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GREEN SYNTHESIS OF AGNPS AND THEIR EFFECTS ON SEED GERMINATION AND SEEDLING VIGOR IN *ACACIA SENEGAL* AND *ACACIA MELLIFERA*

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The biosynthesis of silver nanoparticles (AgNPs) was purposively performed and their effects on seed germination and seedling vigor in *Acacia senegal* and *Acacia mellifera* were assessed in the experiment. The structural properties of the synthesised AgNPs were characterised using powder X-ray diffraction. The average particle was size at 26.51 ± 8.72 nm using Transmission Electron Microscopy, and the sharp color change and the emergence of a characteristic peaked at 415 nm in the UV-Vis Spectrum. Fourier Transform Infrared Spectroscopy was used to detect functional groups, morphological properties, size and shape of AgNPs which were visualised using Scanning Electron Microscope. A completely randomized design was used for the experiment with four replicates and four pre-sowing treatments consisting of silver nanoparticles at different concentrations of (control, 25, 50 and 75 mg L^{-1}) to examine seed germination and seedling growth characteristics. AgNPs treated seeds showed a high germination rate with early growth of seedlings at concentration (75 mg L^{-1}) in *A. mellifera* (100%) and *A. senegal* (72.5%) at the greenhouse condition, compared to the control, which recorded (50%) and (42%) for *A. mellifera* and *A. senegal*, respectively. Additionally, the seedlings growth reached maximum shoot height of 75 cm and 60 cm with the 75 mg L^{-1} AgNPs treatment, compared to 40 cm and 32 cm in the control group for A. mellifera and A. senegal, respectively. Silver nanoparticles had successfully improved seed germination and seedling growth in the two studied Acacia species.

Keywords: Silver nanoparticles, AgNPs, *Acacia mellifera*, *Acacia senegal*, germination, seedling growth

INTRODUCTION

Silver nanoparticles have been extensively exploited, because of their biological and biomedical applications and functions (Rasheed et al. 2017). Comparing with other methods, silver nanoparticle is environmentally friendly, chemically stable and low cost-effective when prepared through green technology, making it a cheaper alternative when it available with a high potentiality (Saheed 2021). Numerous studies in recent years have considered the use of silver nanoparticles (AgNPs) as a possible pre-sowing treatment or nano-fertiliser (Nejatzadeh 2013). The effect of AgNPs appears to be dependent on the species and age of the given plant, the size and concentrations of the nanoparticles themselves, as well as the experimental conditions, which include temperature, duration and method of application (Krishnaraj et al. 2012). Several studies have found that the use of AgNPs improved seed germination, plant growth and development. Because of their small size and unique physicochemical properties, nanoparticles are an effective seed priming technique (Dasgupta et al. 2017, Siddique & Bose 2007). Seed priming can accelerate germination rate, speed of germination, vigor index of seedling, root and shoot elongation, photosynthetic rate and other growth traits of plants (Siddique & Bose 2007). Seed priming is a pre-sowing treatment which puts seeds in a specific concentration of defined solution for a specified period, creating a physiological state

in the seed that strengthens its growth capacity against biotic or abiotic stresses (Conrath 2011).

Acacia senegal (L. Willd) is a multipurpose small tree in the Mimosaceae family, usually 2–6 m in height, occasionally reaching to 10 m under optimum condition that grows in Africa's Sahel zone, which stretches from Senegal to the Red Sea (Siam et al. 2022). Nearly 90% of the renowned commercial gum arabic is produced by this species (Verbeken et al. 2003). Approximately 80–90% of global arabic gum exports come from Sudan, where the tree known locally as hashab contributes to the national economy and the revenues of farming communities (Mohammed et al. 2016). Gum arabic is extracted from the tree and a significant source of income for rural populations. Its ability to fix nitrogen and withstand drought, *Acacia senegal* is an essential component of dryland agroforestry systems and the species is widely recommended in Sahel reforestation programme (Diallo et al. 2015).

