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Biosynthesis of Silver Nanoparticles from Peanut Shell Waste and its Impact on Plant Growth, Biochemicals and Antidiabetic Activity of Raphanus Sativus L.

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Abstract: Silver nanoparticles were synthesised using peanut shell waste, to assess it's effect on growth, biochemical and anti diabetic activity of Raphanus sativus L. The synthesized AgNPs were analyzed by various physicochemical techniques such as UV-Visible spectroscopy, Fourier transform infrared spectroscopy and Scanning electron microscopy.UV absorption showed a characteristic absorption peak of silver nanoparticles at 245nm.FTIR measurement was carried out to identify the possible Hydroxyl, carbonyl and CH groups The effect of silver nanoparticles sprayed with a different concentration (25%, 50%) led to increased the growth parameters, biochemical activity such as photosynthetic pigment, folic acid, vitamin C contents and anti diabetic activity of radish plant. The concentration of 25% sprayed AgNPs was found to be the most effective in the growth of radish plants.

Keywords: Peanut shell, silver nano particles, FTIR, SEM, plant growth, radish plant

Introduction

Groundnut is a nutritious leguminous crop, grown mainly for seed and oil worldwide. Groundnut shells are the leftover product obtained after the removal of groundnut seed from its pod and the abundant agro-industrial waste product which has a very slow degradation rate under natural conditions (Ahmed Moosa, Ali Mousa Ridha, Mustafa Hameed Allawi 2015). However, groundnut shells contain various bioactive and functional components which are beneficial for mankind. Commercially, it is used as a feedstock, food, filler in fertilizer and even in bio-filter carriers. But most of the deserted groundnut shells are burnt or buried resulting in environmental pollution (AL-Hachamii M, Baqir SJ, Aowda SA, Hussein FA, Alasedi MK. 2010). Silver nanoparticles are the currently most produced engineered nanomaterials found in a wide range of commercial products. Silver nanoparticles have been implicated in agriculture for improving cropsfor enhancing the plant growth (Aslani F, et al. 2014). The green synthesis approach has been subjected to a reliable, sustainable, and eco-friendly protocol to synthesize a variety of nanoparticle metal oxides and metal oxides in some cases under exposure to solar radiation to accelerate the synthesis of nanoparticles. Nanotechnology has the potential to be a useful tool for delivering pesticides and fertilizers to the target site with accuracy and precision. Nanoparticles injected into plants could have a significant impact, in improving the plant growth and yield in agricultural applications (Baenas, N., Ferreres, F., García-Viguera, C., and Moreno, D. A. 2015). Appropriate concentrations of Silver nano particles plays a crucial role in improving photosynthetic quantum efficiency and chlorophyll content and enhancing seed germinations.and increasing water and fertilizer use efficiency.The development and continuous improvement of AgNPs to regulate plant growth, soil quality, diseases, agrochemical usage, and environmental degradation have substantially strengthened soil quality, crop yield, and ecosystem security, which contribute greatly to sustainable farming and agro-ecosystems (Chen Y.W., Lee H.V., Juan J.C., Phang S.-M. 2016).

Radish (Raphanus sativus L.) sprouts are widely consumed worldwide due to their nutritional content and antioxidant properties (Danish M, Altaf M, Robab MI, Shahid M, Manohara das S, Hussain SA, et al. 2021). Radish was proposed as a model plant for the study of environmental stresses, mainly atmospheric contaminants but due to its short growing period, this plant has been considered as a model of edible roots for the study of the interaction of plants with soil contaminants (Davies JC .2009). Therefore, the aim of this work is to synthesize silver nanoparticles using peanut shell extract, an agricultural waste and study the impact of using AgNPs in increasing growth, biochemical activity, antidiabetic activity of radish plant.

Materials and Methods

Peanut shells

Peanut [Arachis hypogaea L] shells were collected from Gummidipoondi local market, Tamilnadu, India. The peanut shells were thoroughly cleaned with distilled water to remove the contaminated contents and it was dried in sunlight.

