Plant Archives Vol. 26, Supplement 1, 2026 pp. 652-658 e-ISSN:2581-6063 (online), ISSN:0972-5210

Plant Archives

Journal homepage: http://www.plantarchives.org
DOI Url : https://doi.org/10.51470/PLANTARCHIVES.2026.v26.supplement-1.089

EXTRACTION, CHARACTERIZATION AND UTILIZATION OF SILVER NANOPARTICLES (AGNPS) FROM THE FRUITS OF *SOLANUM XANTHOCARPUM* SCHRAD. & WENDL.

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(Date of Receiving : 28-09-2025; Date of Acceptance : 11-12-2025)

ABSTRACT

The present investigation is on obtaining silver nanoparticles from the fruit extracts of *Solanum xanthocarpum* characterising them and identifying suitable concentration and timing as priming agent on germination and early growth of seedlings of brinjal. Through green synthesis the nanoparticles obtained are spherical in shape and 70 nm in size. The seeds were separately treated in AgNPs solution for 15 and 30 mins at different concentrations (20, 40, 60, 80, and 100 ppm) and their effect on seed germination (%), root length (cm), shoot length (cm), seedling length (cm), seedling dry weight (mg/10 seedlings), seedling vigour index-I and II were investigated. Different concentrations on solanaceae crop showed a positive impact on seed germination and seedling vigour index compared to the control.
***Keywords*:** Silver nanoparticles, germination, brinjal, *Solanum xanthocarpum.*

Introduction

Nanotechnology is the application of nanoscience leading to the use of particles in the range of 1 to 100 nanometers. In the International System of Units, one nanometer is one billionth of a meter (10^{-9}). Nanoparticles exhibit significantly different physical and chemical properties to their larger material counterparts. The most common types of metal nanoparticles include silver, gold, palladium, titanium, zinc and copper nanoparticles. They are characterized by extremely large surface area, high reactivity, strength, sensitivity and stability. Their functional properties include transparency, hydrophobicity, photoluminescence, chemical sensing and bioavailability. Such unique properties are due to their high surface area-to-volume ratio.

Currently there is a growing need to develop sustainable preparation of nanoparticles that get rid of using harmful organic and chemical substances, since NPs (nanoparticles) are widely applied to areas of human contact. Therefore plant mediated biosynthesis of NPs is considered a widely acceptable technology for rapid production of metallic NPs. Such synthesis includes three main phases (Makarov *et al*., 2014). The activation phase in which the reduction of metal ions and nucleation of reduced metal atoms occur. The growth phase referring to the spontaneous coalescence of the small adjacent NPs into particles of a larger size accompanied by an increase in the thermodynamic stability of NPs (ripening process) and the termination phase in which the final shape of the NPs are formed.

Principally this kind of synthesis envelops plant extracts in aqueous forms for the reason that the availability of reducing agent is higher in the extract than the whole plant. The obtained NPs are also more stable, eco-friendly, reproducible and less expensive (Pasupuleti *et al*., 2013). This method is eco-friendly and does not harm human health (Keat *et al*., 2015).

Silver nanoparticles are of particular interest in the modern research of nanotechnology due to its unique properties, which can be incorporated into a wide range of extensive application in medical, cosmetic, food packaging, bioengineering, electrochemistry and agriculture industries.

They have broad range of applications, in agriculture and plant biotechnology. They have been shown to enhance seed germination, plant growth and photosynthetic efficiency, whereas also acting as safe and effective nano-pesticides and fertilizers (Khan *et al*., 2023). Several reports suggest that proper concentration of AgNPs (Silver nanoparticles) are vital in enhancing seed germination, plant growth, improving chlorophyll content and increasing fertilizer and water efficiency (Abd El-Aziz *et al*., 2022). Hence an attempt was made to synthesise AgNPs from the fruits of *Solanum xanthocarpum* and used in solanaceae crop for obtaining higher germination (%) and seedling vigour index.