Acacia mellifera (Vahl) Benth or locally known as kitir is a 6 m tall tree or shrub. It is common in the arid African savanna. It grows on hard clay soil in the central parts of Sudan and extends westward to near Lake Chad (Eisa 2022). *Acacia mellifera* has the ability to fix nitrogen, which is thought to improve soil fertility and ultimately, herbage quality under canopy and beyond (Hagos & Smit 2005). The growth and conservation of acacia trees in arid and semi-arid areas is essential for agricultural lands amelioration and animal survival, during periods of drought when cereal crops and grass may fail to grow, acacia trees can be relied on to provide fodder for livestock and various products for mankind and environmental services in sub-Sahara Africa (Siam & Abdalkreem 2019).

The study was undertaken to perform green synthesis of AgNPs by reducing silver nitrate with phytochemicals found in the extract of *Eucalyptus camaldulensis* leaves and to assess the variability and differences in the effects of biosynthesised silver nanoparticles on overcoming seed dormancy, improving seed germination and seedling vigor issues in *Acacia senegal* and *Acacia mellifera* trees. The specific objective of this study was to evaluate *Acacia senegal* and *Acacia mellifera* seed germination properties and early seedling growth following germination.

MATERIALS AND METHODS

Study area and seed procurement

The experiment was conducted during two growing seasons (2021 and 2022), from May to December each year. It took place in the laboratory and nursery of the Forestry and Wood Technology Department, Faculty of Agriculture, University of Alexandria. Seeds from two species (*Acacia senegal* and *Acacia mellifera*) were donated by the Agricultural Research Corporation (ARC) in Sudan, Tree Seed Center, located at Soba, Khartoum.

Preparation of plant extract

Leaves of *Eucalyptus camaldulensis* were collected from Antoniadis Garden, Agricultural Research Center, Ministry of Agriculture, Alexandria, Egypt. They were washed with sterile distilled water, and 25 g of the leaves was taken then mixed with 100 mL of the prepared phosphate buffer saline (pH 7.4). The suspended plant material was heated for 30 min at 70 ˚C. The extract was stirred (150 rpm) at 24 ˚C. The supernatant was filtered and collected in a clean tube.

Biological synthesis of silver nanoparticles (AgNPs)

The preparation of biosynthesised silver nanoparticles in the Department of Genetics, Faculty of Agriculture, Alexandria University, was carried out according to the method described by Nehal et al. (2015). In the initial preparation, 100 mL of *Eucalyptus camaldulensis* leaf extract was mixed with 8 mM silver nitrate ($AgNO_3$) in a 250 mL conical flask. The reaction mixture was stirred at 150 rpm at 24 ˚C. The extract was removed after one hour and scanned with a UV-Visible spectrum spectrophotometer to confirm the formation of silver nanoparticles AgNPs. After four days, the colloidal suspension of the prepared particles was subjected to centrifugation at 11963g for 30 min at 4˚C. The resulted product was washed with deionised water to remove any unbound silver and plant extract, as seen in Figure 1. Then biosynthesised silver nanoparticles were dried into powdered form and stored at room temperature.

AgNPs characterisation

The morphology of AgNPs was analysed using UV-Visible spectrum spectrophotometer in the wavelength range of 350–900 nm. Dimensional analysis and structural characterisation of the obtained AgNPs were carried out by Transmission Electron Microscopy (TEM) with high-resolution, operated at 120 kV at the Electron Microscope Unit, Alexandria University, Faculty of Science and with the aid of X-ray Diffraction using a DRON 2.0 Diffractometer with Cu tube. Chemical composition of the green synthesised sliver nanoparticles were further characterised using FTIR and observed through Scanning Electron Microscope. After 7 days of the addition of $AgNO_3$ to the extract, SEM slides were prepared by smearing the solute extracts on slides.

Effect of synthesised AgNPs on seed germination and seedling growth

Seeds were brought to the laboratory at room temperature and greenhouse and soaked in four different concentrations of AgNPs (0, 25, 50, and 75 mg L^{-1}) for six hours for seed dormancy. Deionised water (DI) was used as the control (Abbasi et al. 2019). After priming, 100 treated seeds were sown in a Petri dish lined with filter paper for a room temperature experiment at 25 °C. Similarly, in laboratory all petri dishes containing seeds were placed in a seed incubation at 35 °C, under continuous LED lighting for 24 hours and 1 mL water was sprayed to keep filter papers moist, seed germination was observed and counted daily for two weeks, as shown in Figure 1. In greenhouse at 38 °C, all the treated and untreated seeds were then sown in polyethene bags (10 × 20 cm size, 2 seeds per bag). The bags were filled with a mixture of sand, clay soil, and compost (2:1:1), with each bag weighing 1.25 kg. The planted bags were watered daily during the first two months.

