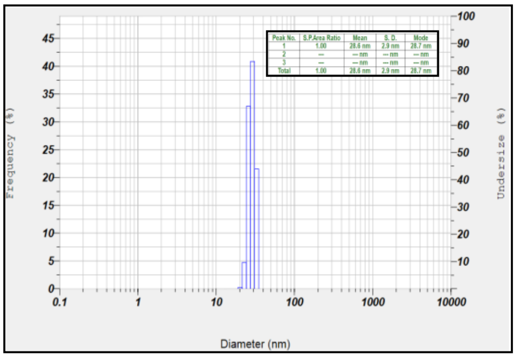

Fig. 3. Particle size of AgNPs

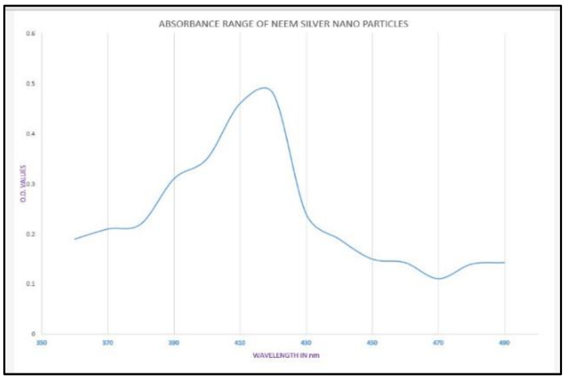

Fig. 4. UV-Vis spectroscopy of AgNPs

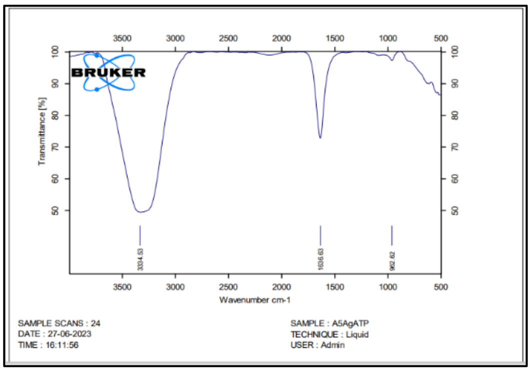

Fig. 5. FTIR spectrum of AgNPs

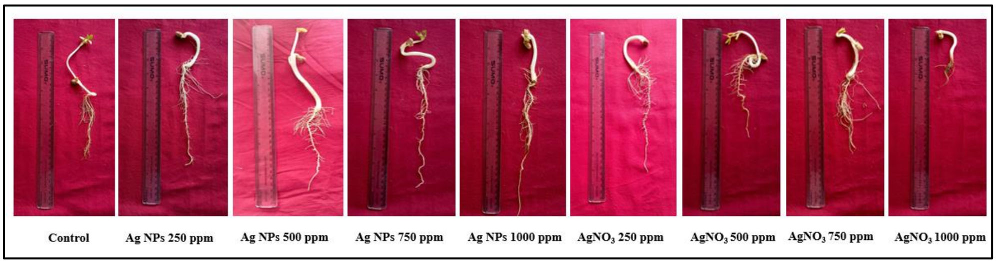

Fig. 6 Seedlings of silver nano and bulk treated seeds

Table 1. Impact of silver nanoparticles on germination, root length, shoot length and seedling length

Treatments	Germination %	Root length (cm)	Shoot length (cm)	Seedling length (cm)
Control	65.33	9.67	9.58	19.25
AgNPs 250 ppm	62.33	16.33	10.53	26.86
AgNPs 500 ppm	71.00	16.36	11.17	27.53
AgNPs 750 ppm	72.00	16.42	11.30	27.72
AgNPs 1000 ppm	72.66	16.80	11.98	28.78
$AgNO_3$ 250 ppm	70.00	14.15	11.17	25.32
$AgNO_3$ 500 ppm	67.66	10.98	10.31	21.29
$AgNO_3$ 750 ppm	48.00	10.93	9.82	20.75
$AgNO_3$1000 ppm	26.00	10.83	8.93	19.76
Mean	61.66	13.60	10.53	24.14
S E m±	1.68	0.33	0.29	0.46
CD (5%)	5.37	0.99	0.89	1.39
CV (%)	1.681	4.14	4.9	3.29