Materials and Methods

Preparation of AgNPs

Twenty grams of freshly weighed *Solanum xanthocarpum* berries were cut, lightly crushed in pestle and mortar. This was heated to read 60°C adding 100 ml of double distilled water. Then it was retained at 60°C for 5 mins. Finally filtered and stored at 4°C until further use. 10 ml of plant extract was added to 100 ml of 1 mM $AgNO_3$, continuously stirred at 60°C, until the colour of solution turns reddish brown indicating the formation of nano silver. The solution was centrifuged at 20,000 rpm for 15 mins, to collect the samples. The collected samples were thoroughly washed with ethanol and double distilled water and dried for characterization.

Characterization

Field Emission Scanning Electron Microscopy (FESEM)

The surface morphology or structural analysis of the synthesized AgNPs was inspected using SEM, in the 'ZEISS' model analytical scanning electron microscope, available at Centralised Instrumentation and Service Laboratory (CISL), Annamalai University. This was operated at 40 KX magnification and a voltage of 20 kV and a working distance of 7.4 mm. The sample for SEM analysis was prepared by placing AgNPs in water on a carbon-coated copper grid and drying them completely using blotting paper, followed by placing them under a mercury lamp for 5 min. Images of the sample were taken and a size distribution histogram of the AgNPs was plotted fig.1.

Energy Dispersive X-ray Analysis (EDAX)

The presence of metallic silver ions or elemental analysis was examined by using an EDAX detector. The synthesized AgNPs were poured on the carbon film and dried. The spectrum obtained from the sample was then analysed by using a semiconductor for the detection of X-rays together with electronic processing.

Collection of seeds and seed treatment

Seeds were extracted from well ripen fruits of brinjal, were primed with AgNPs. Before executing seed treatments, the seeds were air dried to ensure their viability. Seeds were immersed in the priming media for 15 and 30 mins at ten different concentrations (20, 40, 60, 80 and 100 ppm). The unprimed seeds were kept as control. As per the above schedule of treatments, the seeds were subjected to dipping in solutions prepared for specific time and then sown immediately in the protrays filled with nursery soil. This was laid out in the completely randomized design (CRD) with three replications.

Statistical analysis

The experimental data recorded on various characteristics during the investigation were analysed statistically using the method of analysis of variance (ANOVA) for complete randomized design (CRD) by Fisher and Yates (1963). Analysis of variance (one-way classified data) for each character was performed using WASP 2.0 software. The significance of various treatments was judged with the help of 'F' value (test) at 5% level of significance. The respective results of the data are presented in tables and graphs.

Observations

Germination percentage (%)

The total germinated seeds in each treatment of each replication were counted after the completion of germination. It was calculated by dividing the total number of seeds sown with the number of seeds germinated and germination per cent was calculated as per the following formula:

$$\text{Germination percentage} = \frac{\text{Number of seeds germinated}}{\text{Number of seeds sown}} \times 100$$

Root length

Ten normal seedlings taken at random from the germination test were used for measuring root length. It was measured from the collar region to the tip of the primary root and the mean value was expressed in cm.

Shoot length

Ten normal seedlings used for root measurement were measured for shoot length from the collar region to the tip of the plumule and the mean value was expressed in cm.

Seedling length

Ten seedlings from each replication after germination were taken at random on final count. The seedling length was measured from the tip of the primary root to the tip of the primary leaf and the mean value was expressed in cm.

Dry matter production (mg/10 seedlings)

Ten normal seedlings used for growth measurement were placed in a paper cover and shade dried for 24 hours, then kept in the hot air oven maintained at 85^0C for 24 hours. The dried seedlings were cooled in a desiccator for 30 minutes and expressed as mg/10 seedlings.

Seedling vigour index-I

The seedling vigour index-I was computed adopting the procedure of Abdul Baski and Anderson (1973) and expressed as whole number.

Seedling vigour index-I = Germination percentage (%) x Total seedling length [Mean root length (cm) + Mean shoot length (cm)]

Seedling vigour index-II

The seedling vigour index-I was computed adopting the procedure of Abdul Baski and Anderson (1973) and expressed as whole number.