Seeds were observed daily throughout six weeks for germination. The seed germination rate and seedling vigor index in each treatment were determined by counting the number of emerged seeds each day (Kharnaior & Thomas 2023). Two months after germination, seedlings were transplanted from small bags into larger polythene bags measuring (20 cm in diameter and 30 cm in height) containing 5 kg of soil per bag. After six months, the seedlings were separated from the soil and measured for shoot and root length using a centimeter-graduated ruler. (Kumar et al. 2016). Calculations were as shown in the following equations:

$$GP=\frac{\text{Number of germinated seeds per day}}{\text{Number of total seeds}} \times 100 \quad (1)$$

where GP is germination percentage.

$$SVI=GP \times \frac{\text{mean (shoot length + root length)}}{100} \quad (2)$$

where SVI is seedling vigor index.

Experimental design and statistical analysis

The experiment design by the completely randomized design (CRD) with four replicates and four treatments was adopted. Calculation of one-way ANOVA, P value < 0.05 is considered statistically significant. The data were analysed using SPSS 22 software.

RESULTS AND DISCUSSION

Biosynthesis and characterisation of silver nanoparticles

Figure 2B and C displays the color change from yellow to blackish brown which was observed in the reaction mixture solution of ($AgNO_3$ + extract) indicating the bioreduction of the Ag^{+1} into Ag^{+0} and then into nanoparticles served as evidence of the formation of AgNPs. Thus, the plant extracts acted as reducing agent. The primary indicator of nanoparticles synthesis is the changing color of the reaction mixture (visual observation) during the reaction time (Patil & Kim 2016). Color changes can vary from plant to plant and depending on the method of synthesis (Pourmortazavi et al. 2015). The phytochemicals act as a reducing agent by adhering to the nanoparticles and preventing agglomeration, but they also act as a stabilising agent, which is difficult to obtain when the chemical procedure is used (Syafiuddin et al. 2017). Weed plant extracts are widely used because their internal secondary metabolites, such as protein, terpenoids, and flavonoids, have the potential to act as bio

