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Influence of biosynthesized silver nanoparticles on pollen germination and tube growth in *Catharanthus roseus* (L.) G. Don

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ABSTRACT

This study examined how pollen germination and pollen tube growth in *Catharanthus roseus* (L.) G. Don was affected by biosynthesized silver nanoparticles (AgNPs). *Pteris vittata* L. frond extract was used to synthesize AgNPs, which were then characterized using UV-Vis spectroscopy, FTIR, EDS and SEM. There have been measurements of silver nanoparticles of different sizes (17 nm to 120 nm) with a mean size of 46 nm. Different concentrations of AgNP (0-100 µg/ml) were applied to pollen grains in a basic germination medium consisting of 5% sucrose solution added with boric acid and magnesium sulphate. The results revealed that addition of AgNPs had a substantial impact on pollen germination and pollen tube growth where basic germination media served as a control. Optimum concentration of 75µg/ml of AgNP was shown to be the most effective which improved percent pollen germination and pollen tube length. Microscopic analysis revealed changes in pollen tube morphology and cell wall integrity. The findings suggest that biosynthesized AgNPs can modulate pollen germination and tube growth in *Catharanthus roseus*, potentially impacting plant reproduction and fertility. Thus, impact of AgNP treated pollen grains might be breakthrough in the field of agriculture against pollen sterility as well as in hybridization experiments.

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INTRODUCTION

With several applications in plant biotechnology, agriculture and environmental science, nanotechnology has become a game-changing field. Among many other nanomaterials, silver nanoparticles (AgNPs) are known for their exceptional physicochemical and biological characteristics, such as their antibacterial activity, catalytic potential, and ability to modulate plant growth (Sharma *et al.*, 2009). Environmental issues arise from the use of hazardous chemicals and energy intensive processes in traditional chemical and physical ways of creating nanoparticles. As a result, biological synthesis, popularly known as "green synthesis," employing plant extracts has grown in popularity as a sustainable, eco-friendly beneficial method (Aboyewa *et al.*, 2021). Phytochemicals including terpenoids, flavonoids, and phenolics work as natural stabilizing and reducing agents during biosynthesis, producing nanoparticles with biocompatible surfaces that improve their ability to interact with plant tissues (Das *et al.*, 2017).

A key factor in influencing the success of fertilization and fruit set is plant reproduction, namely pollen germination and tube expansion. Environmental and chemical signals, including as ions, hormones, and nanoparticles, have a significant impact on pollen tube elongation (Boavida *et al.*, 2005). Nanoparticles can change the physiology of pollen by modifying enzymatic activity, reactive oxygen species (ROS) levels, and membrane integrity, into the reproductive environment (Sosan *et al.*, 2016). Nanoparticles can have both stimulatory and inhibitory effects on pollen viability depending particle size, shape, optimum dosage and synthesis technique. However, the majority of research has been on vegetative development or seed germination, with comparatively little investigation into the impact of nanoparticles on pollen–pistil interactions and reproductive physiology.

The famous Madagascar periwinkle, *Catharanthus roseus* (L.) G. Don, is a medicinally significant species that is prized for its bioactive alkaloids, including vincristine and vinblastine, which have strong anticancer and antihypertensive effects (Moudi *et al.*, 2013). Given its importance in the pharmaceutical sector, knowledge of periwinkle reproductive reactions to nanomaterials may shed light on the ways in which nanoparticles affect pollen viability and overall plant fertility. Furthermore, biosynthesized AgNPs that are enhanced with metabolites generated from plants may have unique biological effects (Arruda *et al.*, 2015). The safe and efficient application of nanoparticles in plant biotechnology can be facilitated by examining these effects at different nanoparticle concentrations in order to determine the boundaries between advantageous and harmful reactions.

The present study aims to evaluate the influence of biosynthesized silver nanoparticles on pollen germination and tube length in *Catharanthus roseus*. By assessing pollen germination percentage, tube length under controlled conditions, this research seeks to elucidate the responses of pollen to biosynthesized AgNP exposure (Fayant *et al.*, 2010). The findings are expected to contribute to a better understanding of nanoparticle–plant reproductive interactions and may offer potential applications in enhancing pollination efficiency, seed production, and controlled fertilization in economically valuable crops.