Aqueous extract preparation

The dried peanut shell was grind into a fine powder using a mixer grinder and then stored in an air tight container for further use. 25g of powdered peanut shell was boiled for 15 mins. The shell extract was cooled and filtered using a whatman No. 1 filter paper.

Phytochemical screening of peanut shell extract ((Raaman N, 2006))

The different chemicals tests were performed for screening the phytochemical profile of the peanut shell extract using standard procedures.

Biosynthesis of AgNPs ((Moosa et al. 2015)

$AgNO_3$ crystals were dissolved in double distilled water to prepare 1 mM $AgNO_3$ solution. The $AgNO_3$ solutions were mixed with the peanut shell extract at a ratio of 150: 100 (v/v) The flask was wrapped with an aluminium foil and then allowed to react in sunlight for 5 min. Samples were then monitored for the formation of AgNPs by monitoring the change in the color of the solution .

Optical analysis of AgNPs by UV-visible spectrophotometer

The synthesized AgNPs were subjected to optical measurements, which were carried out by using a UV-Vis spectrophotometer (UV PLUS) instrument and scanning the spectra between 200-800 nm.

FTIR analysis

FTIR analysis was performed to investigate the various functional groups of synthesized AgNPs. The FT-IR spectra of silver nanoparticles of peanut shell were measured by using IR Affinity-1 instrument.

SEM analysis

A SEM is a type of electron microscope used to analyze the sample images with a focused beam of electrons. Scanning Electron Microscope (TESCAN VEGA3) was used for morphological analysis of silver nanoparticles. The size and shape of AgNPs were confirmed by the SEM.

Impact of different concentration of AgNPs on the Raphanus sativus L. plant growth

Seeds of radish plant were obtained from Green choice Nursery, Chennai .The seeds of radish plant was sterilized with 0.1% sodium hypochlorite for 5 min and then washed with distilled water twice. In this experiment, garden soil and river sand used, which was collected from the fields of Gummidipoondi, Tamilnadu, India. The soil was autoclaved for 20-25 min, before planting and the seeds were grown in the fibre bags.Different concentration (25%, 50%, 100%) of AgNPs emulsion were sprayed after 5 days of sowing. One set of plants without spraying of AgNPs served as control The experiment results were stopped 30 days of spraying silver nanoparticles . Samples (radish leaves) was taken after 30 days of sowing to study the crop performance in terms of growth parameters and to estimate the photosynthetic pigment (chlorophyll a, chlorophyll b, total chlorophyll and carotenoids) , folic acid , vitamin C contents and to study antidiabetic activity. The quantitative estimation of chlorophyll-a, chlorophyll-b, and total chlorophyll of fresh radish leaves was carried out by the method of (Arnon,1949), while carotenoids were determined by following(Duxbury and Yentsch,1956; Macalacham and Zalik,1963) . Folic acid content of radish leaves were estimated using the method given by (Settaluri et al. 2015). Vitamin C content of radish leaves were estimated using the method of (Hachamii M et al.

2010). Antidiabetic activity of radish leaves were determined by using the method of (Hansawasdi Cet al. 2000).

Results and discussion

The phytochemical screening test showed that ethanol extract of peanut shell contains Tannin, flavonoid and saponin(Risha Fillah Fithria et al . 2019). Flavonoids and quinones were found positive in Arachis hypogaea shells extract (Bella Stevanny et al. 2020).

Table 1: The results of Phytochemical screening of the shell extract of Arachis hypogae

S.No	Phytochemical	Test	Result
1.	Alkaloids	Dragendroff's test	_
2.	Terpenoids	Salkowski test	+
3.	PhytoSterols	Libermann- Burchard,s test	+
4.	Phenolic compounds	Ferric chloride test	+
5.	Flavonoids	Alkaline reagent test	+
6.	Tannins	Lead acetate test	+
7.	Glycosides	Legal's test	_
8.	Carbohydrates	Molisch test	+
9.	Quinones	H_2SO_4 test	_
10.	Saponins	Foam test	+
11.	Proteins	Xanthoproteic test	+