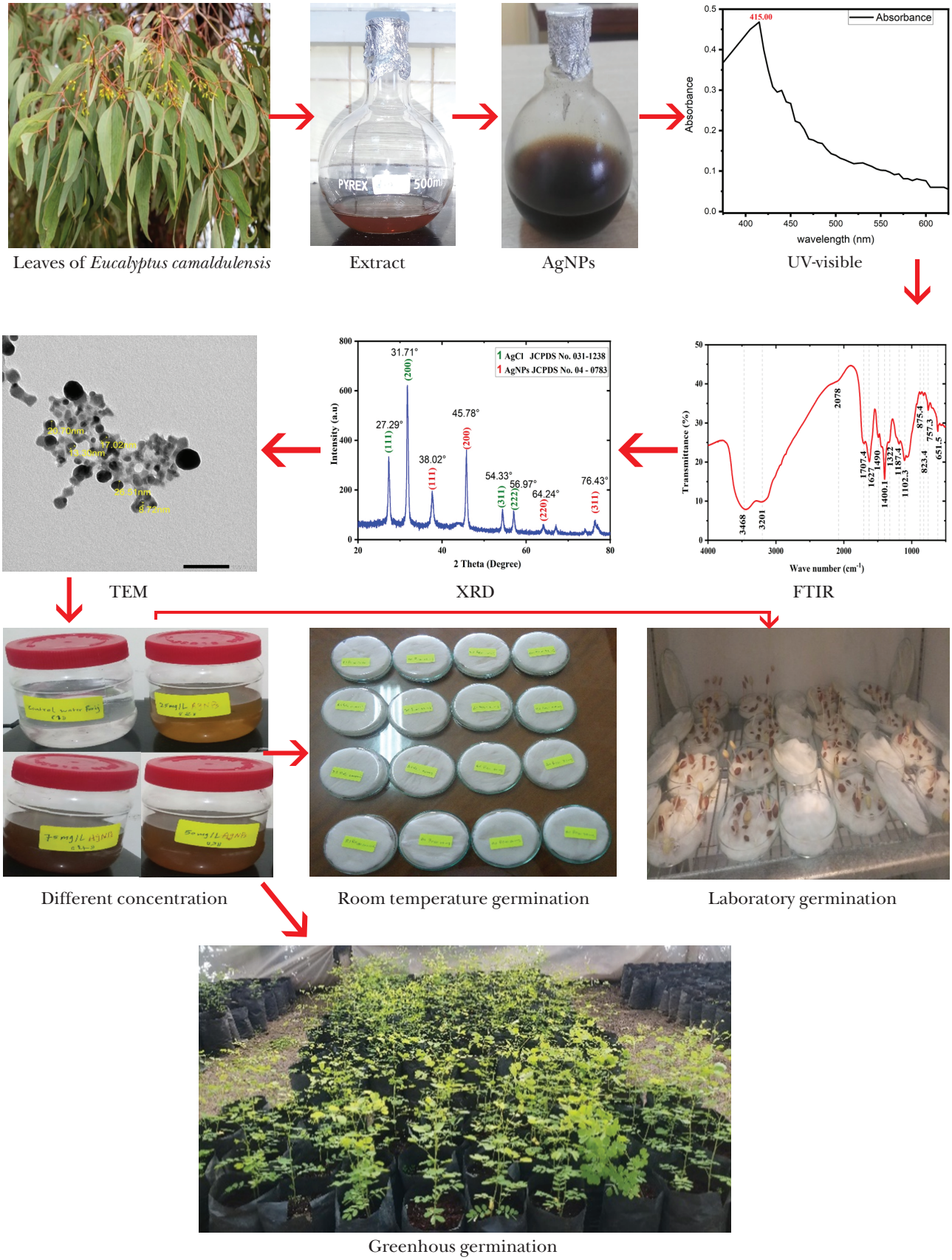
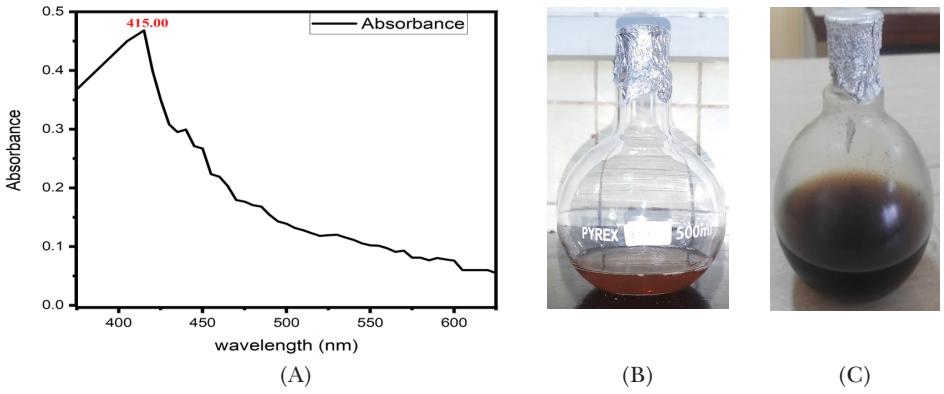


Figure 1 The experimental steps involved in the biological synthesis, characterisation, application of AgNPs, and germination of *A. senegal* and *A. mellifera* seeds.

reductant (Dhar et al. 2017). Figure 2A shows the absorbance of AgNPs colloidal suspension by UV-Vis spectrum the emission peak at 415 nm due to Surface Plasmon Resonance (SPR) value in the visible region of the spectrum and the presence of dispersed particles was indicated. Due of the excitation of the localised Surface Plasmon Resonance, AgNPs absorb the spectrum in the visible region around 400 nm (Dhand et al. 2016). Furthermore, an increase in nanoparticle size can be identified by their maximum absorbance occurring at a higher wavelength range (Sulaiman et al. 2013, Tan & Cheong 2013).