MATERIALS AND METHODS

Collection and identification of plant material

Fresh and healthy flowers of *Catharanthus roseus* (L.) G. Don were collected from the botanical garden of Nowrosjee Wadia College, Pune, during the flowering season. It was authenticated by Botanical Survey of India, WRC and a voucher specimen was deposited in the departmental herbarium for future reference.

Preparation of plant extract for nanoparticle synthesis

Fresh fronds of *Pteris vittata* L. were washed thoroughly under running tap water. About 1 gm of finely chopped fronds were extracted with 10 ml of de-ionized water so as to get homogenate. Further, it was centrifuged at 6000 rpm for 10 min at room temperature and the clear supernatant was stored at 4°C and used as a reducing and stabilizing agent for silver nanoparticle synthesis.

Biosynthesis of silver nanoparticles (AgNPs)

Biosynthesis of AgNPs was carried out where an aqueous solution of 1 mM silver nitrate ($AgNO_3$) was prepared and 95 ml of $AgNO_3$ was added in a 5 ml of supernatant of frond extract under continuous stirring. The reaction mixture was incubated at room temperature for 48 to 72 hrs. Colour change from pale yellow to brown-black indicated nanoparticle formation. The colloidal suspension was centrifuged at 10,000 rpm for 10 min, and the pellet was washed thrice with distilled water followed by ethanol to remove unspecific bindings.

The crystalline nanoparticles were dried at 40°C and stored for further characterization and biological assays.

Characterization of biosynthesized silver nanoparticles

The synthesized AgNPs were characterized to confirm their formation and stability using standard techniques like UV–Visible Spectroscopy, Fourier Transform Infrared Spectroscopy (FTIR), Scanning Electron Microscopy (SEM) and X-Ray Diffraction (XRD) so as to study their size, shape, morphology and other stability parameters.

Collection and preparation of pollen grains

Fresh, mature anthers of *C. roseus* (L.) G. Don were collected early in the morning (around 8 am) from newly opened flowers. The anthers were carefully tapped over clean glass slides to release viable pollen grains. The collected pollen was used immediately for germination assays to ensure high viability.

Pollen germination medium

Pollen germination was carried out in Brewbaker and Kwac medium (Brewbaker and Kwack, 1963) with little modifications which contained 05% sucrose, 0.01 gm Boric acid (H_3BO_3), and 0.02 gm Magnesium sulfate heptahydrate ($MgSO_4 \cdot 7H_2O$). The final volume of the medium was adjusted at 100 ml. It was sterilized by filtration and the pH was set at 6.8 before use.

Treatment of pollen with biosynthesized AgNPs

Stock solution of biosynthesized AgNPs (1mg/ml) was prepared in sterile distilled water and sonicated so as to get uniform dispersion. Different concentrations (25ug, 50ug, 75ug and 100ug/ml) were added in aforesaid traditional medium. Only traditional medium without addition of AgNP was served as control. The slides were incubated in a humid chamber at 25 ± 2°C for 1 hr under diffused light.

Assessment of pollen germination and tube growth

After incubation, slides were observed under a light microscope. A pollen grain was considered germinated when the pollen tube length exceeded the diameter of the pollen grain.

Pollen germination percentage was calculated using the formula:

$$\text{Germination (\%)} = \frac{\text{Number of germinated pollen grains}}{\text{Total number of pollen grains observed}} \times 100$$

Pollen tube length was measured using a calibrated ocular micrometer. The experiments were performed in triplicates and the mean tube length was recorded.

Statistical analysis

All experiments were conducted in triplicate, and data were expressed as mean ± standard deviation (SD).

RESULTS AND DISCUSSION

Characterization of biosynthesized silver nanoparticles

The biosynthesis of silver nanoparticles (AgNPs) using *Pteris vittata* L. frond extract was confirmed by a visible colour change from pale yellow to brown,-black indicating the reduction of Ag^+ ions to Ag^0 nanoparticles (Fig. 1). The UV–Visible spectrum of the colloidal solution exhibited a distinct surface plasmon resonance (SPR) peak at 435 nm, characteristic of silver nanoparticles (Brewbaker and Kwack, 1963) (Fig. 2).