Figure-1(A-C): Figures describing (A) Dried peanut shell, (B) Powdered peanut shell, (C) Boiled with dis.H_2O

Biosynthesis of silver nanoparticles

In the present study, silver nanoparticles were synthesized using the aqueous extract of peanut (Arachis hypogaea L) shells. The formation of silver nanoparticles after 10 mins of the synthesis process, was visually confirmed by the colour change from yellow to dark brown colour as shown in the (Fig: 2). (Palanivel Velmurugan et al. 2015) has also reported the same colour change while in the synthesis of silver nanoparticles from Arachis hypogaea L shell.

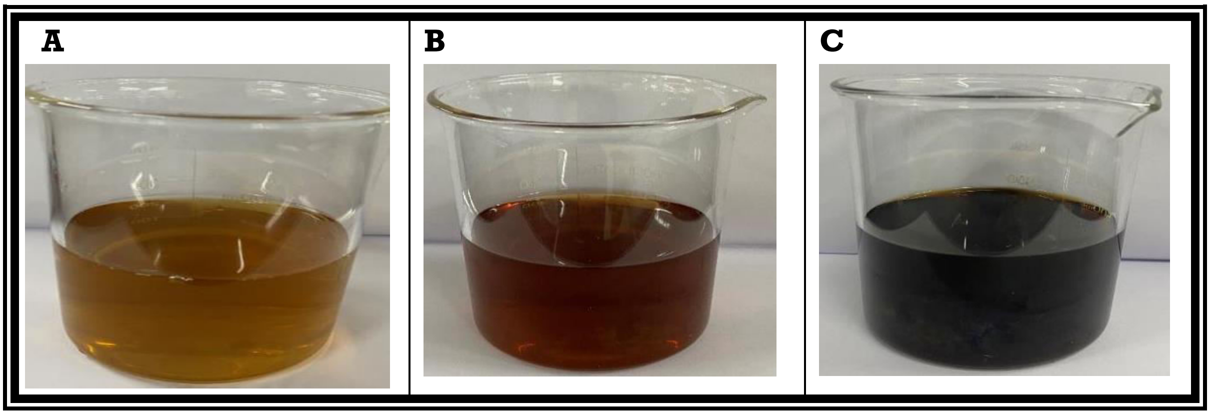


Figure-2(A-C): Figures describing (A) Shell extract + $AgNO_3$, (B) Reaction of Shell extract + $AgNO_3$ after 10 mins, (C) Reaction of Shell extract + $AgNO_3$ after 24 hrs

Optical analysis of AgPs by UV-visible spectrophotometer

UV visible absorption spectra for synthesized silver nanoparticle was observed. The reaction mixture of aqueous extract of peanut shell and the silver nitrate in the solution shows the colour change after 24 hours of incubation. The observed colour change of synthesized silver nanoparticle is due to the excitation of surface plasmon vibrations. The UV spectrum showed peak λ at 245 nm reveals the formation of silver nanoparticles (Fig,3).