3.2.3 Root dry weight, shoot dry weight and seedling dry weight

The seed treatment of silver nanoparticles on groundnut seeds at different concentrations resulted in a significant increase in dry weights (Table 2). Root dry weights of the groundnut seeds treated with AgNPs showed significant variation among all the treatments. The treatment AgNPs 1000 ppm has the highest root dry weight (0.06 g), shoot dry weight (0.32 g) and seedling dry weight (0.38 g) were recorded. The results are in agreement with Smitha *et al*. (2019) that priming of silver nanoparticles on the groundnut seeds at different concentrations resulted in seedling dry matter. Comparable findings were reported by Khalaki *et al*. [24] when employing silver nanoparticles on *Thymus kotschyanus*. The seeds treated with AgNPs displayed enhances seedling dry weight, potentially attributed to improved seedling length [25]. Moreover, the stimulating effect of AgNPs on the biometric parameters of bean seedlings such as height, fresh and dry weights of shoots observed by Prazak *et al.* [26].

3.3 Seed Vigour Index I & II

Vigour index showed significant variation as a result of nano treatment and their dosages. The groundnut seeds treated with AgNPs 1000 ppm showed maximum SVI I & II i.e., 2092.10 and 27.61 respectively. The results are in agreement with by Shyla and Natarajan [2] that the application of AgNPs on groundnut seeds increased SVI by about 40.35% over control.

Table 2. Effect of AgNPs and bulk suspensions on root dry weight, shoot dry weight, seedling dry weight, SVI I& II

Treatments	Root dry weight (g)	Shoot dry weight (g)	Seedling dry weight (g)	SVI-I	SVI-II
Control	0.04	0.29	0.33	1282.74	21.55
AgNPs 250 ppm	0.04	0.27	0.31	1674.27	19.32
AgNPs 500 ppm	0.05	0.30	0.35	1989.06	24.85
AgNPs 750 ppm	0.05	0.30	0.35	2030.05	25.20
AgNPs 1000 ppm	0.06	0.32	0.38	2092.10	27.61
$AgNO_3$ 250 ppm	0.05	0.30	0.35	1839.47	24.50
$AgNO_3$ 500 ppm	0.04	0.24	0.28	1441.61	18.94
$AgNO_3$ 750 ppm	0.03	0.20	0.23	993.93	11.04
$AgNO_3$1000 ppm	0.02	0.15	0.17	513.76	4.42
Mean	0.04	0.26	0.30	1539.66	18.84
S E m±	0.01	0.07	0.004	42.09	0.48
CD (5%)	0.04	0.25	0.01	126.02	1.55
CV (%)	4.56	5.11	2.55	4.71	4.32

Increase in SVI of groundnut seeds when primed with silver nanoparticles was observed by Smitha *et al.* [5]. Similar reports were given by Sharma *et al.* [7] that the vigour index of the silver nano treated seedlings was significantly higher than the control seedlings and also observed that silver nanoparticle treatment improves the overall growth profile of the treated seedlings.

4. CONCLUSION

In this study, the application of environmentally synthesized silver nanoparticles was observed to markedly improve the seed quality parameters of groundnut. Notably, the treatment at a concentration of AgNPs 1000 ppm showed substantial improvements in various parameters such as germination percentage, root length, shoot length, seedling length, root dry weight, shoot dry weight, seedling dry weight and SVI I & II. The impact of AgNPs 750 ppm and 500 ppm concentration were on par with AgNPs 1000 ppm concentration. Bulk $AgNO_3$, in contrast to green synthesized silver nanoparticles, exhibited detrimental effects on seed quality parameters as its concentration increased.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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