Fig. 1. Change in reaction colour representing synthesis of AgNP

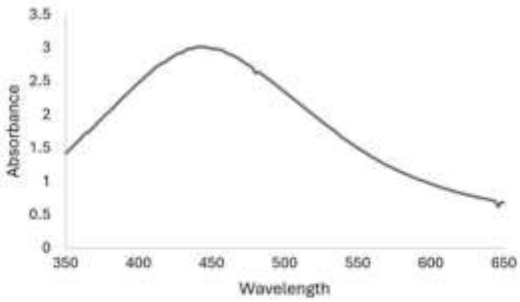

Fig. 2. UV-Vis spectrum of biosynthesized AgNP

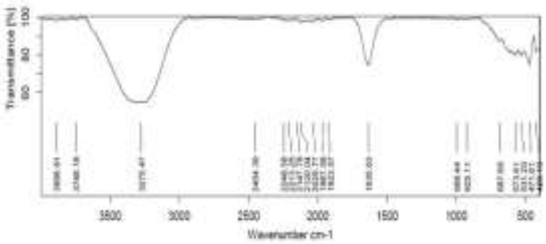

Fig. 3. FTIR analysis of biosynthesized AgNP

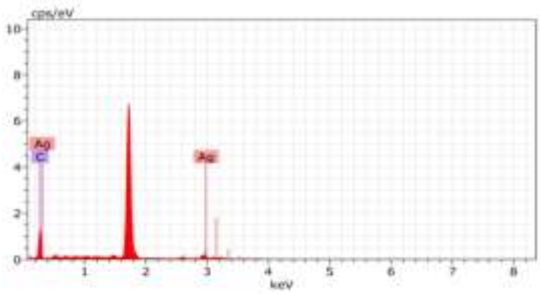

Fig. 4. EDS spectrum of biosynthesized AgNP

Fourier Transform Infrared (FTIR) analysis revealed two noticeable peaks at wave numbers 3275.47 cm^{-1} and 1635.63 cm^{-1} these representing the polymeric O-H stretch and amide (C=O stretching) peaks, respectively (Fig. 3). This confirms the involvement of phytochemicals such as flavonoids, etc. in the reduction and stabilization of AgNPs.

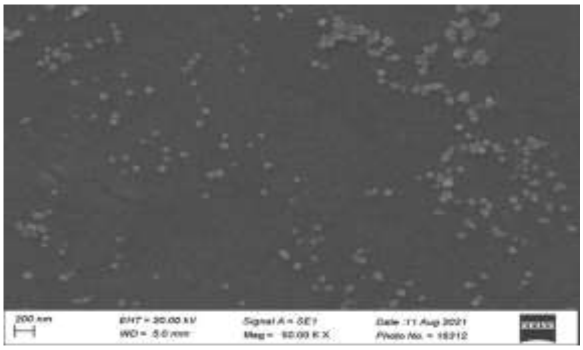

Fig. 5. SEM image of biosynthesized AgNP

SEM verified that the biosynthesized nanoparticles had a spherical shape (Fig. 5). The characteristic peak for Ag was visible in the EDS spectrum at about 3 keV. A second peak that corresponded to C was seen. Ag (50.59) and C (49.41) were the percentage relative compositions found by EDS analysis (Fig. 4). The capping elements linked to phytosynthesized AgNP are responsible for the presence of C.

Effect of AgNP concentration on percent pollen germination and pollen tube length

Pollen germination percentage varied significantly with AgNP concentration (Table 1). The control (Basic medium) showed a mean germination rate of 71.2%, representing normal physiological activity. A moderate increase in germination was observed (78.6%) when a basic medium was modified with 25ug/ml AgNP. Further increases in concentration (basic medium in addition with 50ug/ml and 75ug/ml) resulted in a progressive increase in germination rate that is 85.3 % and 82. 4 % respectively. However, at 100ug/ml concentration a sudden decline of germination percentage was observed. This biphasic response indicates that biosynthesized AgNPs exert a stimulatory effect at low concentrations but become inhibitory at higher levels (Kummara *et al.*, 2016). This dose-dependent behavior of silver nanoparticles has been reported widely where moderate nanoparticle exposure enhanced pollen viability and metabolic activity, while excess nanoparticles induced oxidative stress and cellular damage (Manchanda *et al.*, 2022).