Seedling vigour index-II = Germination percentage (%) x Dry matter production (mg)

Results and Discussion

Nanoparticle characterization

FESEM

Figure 1 shows the FESEM image of silver nanoparticles reduced with *Solanum xanthocarpum* extract using green synthetic route. Here most of the AgNPs are spherical in shape with the size of 70 nm. All the nanoparticles were well formed and found to be clustered.

EDAX

Figure 2 shows the EDAX spectrum which strongly reveals the presence of silver in the synthesized nanomaterial. No impurities from any other material were found. This shows the purity of the synthesized material.

Colour mapping

Figure 3 shows the colour mapping of AgNPs to establish its presence and entire distribution.

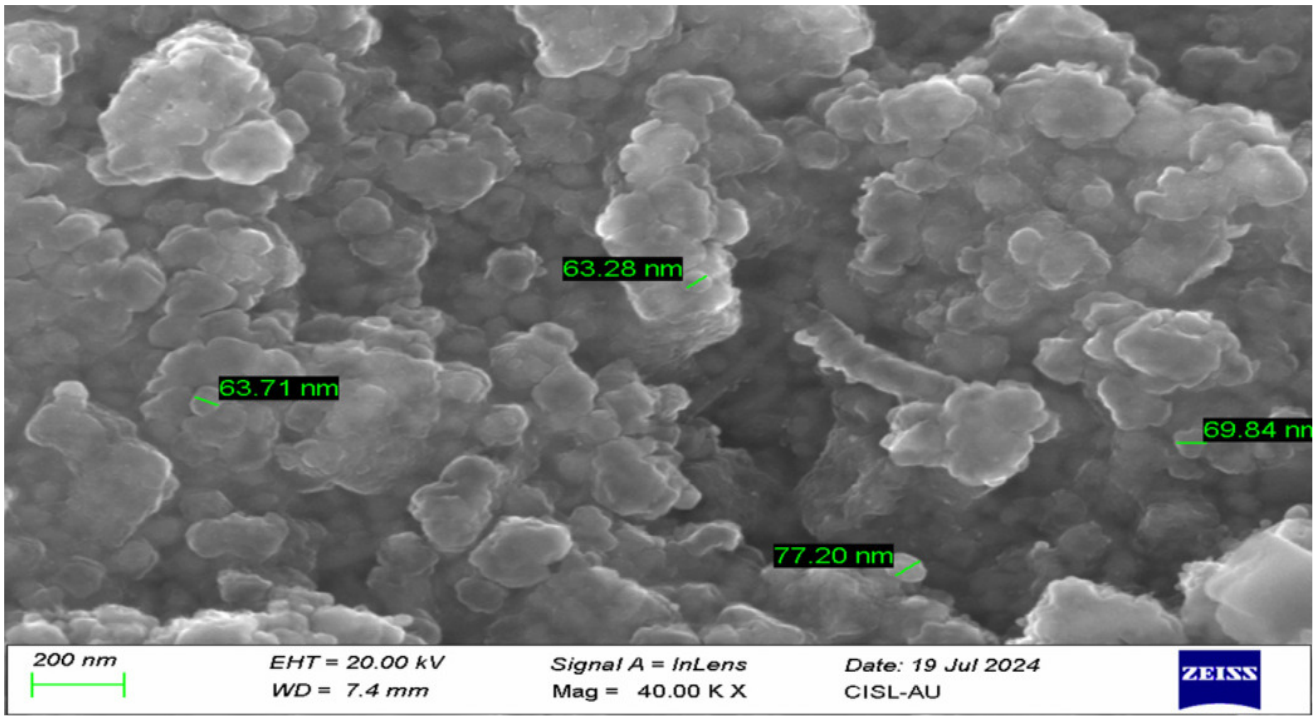

Fig. 1 : Field Emission Scanning Electron Microscopy (FESEM) image of AgNPs

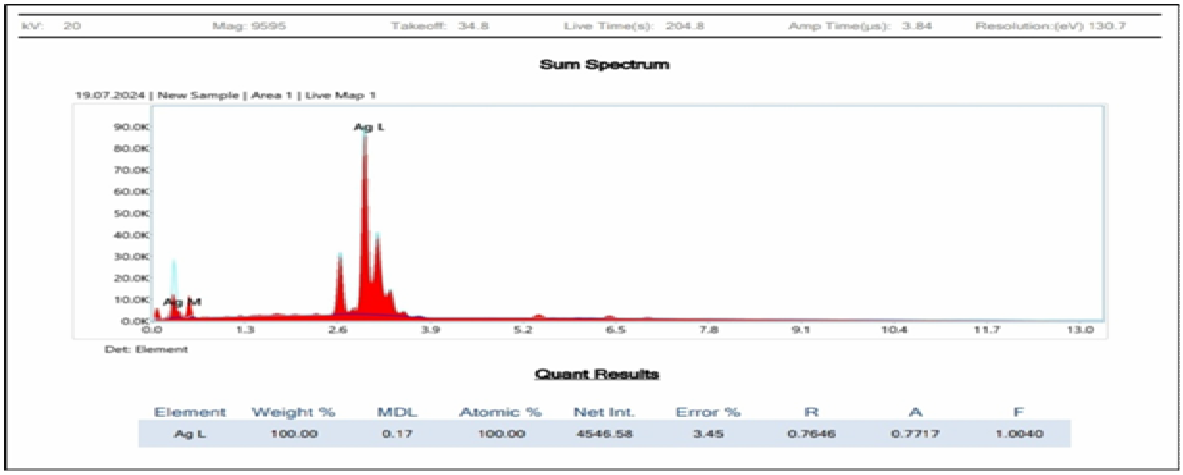

Fig. 2 : EDAX spectrum

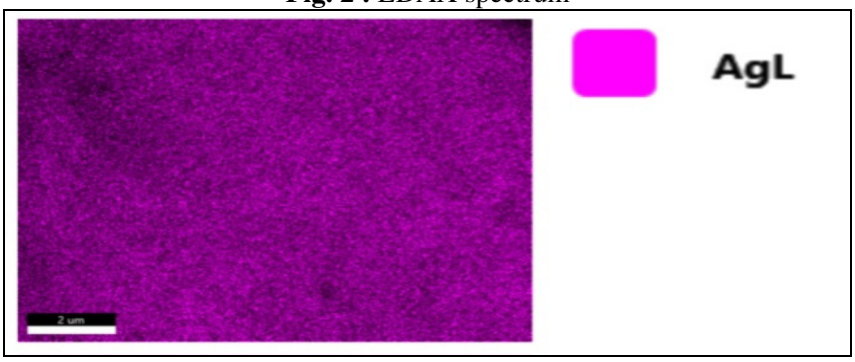

Fig. 3 : Colour mapping of AgNPs

Table 1 : Effect of AgNPs as priming agent on germination percentage (%), seedling growth and seedling vigour index of brinjal