In order to confirm the crystalline nature of the silver nanoparticles, X-ray diffraction analysis was performed and the prepared silver nanoparticles XRD patterns were recorded from *Eucalyptus camaldulensis* leaves at 2θ value ranging from 20–80 as shown in Figure 3A. These peaks showed a good match with pure crystalline silver and silver chloride structures by the Joint Committee on Powder Diffraction Standards (JCPDS) under file numbers 04-0783 and 31-1238, respectively. There were 8 peaks observed by XRD analysis: 4 peaks for silver nanoparticles and 4 peaks for silver chloride. AgNPs were present in the diffraction pattern at peaks (111), (200), (220), and (311), which corresponded to reflections at angles of 38.02°, 45.78°, 64.24°, and 76.43°, respectively. This described the face cubic center structure confirming the crystalline nature of the silver nanoparticle. The XRD spectra for AgNPs showed four strong reflections at 38.45°, 44.25°, 64.4°, and 77.4° which corresponded to the crystallographic planes of the face-centered cubic silver (Alghoraibi et al. 2020, Kathiravan et al. 2015, Patil et al. 2018). Figure 3B shows the Transmission Electron Micrographs (TEM). It was observed that particle sizes ranged from 8.72 to 26.51 nm. The particles were spherical, and minimal agglomeration was noted, indicating that the *Eucalyptus camaldulensis* leaves extract was well-suited for reducing and stabilizing the AgNPs. This observation was consistent with (Oliveira et al. 2023); who synthesised silver nanoparticles (AgNPs) with a spherical shape and a size of 9.7 ± 0.3 nm using the aqueous leaf extract of *Eucalyptus grandis*. The silver nanoparticles that are spherical in shape, with particle sizes ranging from 20 to 35 nm (Singh et al. 2020).

The functional groups of AgNPs powder were investigated using an IRAffinity-1S Spectrometer. The FTIR spectrum of green synthesised silver nanoparticles was recorded to identify potential interactions between silver and bioactive molecules**.** The FTIR spectrum shows strong absorption peaks Figure 3C at 3468, 3201, 2078, 1627, 1102.3 and 875.4 cm^{-1}. The absorption bands at 3468, 3201and 2078 cm^{-1} are due to the hydroxyl (OH) stretching vibration of the alcohol and phenols in the nanomaterial, C–H stretching. This is in agreement with report that, the peak observed in the FTIR spectrum at 3591–3269 cm^{-1} indicates the presence of the O-H group in alcohol and phenols, as well as the N-H stretching vibration of protein amide (Khan et al. 2022). The bands at 1627, 1102.3 and 875.4 cm^{-1} indicate the presence of the C-N stretching, C–C stretching



(A) (B) (C)

Figure 2 UV-visible spectroscopy of biogenic silver nanoparticles (A), *Eucalyptus camaldulensis* plant extract (B), The formation of a blackish-brown color indicates the formation of silver nanoparticles (C)

vibrational modes, N=O symmetry stretching in the nitro compounds and the bonding of metal particles with oxygen, respectively. The presence of proteins is indicated by the absorption peak at 1648 cm^{-1} (Prakash et al. 2013; Singh et al. 2020). Furthermore, the study revealed that there were significant interactions between the AgNPs and the extract's biomolecules. The current study supported the findings of other studies, which revealed that biomolecules present in extracts could reduce Ag^{+} ions. The morphological properties, size and shape of the synthesised nanoparticles were visualised using Scanning Electron Microscopy. The zoomed at ×35000 micrographs of silver nanoparticles by green synthesised were found to be in irregular shapes with diameters of 25.41, 26.51, and 27.57 nm the aggregation as shown in Figure 3D. Other researchers have reported that *Eucalyptus camaldulensis* nanoparticles come in a variety of shapes and sizes (Alghoraibi et al. 2020, Syukri et al. 2020).