Table 1. Effect of biosynthesized AgNPs on percent pollen germination and tube length in *Catharanthus roseus* (L.) G Don

Type of medium	Pollen germination in (%)	Pollen tube length	Observations on tube formation
Basic medium (5% Sucrose + H_3BO_3 + $MgSO_4$)	71.2 ± 1.9^{d}	114.8 ± 3.4^{e}	Moderate tube formation
Basic medium + 25ug/ml AgNP	78.6 ± 2.1^{c}	150.2 ± 5.1^{d}	Very less tube formed
Basic medium + 50ug/ml AgNP	85.3 ± 2.0^{a}	165.1 ± 4.8^{c}	Small tube formed
Basic medium + 75ug/ml AgNP	82.4 ± 1.7^{a}	210.1 ± 3.9^{a}	Large tube formation
Basic medium + 100ug/ml AgNP	70.0 ± 1.9^{b}	180.3 ± 4.5^{b}	Moderate tube formation
p = .05	1.94×10^{-25}	2.99×10^{-40}	

Values are mean ± SD (n = 10). Different superscript letters (a–e) within a column indicate significant differences at $p<0.05$.

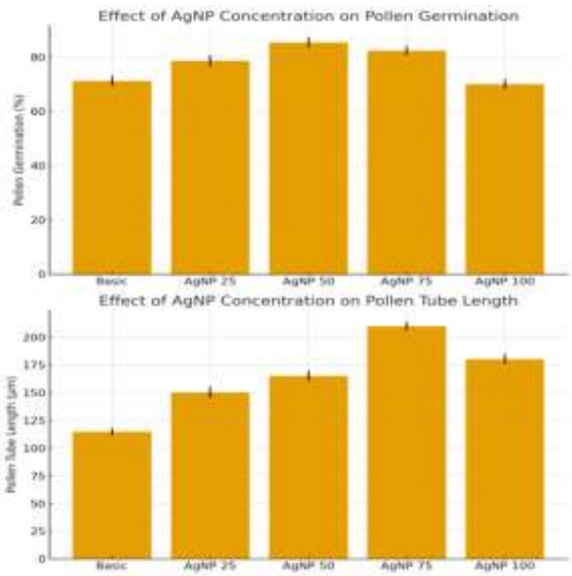

Fig. 6. Graph representing an effect of AgNP with the gradual changes in pollen germination and pollen tube length

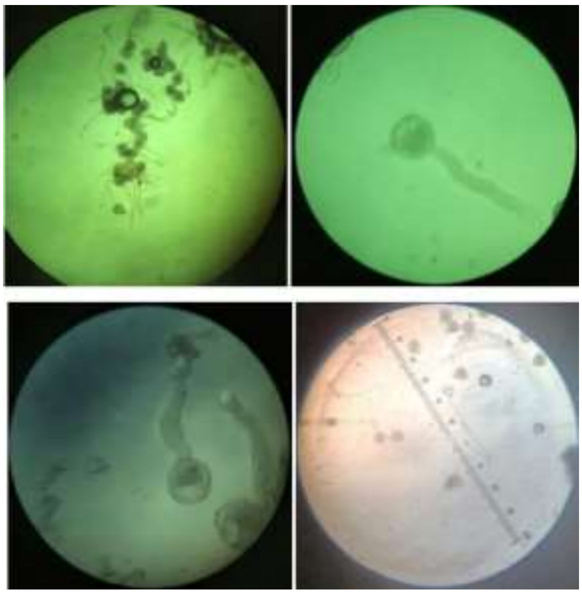

Fig. 7. Microscopic observations of effect of AgNP on pollen germination

Pollen tube elongation followed a similar trend to germination rate. The average tube length increased significantly at 25 ug/ml, 50 ug/ml and 75 ug/ml concentrations, suggesting enhanced metabolic activity and calcium ion flux essential for tube elongation (Brewbaker and Kwack, 1963). The optimum response was recorded at 75ug/ml where tube length reached 210.1 µm compared to 114.8 µm in the control. Beyond this concentration (at 100ug/ml), tube elongation was markedly inhibited. Microscopic images revealed stunted, swollen, and irregular in shape at higher concentrations (Fig. 6&7). Such abnormalities are likely caused by excessive accumulation of AgNPs on pollen walls, leading to disruption of cell membrane integrity and alteration of cytoplasmic streaming. The inhibitory effect at higher concentrations may also be attributed to nanoparticle-induced oxidative stress, generation of reactive oxygen species (ROS), and interference with calcium-mediated signaling pathways necessary for directional pollen tube growth (Sosan *et al.*, 2016).