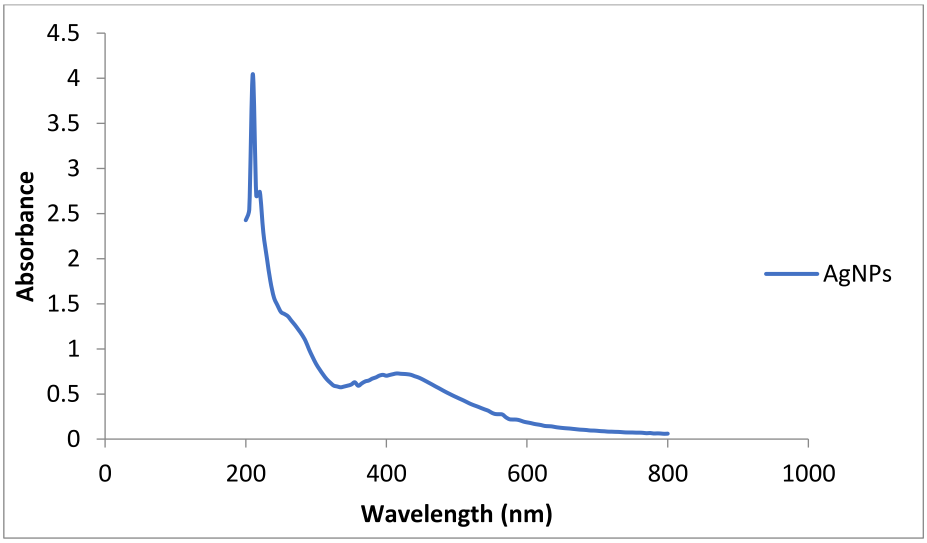


Figure 3: UV-VIS analysis of synthesized AgNPs

FTIR analysis

FTIR were carried out to determine the potential functional groups of biomolecules that are responsible for the formation of AgNPs. (Fig,4) shows the FTIR spectra of AgNPs derisved from peanut (Arachis hypogaea L) shell extract after reaction with $AgNO_3$. The signal that appeared at 3272 cm^{-1} could be attributed to the hydroxyl group and the peak at 1634 cm^{-1} might be due to the C=Ostretching. The peak at 1545 cm^{-1} could be due to the C=C aromatic stretching.The other signals at 1382 cm^{-1}, 1254 cm^{-1} and 1032 cm^{-1} belonged to the C-O-Cstretching,C-N stretching and Ag-Ostretching, respectively.

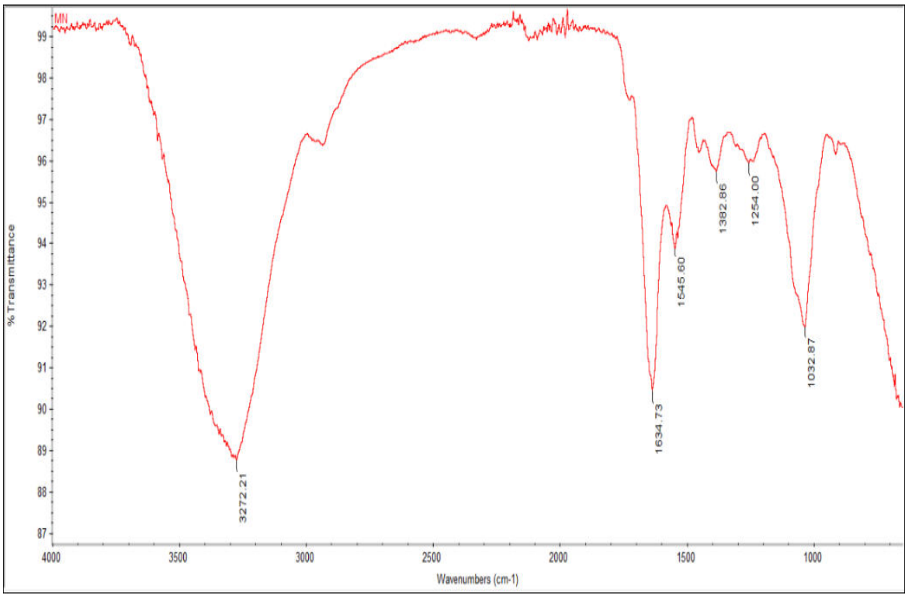


Figure 4: FTIR analysis of synthesized AgNPs

SEM analysis

The morphology of the synthesized AgNPs of peanut shell was examined using SEM. The Size of the AgNPs of peanut shell from SEM analysis was found to be in the range of 47- 96nm (Fig:5).