Germination properties and seedling survival

The results revealed significant differences among conditions in germination capacity between AgNPs concentration within each species, as well as total survival rate (Table 1). The highest total germination percentage was recorded by greenhouse (100%), (72.5%) and room temperature (95%), (71.5%) and laboratory condition (67.5%), (57.5%) in *Acacia mellifera* and *Acacia senegal*, respectively. Seeds that underwent an electrical needle test and boiling water treatment exhibited the highest germination rate at 60%, whereas those treated with H_2SO_4 25% showed the lowest rate at 20% in overcoming dormancy in *Acacia polycantha* seeds (Hamdon et al. 2014). At all conditions the germination capacity at concentration of 75 mg L^{-1}, was significantly high compared to the control for both species. The results are in a line with those of (Abbasi et al. 2019); who found

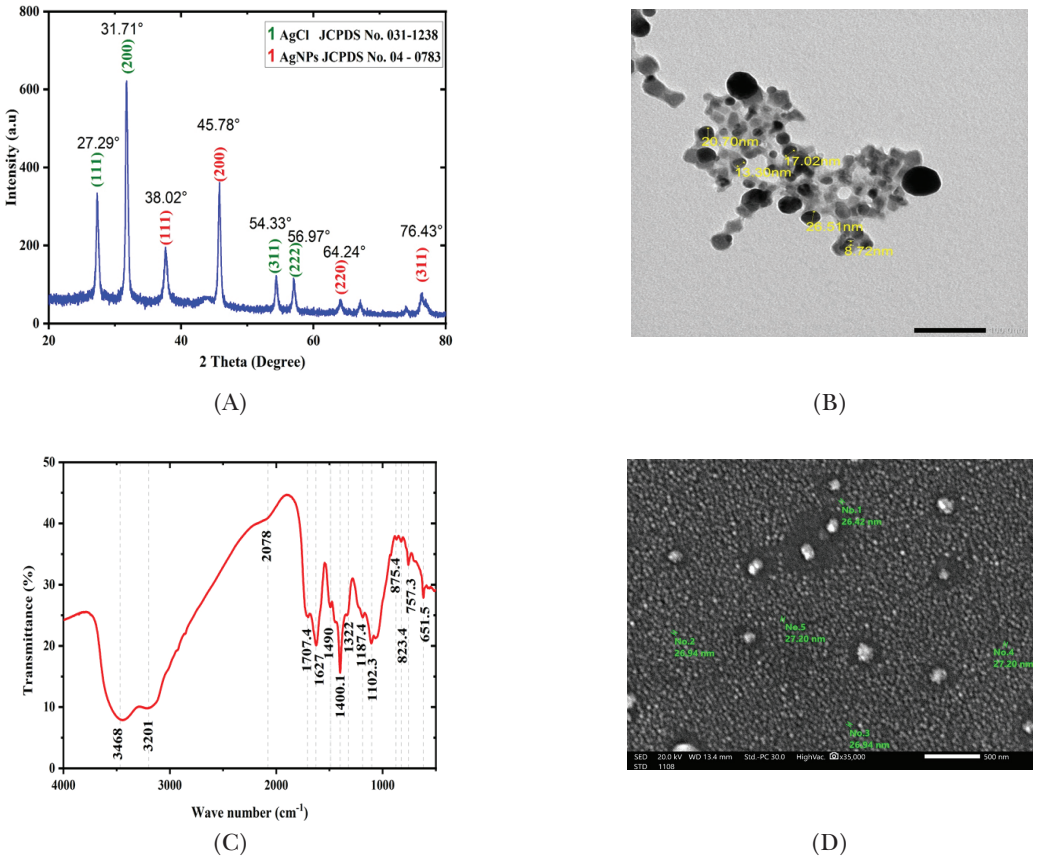


Figure 3 Characterisation of biogenic silver nanoparticles (AgNPs). (A) X-ray pattern with peak indices and 2-theta position; (B) Transmission Electron Microscopy TEM, (C) Fourier Transform Infrared Spectroscopy FTIR and (D) Scanning Electron Microscopy SEM

Table 1 Influence of silver nanoparticles treatment on seed germination and seedling survival of *Acacia senegal* and *Acacia mellifera* by three conditions (Laboratory, greenhouse, and room temperature)