T. No.	Treatment details	Germination percentage (%)	Root length (cm)	Shoot length (cm)	Seedling length (cm)	Seedling dry weight (mg/10 seedlings)	Vigour index-I	Vigour index-II
T_0	Control	75.00	4.73	4.79	9.52	15.03	714.00	1.12
T_1	AgNPs @ 20ppm for 15 minutes	91.00	9.01	5.86	14.87	18.67	1353.17	1.70
T_2	AgNPs @ 40ppm for 15 minutes	95.00	9.89	6.03	15.92	19.14	1512.40	1.81
T_3	AgNPs @ 60ppm for 15 minutes	84.00	7.57	5.41	12.98	17.57	1090.32	1.47
T_4	AgNPs @ 80ppm for 15 minutes	80.00	6.90	5.20	12.10	17.09	974.05	1.37
T_5	AgNPs @ 100ppm for 15 minutes	77.00	6.07	4.97	11.04	16.49	850.08	1.27
T_6	AgNPs @ 20ppm for 30 minutes	86.00	7.92	5.54	13.46	17.99	1157.56	1.55
T_7	AgNPs @ 40ppm for 30 minutes	89.00	8.53	5.74	14.27	18.43	1270.03	1.64
T_8	AgNPs @ 60ppm for 30 minutes	83.50	7.52	5.39	12.91	17.53	1077.98	1.46
T_9	AgNPs @ 80ppm for 30 minutes	80.00	6.81	5.16	11.97	17.04	957.60	1.36
T_{10}	AgNPs @ 100ppm for 30 minutes	76.50	5.86	4.92	10.78	16.41	824.67	1.25
S.Ed.		0.19	0.07	0.01	0.06	0.01	7.23	0.003
CD at 5% (p=0.05)		0.39	0.14	0.02	0.13	0.03	14.54	0.007