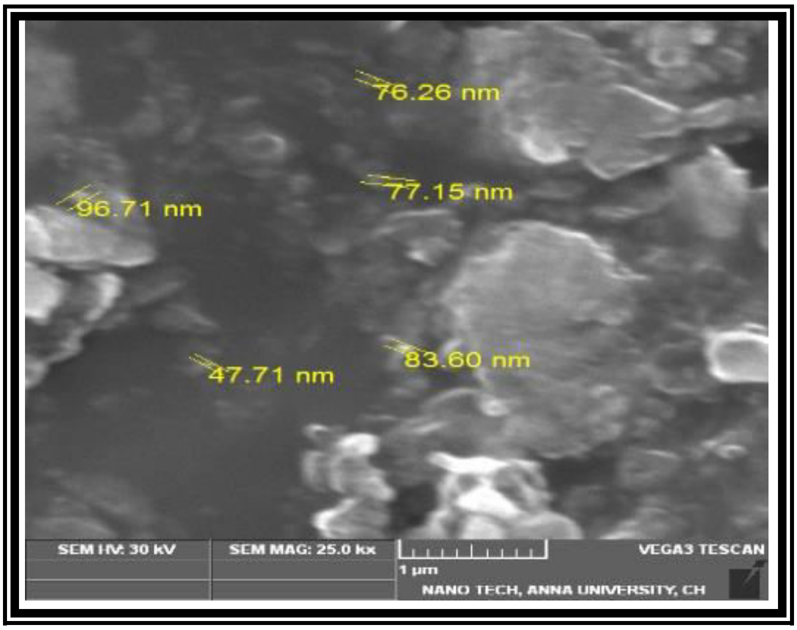


Figure 5: SEM analysis of synthesized AgNPs

Impact of different concentration of silver nanoparticles on the growth of radish plant (Raphanus sativus L)

Plant growth

AgNPs have been prepared from the peanut shell waste and their impact on the Raphanus sativas plant was studied (Fig:6,7). The data presented in the (Table:2) shows the effect of different concentrations of AgNPs on the number of root hairs and length of arial, root, tuber parts of radish plants. Foliar application of radish plants with the concentration (25%, 50%) of AgNPs increased all these growth parameters as compared with the untreated plant. At the concentration of 100 % AgNPs , all these growth parameters are decreased when compared to the control. The highest response in all the growth criteria was obtained by using 25% concentration of AgNPs. Higher concentration of silver nanoparticles has resulted in a reduction of biomass in the Arabidopsis plant, according to (Kaveh et al.2013) . (Thuesombatet al.2014) identified that increased AgNPs concentrations in jasmine rice decreased seed germination and subsequent seedling growth. (Sadak MS, 2019) founded that foliar application of silver nanoparticles at different concentrations (20, 40, and 60 mg/L) was found to increase the fenugreek plant growth parameters. (Monica RC andCremoniniR,2009) investigated that AgNPs were developed as plant-growth stimulators in agriculture. (Danish et al.2021) has studied synthesized silver nanoparticles using plants and found that the 50 ppm AgNP treatment in Trachyspermum ammi L. plant increased plant growth, biochemical, and antioxidant enzyme activities.