Species	Treatment	Germination percentage at Laboratory condition (%)	Germination percentage at room temperature (%)		Germination percentage at greenhouse condition (%)		Survival percentage after six months for greenhouse condition (%)	
		1st	1st	2nd	1st	2nd	1st	2nd
Acacia senegal	Control	27^{c}	37.5^{c}	38.5^{c}	47.5^{c}	42^{bc}	18^{c}	20^{cd}
	25 mg L^{-1}	52.5^{ab}	57.5^{b}	55^{b}	62.5^{ab}	48^{bc}	50^{ab}	33^{c}
	50 mg L^{-1}	56.5^{a}	60^{ab}	59^{b}	62.5^{ab}	55^{ab}	52^{ab}	45^{ab}
	75 mg L^{-1}	57.5^{a}	70^{a}	71.5^{a}	72.5^{a}	62^{a}	68^{a}	51^{a}
Acacia mellifera	Control	50^{bc}	67.5^{c}	55^{c}	82.5^{b}	50^{c}	24^{c}	36^{d}
	25 mg L^{-1}	62.5^{ab}	85^{b}	86^{ab}	87.5^{b}	78^{b}	72^{b}	52^{bc}
	50 mg L^{-1}	62.5^{ab}	87.5^{b}	80^{ab}	90^{ab}	85^{ab}	81^{ab}	64^{b}
	75 mg L^{-1}	67.5^{a}	95^{a}	93.5^{a}	100^{a}	97^{a}	93^{a}	88^{a}

Means in the same column within each condition for a given species having different superscript are significantly different ($p< 0.05$).

an increase in seed germination in *Festuca ovina* using nanoparticles was likely attributed to the small size and dimensions of the materials, enabling them to rapidly and efficiently affect the seeds. Germination began earlier during the third day and peaked on the sixth day for all concentrations of AgNPs in *A. mellifera* and *A. senegal*, whereas peaked on the ninth day in the control. Germination started during the first week and peaked in second week for *A. mellifera* seeds (Abdalkreem et al. 2017). The *Acacia senegal* seeds from the Buram provenance demonstrated a high overall germination rate of 61% during the six-week assessment period (Nusiba & Abubakr 2017). The delay in seed germination in *A. senegal* may be attributed to the hardness of the seed coat and, as a result, reduced water absorption (Nusiba & Abubakr 2017). The impact of using AgNPs during germination varied from beneficial to neutral or inhibitory, primarily depending on their dosage. Applying AgNPs at a concentration of 50 mg L^{-1} enhances the germination percentage of *Brassica nigra*, while at higher dosages ranging from 100 to 1600 mg L^{-1}, there is a decrease in germination rates (Amooaghaie et al. 2015).

High survival rates of (93%), (68%) for first season and (88%), (51%) in second season after one years in greenhouse at concentration of 75 mg L^{-1}, and low survival rates in control at (20%), (18%) for first season and survival rate at (36%), (20%) for second season in *A. mellifera* and *A. senegal*, respectively were detected**.** In general, the treatment of AgNPs exhibited substantial survival capacity of seedlings of both species relative to control ones. These results are in line with findings of (Sarkhosh et al. 2022); who reported that the highest concentrations of zinc oxide nanoparticles (ZnONPs) have been observed to positive influence the speed of germination and survival rates in *Brassica napus* and *Camelina sativa* plants. High seedling survival caused by AgNPs results in more new pores that remain useful in carrying nutrients efficiently, resulting in seedlings with fast germination, growth rate ability and survival, as indicated in Figure 4. Silver nanoparticles (AgNPs) exhibited a greater enhancement in the final germination percentage compared to other types of nanoparticles, while zinc nanoparticles (ZnNPs) notably promoted root elongation (Guo et al. 2022). The improved growth in seedlings may be attributed which increased water and nutrient absorption by growth traits with AgNPs, that suggests a positive correlation between seeds and silver nanoparticles. Silver nanoparticles treatments significantly boosted α-amylase activity in rice seedlings compared to the control. Additionally, AgNPs regulates aquaporin genes in germinating seeds, corresponding with the accelerated germination observed in nanoprimed seeds (Mahakham et al. 2017).