The use of nano materials is expanding in every field of science including agriculture. When the materials are transformed to nano, they change their physical, chemical and biological characteristics as well as catalytic properties and even more increase the chemical and biological activities (Mazaherinia *et al*., 2010). So far AgNPs has been reported to be extracted from medicinal plants like *Foeniculum vulgare* (Showmya *et al*., 2012), *Andrographis paniculata*, *Phyllanthus niruri* and *Tinospora cordifolia* (Sharma *et al*., 2019) and ashwagandha (Saleh and Mahdi, 2021). Medicinal plants are rich source of bioactive

compounds including various secondary metabolites like alkaloids, flavonoids, phenolics, saponins, terpenoids and tannins. Therefore, use of medicinal plants for the approach of nanoparticles generation are cost effective, eco-friendly, non-toxic and safer than synthetic drugs (Tang *et al*., 2012). The green synthesized AgNPs has gained attention in agriculture as a potential seed priming agent (Abou-Zeid and Ismail 2018). Seed priming involves pre-soaking seeds in a solution to enhance germination, seedling vigour and overall plant performance. Silver nanoparticles can increase the rate and uniformity of seed germination, resulting in faster seedling emergence. Priming with NPs can lead to stronger and healthier seedlings with better shoot and root development (Singh *et al*., 2020). It helps seedlings to withstand abiotic stresses such as drought, salinity, temperature fluctuations and enhancing survival rates. NPs stimulate the production of antioxidants in seedlings helping to mitigate oxidative stress caused by environmental factors and also enhance photosynthetic efficiency and metabolic activity, leading to improved growth rate.

Characterization of silver nanoparticles from fruit extract of *Solanum xanthocarpum* is done under SEM and EDAX. Here the AgNPs are spherical in shape with the size of 70 nm and the nanoparticles were found to be clustered and the EDAX spectrum reveals the presence of silver and purity of synthesized nanomaterial. Nano priming helps in activating metabolic processes within seeds, leading to the synthesis of enzymes and hormones that promote germination and growth. It improves water retention in seeds, facilitating better hydration and germination under dry conditions. Another reason for faster seed germination is reactive oxygen species (ROS) that result from the entry of nanoparticles in the area between the cell membrane and the intracellular space of the seed coat parenchyma. Elevated levels of ROS in seeds, increases ion penetration as well as water and oxygen absorption, both of which are required for faster seed germination (Mishra *et al*., 2023).