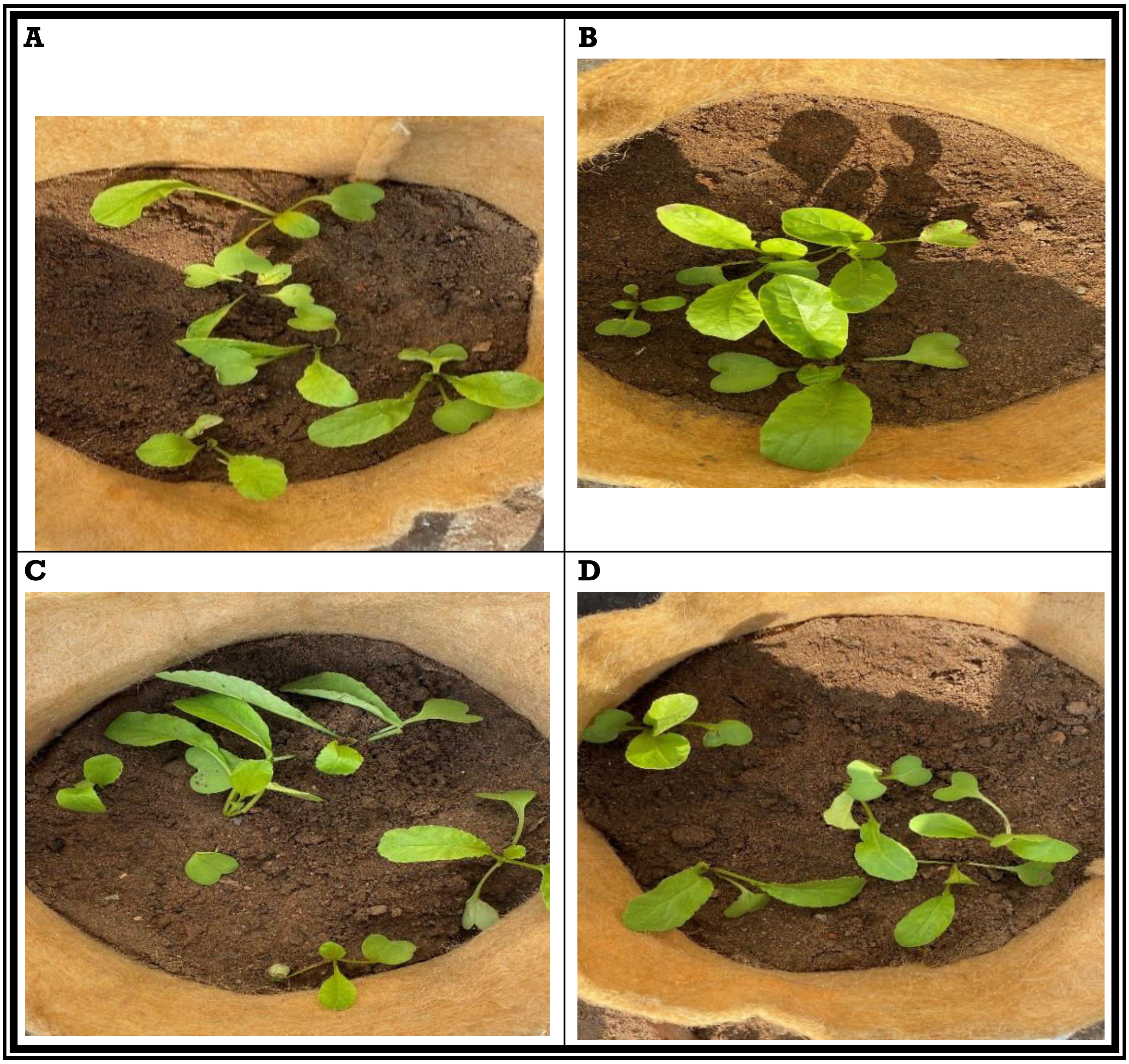
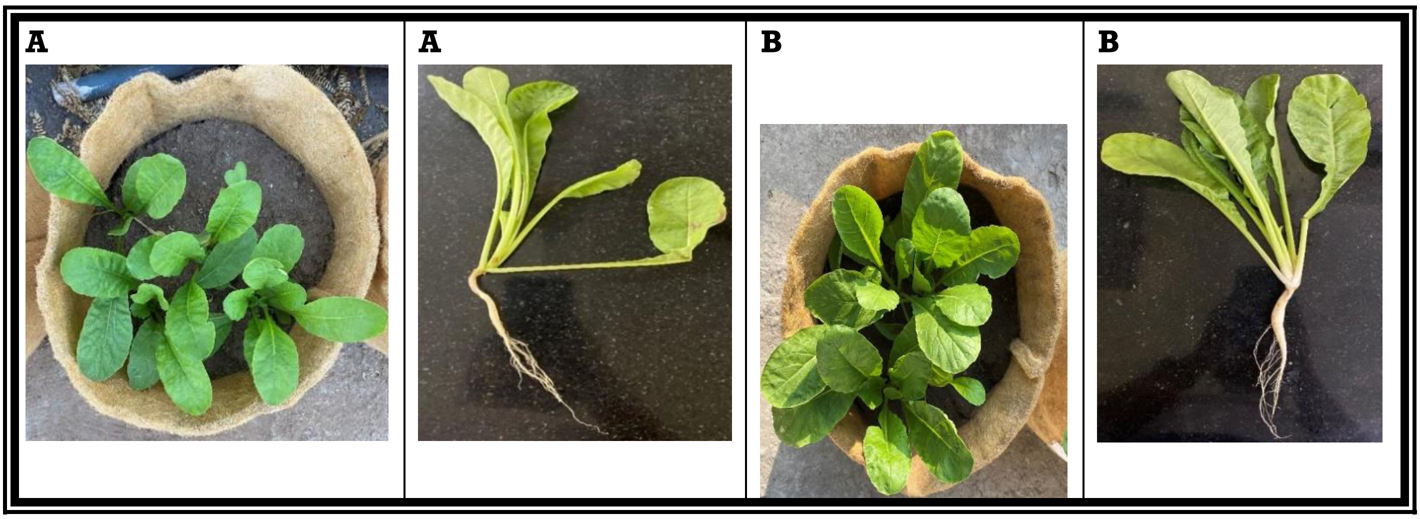