CONCLUSION

The outcome of this study demonstrates that biosynthesized silver nanoparticles significantly influence pollen germination and tube length in *Catharanthus roseus*. Low concentrations of AgNPs (up to 75ug/ml) enhanced pollen performance, likely due to their involvement in activating enzymatic activity, maintaining ionic balance, and facilitating energy metabolism. However, excessive concentrations led to inhibition, reflecting nanoparticle-induced stress. The green-synthesized AgNPs, capped with bioactive phytochemicals, appear to interact more gently with plant tissues than chemically synthesized nanoparticles, highlighting their potential for use in reproductive physiology studies and nano-assisted plant breeding. Thus, synthesized silver nanoparticles showed effective for in-vitro pollen germination but it will be very interesting to find out the exact mechanism. Impact of AgNP treated pollen grains might be breakthrough in the field of agriculture against pollen sterility as well as in hybridization experiments.

Overall, the study provides valuable insight into nanoparticle pollen interactions and supports the potential of biosynthesized AgNPs as a tool for controlled modulation of pollen physiology in medicinally and agriculturally important species.

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REFERENCES

Aboyewa JA, Sibuyi NRS, Meyer M, Oguntibeju OO. 2021. Gold nanoparticles synthesized using extracts of *Cyclopia intermedia* (honeybush) amplify the cytotoxic effects of doxorubicin. Nanomaterials **11**(1), 1–16.

Arruda SC, Silva AL, Galazzi RM, Azevedo RA, Arruda MA. 2015. Nanoparticles applied to plant science: A review. Talanta **131**, 693–705.

Boavida LC, Vieira AM, Becker JD, Feijó JA. 2005. Gametophyte interaction and sexual reproduction: How plants make a zygote. International Journal of Developmental Biology **49**, 615–632.

Brewbaker J, Kwack BH. 1963. The essential role of calcium ion in pollen germination and pollen tube growth. American Journal of Botany **50**, 859–865.

Das RK, Pachapur VL, Lonappan L, Naghdi M, Pulicharla R, Maiti S. 2017. Biological synthesis of metallic nanoparticles: Plants, animals and microbial aspects. Nanotechnology for Environmental Engineering **2**. Springer Science and Business Media Deutschland GmbH.

Fayant P, Girlanda O, Aubin CE, Villemure I, Geitmann A. 2010. Finite element model of polar growth in pollen tubes. Plant Cell **22**, 2579–2593.

Kummara S, Patil MB, Uriah T. 2016. Synthesis, characterization, biocompatibility and anticancer activity of green and chemically synthesized silver nanoparticles: A comparative study. Biomedicine and Pharmacotherapy **84**, 10–21.

Manchanda P, Sharma S, Marni AD. 2022. Effects of various carbohydrates on *in vitro* pollen germination of *Vinca rosea* and *Cucumis melo* var. *utilissimus*. International Journal of Agriculture, Environment and Biotechnology **15**(1), 19–24.

Moudi M, Rusea G, Christiana Y, Mohd N. 2013. Vinca alkaloids. International Journal of Preventive Medicine **4**(11), 1231–1235.

Sharma VK, Yngard RA, Lin Y. 2009. Silver nanoparticles: Green synthesis and their antimicrobial activities. Advances in Colloid and Interface Science **145**, 83–96.

Sosan A, Svistunenko DA, Straltsova D, Tsiurkina K, Smolich I, Lawson T, Subramaniam S, Golovko V, Anderson D, Sokolik A, Colbeck I, Demidchik V. 2016. Engineered silver nanoparticles are sensed at the plasma membrane and dramatically modify the physiology of *Arabidopsis thaliana* plants. Plant Journal **85**(2), 245–257.