Silver nanoparticles (AgNPs) as seed priming agent significantly influenced higher seed germination in brinjal seeds under 40 ppm for 15 mins (Table. 1). AgNPs are likely to seep through the seed coat and have a positive impact on the germination process and stimulates seed germination by breaking seed dormancy and affecting various biochemical processes such as hydrolysis of growth suppressing metabolites, absorption and enzyme activation (Singh *et al*., 2020). The effect of silver nanoparticles on seed germination mechanisms may include increased water absorption by seeds, elevated level of enzyme nitrate reductase, enhanced capacity of seeds to absorb and utilise fertiliser and water, stimulation of seed antioxidant systems, reduction of antioxidant stress through reduction of H_2O_2, superoxide radicals and malonyldialdehyde content and increased activity of certain enzymes (such as superoxide dismutase, ascorbate peroxidase, guaiacol peroxidase and catalase) (Lu ChangMei *et al*., 2002). AgNPs may have a role in promoting plant regulators and enhancing certain enzymes involved in cell proliferation, which would improve physiological parameters and cause higher percentage of seed germination (Syu *et al*., 2014). The concentration and the size of nanoparticles are highly limiting factors considering the effect that appeared on the plants (Ma *et al*., 2010). Results are in line with Hojjat and Hojjat (2015) studied the effect of nano-silver on seed germination and concluded that an accumulation and uptake of nanoparticle was dependent on the time of exposure and concentration in fenugreek.

Silver nanoparticles at different concentrations significantly increased the seedling growth parameters; root, shoot and seedling length. The enhanced root, shoot and seedling length may be due to AgNPs capacity to stimulate cell division regulators, hasten the production of photosynthetic pigment and play major role in root enlargement for enhancing water and nutrient uptake by the treated seeds. It also stimulates the production of growth hormones such as auxin, gibberellins and biosynthesis of phytohormones, which are essential for seedling growth and root development (Guzman-Baez *et al*., 2021). It also possesses antimicrobial activity which can help protect seeds from pathogens during germination and early growth stages. In winged bean, AgNPs increase the mitotic index by affecting the cell division stages and explain the variability in root and shoot lengths (Kumar *et al*., 2020). Enhancement in seedling length can be attributed to the activation of various metabolic pathways essential for seed germination, root and shoot growth, triggered by the nanoparticles accumulated within the seeds (Hemalatha *et al*., 2024). Silver nanoparticles seep into the seeds and encourage the accumulation of silver in the shoots and roots, causing the antioxidant enzyme system to react in different ways. Silver primarily resided in the shoots under low concentrations, compared to the roots, and it lightly enhanced photosynthesis in alfalfa (Song *et al*., 2022). Furthermore, seed soaking can proportionally influence the seedlings vigour *via* triggering beneficial metabolic system which is necessary for seedling growth (Paparella *et al*., 2015). Similar results were obtained by Salih *et al*. (2022) in tomato.

Seedling vigour index - I was obtained by considering seedling length (root + stem length cm) and germination percentage. Seedling vigour index - II was obtained by computing dry matter production (mg/10seedlings) and germination percentage, respectively. AgNPs increased vigour index at appropriate concentration by stimulating various metabolic mechanisms (such as α-amylase activity and antioxidant systems) associated with germination by increasing water and small solute uptake and movement, starch hydrolysis and seed reserve mobilization during seed germination as stated by Sencan *et al*. (2024) in basil.

The effectiveness of AgNPs as seed priming agent is influenced by several factors including different concentration, timing and particle size. Positive impact has been observed at varying concentration and two periods of treatments. Research shows that, AgNPs as seed priming agent improve seed germination and seedling growth, but the advantages differ based on concentration and timings. Lower concentration may not contain enough nanoparticles to have a substantial effect, but greater concentrations may cause toxicity. The results obtained are in consonance with the findings of Sharma *et al*. (2012) in *Brassica juncea*, Parveen and Rao (2015) in *Pennisetum glaucum*, Pallavi *et al*. (2016) in cowpea and Budhani *et al*. (2019) in bean seeds.

Fig. 4 : Comparison of best

Conclusion

The current investigation ascertains that the silver nanoparticles of desired size and shape could be synthesized from aqueous extract of *Solanum xanthocarpum*. The synthesized AgNPs are spherical in shape and 70 nm in size. Application of AgNPs as seed priming agent improved the seed germination (%), seedling growth and seedling vigour index of brinjal seeds @ 40 ppm for 15 mins. The seedling germination and vigour exhibited a positive impact under in vivo conditions. Germination studies conducted in *in vivo* has bad to deeper understanding and accurate conclusions on response to stimuli in a natural environment. In this way these results are more reliable.

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