Figure-6(A-D): Figures describing Effect of silver nanoparticles on radish plant growth (after 15 days) (A) Control (without AgNPs), (B) 25% AgNPs, (C)50% AgNPs, (D) 100% AgNPs



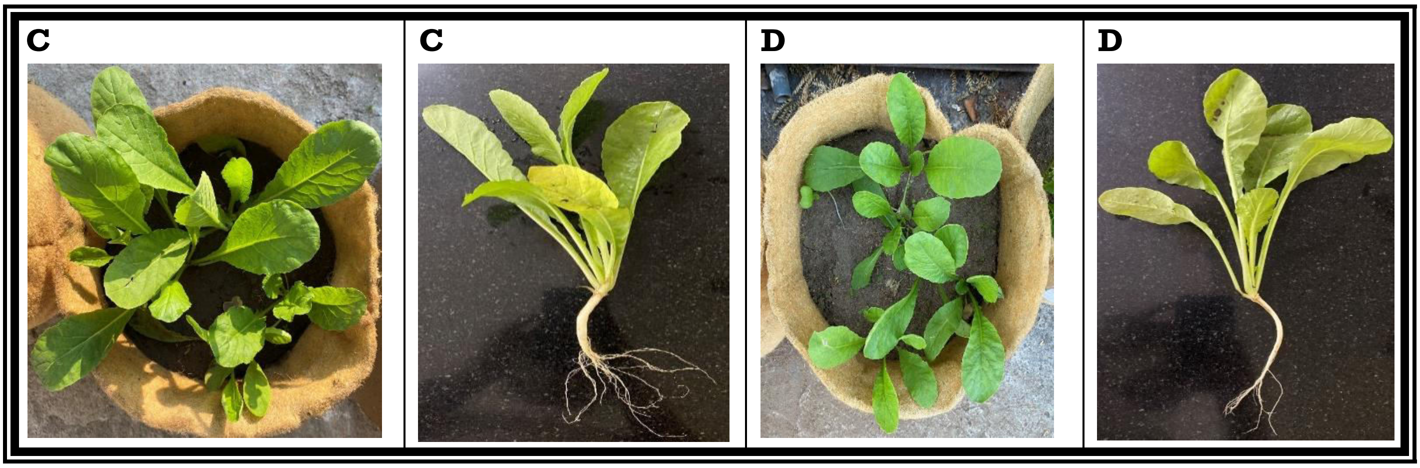


Figure-7(A-D): Figures describing Effect of silver nanoparticles on radish plant growth (after 30 days) (A) Control (without AgNPs), (B) 25% AgNPs, (C)50% AgNPs, (D) 100% AgNPs

Table 2: Effect of different concentration of AgNPs on growth criteria of radish plants

AgNPs (%)	**Plant Growth**			
	Arialpar t (cm)	**Root (cm)**	**Root hair (cm)**	**Tuber (cm)**
0	19.7	7	12	3
25	24.1	11	17	7
50	23	7.5	15	5
100	18.2	6.5	9	2