Acacia mellifera *Acacia senegal*

Figure 4 Six months survival seedling of *Acacia senegal* and *Acacia mellifera* under treated with AgNPs (control, 25,50 and 75 mg L^{-1}) greenhouse conditions

Early growth of seedlings

The result showed significant differences in seedling heights between treatments in each species (Table 2). The three AgNPs concentration treatments have induced significantly higher growth rate of root and shoot in all conditions for *A. senegal* and in greenhouse and room temperature for *A. mellifera* compared to control seedlings. Over two the seasons, *A. senegal* reached maximum shoot height of 60 cm and 32 cm at 75 mg L^{-1} AgNPs and control respectively in greenhouse condition. Moreover, *A. mellifera* exhibited higher height of 75 cm at 75 mg L^{-1} and 40 cm at control in greenhouse condition. These results were congruent with the results of (Ajmal et al. 2023); who indicated that the treatment with Kn-ZnONPs at a concentration of 10 µg mL^{-1} had resulted in the highest shoot length of *Vigna radiata*. The application of AgNPs induced better shoot formation, improved its chlorophyll composition and total/carotenoid ratios (Tejada et al. 2023). The highest root lengths in greenhouse were 34 cm and 33 cm at 75 mg L^{-1} and 22 cm and 19 cm in control for *A. mellifera* and *A. senegal*, respectively. Furthermore, the use of silver nanoparticles at concentrations ranging from 50 to 100 ppm stimulated root elongation in *mustard* seedlings (Tomaszewska et al. 2023).

CONCLUSION

The study successfully synthesised silver nanoparticles (AgNPs) using *Eucalyptus camaldulensis* leaves and silver nitrate solution through a biological synthesis process. These nanoparticles demonstrated stability in solution. The application of silver nanoparticles had positive impact on the seed germination of the studied *Acacia* species. Their small size and dimensions of the nanomaterials enabled them to rapidly and efficiently affect the germination of the tested seeds. In conclusion, the use of nanoparticle-treated seeds showed promising technique for enhancing seedling growth and seed germination in *Acacia senegal* and *Acacia mellifera*. However, further investigations are recommended before definitive conclusions can be drawn.

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Table 2 Length of root and shoot of *Acacia senegal* and *Acacia mellifera* (laboratory and greenhouse conditions)

Species	Treatment	Root and shoot length at laboratory (cm)		Root and shoot length at greenhouse (cm)				Root and shoot length at room temperature (cm)			
		Root	Shoot	Root		Shoot		Root		Shoot	
		1st		1st	2nd	1st	2nd	1st	2nd	1st	2nd
Acacia senegal	Control	10.3^{c}	13.7^{c}	19^{d}	17^{d}	30^{d}	32^{d}	11^{d}	10^{d}	15^{c}	17^{c}
	25 mg L^{-1}	19.3^{a}	15.6^{b}	27^{c}	28^{c}	40^{c}	37^{c}	17^{c}	18^{a}	20^{b}	15^{d}
	50 mg L^{-1}	15.7^{b}	15.3^{b}	30^{b}	31^{b}	49^{b}	50^{b}	18^{b}	15^{c}	20^{b}	22^{a}
	75 mg L^{-1}	15.4^{b}	17.3^{a}	32^{a}	33^{a}	60^{a}	55^{a}	19^{a}	17^{b}	21^{a}	20^{b}
Acacia mellifera	Control	18.3^{b}	25.5^{b}	22^{d}	20^{d}	38^{d}	40^{d}	19^{c}	15^{c}	20^{d}	18^{d}
	25 mg L^{-1}	18.5^{b}	31.6^{a}	25^{c}	21^{c}	47^{c}	46^{c}	17^{d}	14^{d}	27^{c}	22^{c}
	50 mg L^{-1}	15.5^{c}	25.2^{b}	29^{b}	25^{b}	51^{b}	52^{b}	20^{b}	18^{b}	33^{b}	28^{b}
	75 mg L^{-1}	19.4^{a}	24.3^{c}	34^{a}	32^{a}	75^{a}	70^{a}	22^{a}	25^{a}	34^{a}	30^{a}

Means in the same column within each condition for a given species having different superscript are significantly different ($p< 0.05$)

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