Biochemical parameters of radish plant leaves

Estimation of chlorophyll and carotenoids

Data in (Table:3) shows the response of photosynthetic pigments of radish plant leaves sprayed with different concentrations of AgNPs. The results revealed the significant increases in all photosynthetic pigment contents (chlorophyll a, chlorophyll b, carotenoids and total pigments) of radish leaves in response to treatment with different concentrations (25%,50%) of AgNPs. Treatment with 100% AgNPs decreased photosynthetic pigment contents, when compared with other two lower concentrations of AgNPs and untreated control. The most effective treatment was 25% of AgNPs as it gave the highest increases in all photosynthetic pigment.

Table3: Effect of different concentration of AgNPs on photosynthetic pigments of radish plant leaves

AgNPs (%)	Chlorophyll-a (mg/g)	Chlorophyll-b (mg/g)	Total chlorophyll (mg/g)	Carotenoids (mg/g)
0	0.404±0.003	0.465±0.003	0.852±0.002	0.271±0.001
25	0.415±0.004	0.732±0.007	1.146±0.003	0.311±0.001
50	0.406±0.001	0.602±0.003	1.007±0.004	0.285±0.001
100	0.387±0.003	0.435±0.004	0.838±0.003	0.224±0.001

Estimation of folic acid

(Table:4) shows the variation in folic acid content in response to spraying with different concentration of AgNPs. Spraying radish plants with different concentration of AgNPs significantly increased folic acid content in the leaves of radish plant. The treatment with 100% AgNPs decreased the content of folic acid in the leaves of radish plant, when compared with other two lower concentration of AgNPs and the untreated control. The most effective treatment was 25% of AgNPs as it resulted in the highest content of folic acid compared with the corresponding control plant.

Table 4: Effect of different concentration of AgNPs on folic acid content of radish plants leaves

AgNPs (%)	FOLICACID CONTENT (µg/mg)
0	101.78±1.09
25	153.86±3.40
50	110.67±1.45
100	88.61±2.16

Estimation of vitamin C

(Table:5) shows the variation in vitamin C content in response to spraying with different concentration of AgNPs. Spraying radish plants with different concentration of AgNPs significantly increased the vitamin C content in the leaves of radish plant. The treatment with 100% AgNPs decreased the vitamin C content in radish plant leaves , when compared with other two lower concentration and untreated control. The treatment with 25% AgNPs was found to be the most effective, as it resulted in the highest content of vitamin C in the radish plant leaves, when compared with the corresponding control plants.

Table 5: Effect of different concentration of AgNPs on vitamin C content of radish plant leave

AgNPs (%)	**Vitamin-C Content (mg/ml)**
0	0.24±0.01
25	0.27±0.01
50	0.26±0.01
100	0.20±0.01

Anti diabetic activity of radish plant leaves

Alpha-amylase enzyme inhibition assay

Diabetes mellitus is a metabolic disorder characterized by chronic hyperglycemia and type II is the major form of diabetes. The management of the blood glucose level is a critical strategy in the control of diabetes complications. The inhibition of carbohydrate hydrolyzing enzymes such as α-amylase can be an important strategy to lower postprandial blood glucose levels (Tundis et al.2010). In the present study, antidiabetic activity of radish leaves extract from different concentration of AgNPs treated and untreated plants was studied by using alpha-amylase enzyme inhibition assay. The maximum α-amylase enzyme inhibition of 25% AgNPs treated radish leaves extract was 76.08±1.32% , 50% AgNPs treated radish leaves extract was 67.95±1.08%, 100% AgNPs treated radish leaves extract was 55.08±2.67% and no AgNPs treated radish leaves extract was 65.50±2.49% at 120 μg/mL concentration (Fig,8). The IC50 of different concentration (25%, 50%,100%) AgNPs treated and untreated (no AgNPs) radish leaves extract was 38.918, 64.322, 96.394 and 78.140 μg/mL concentration.

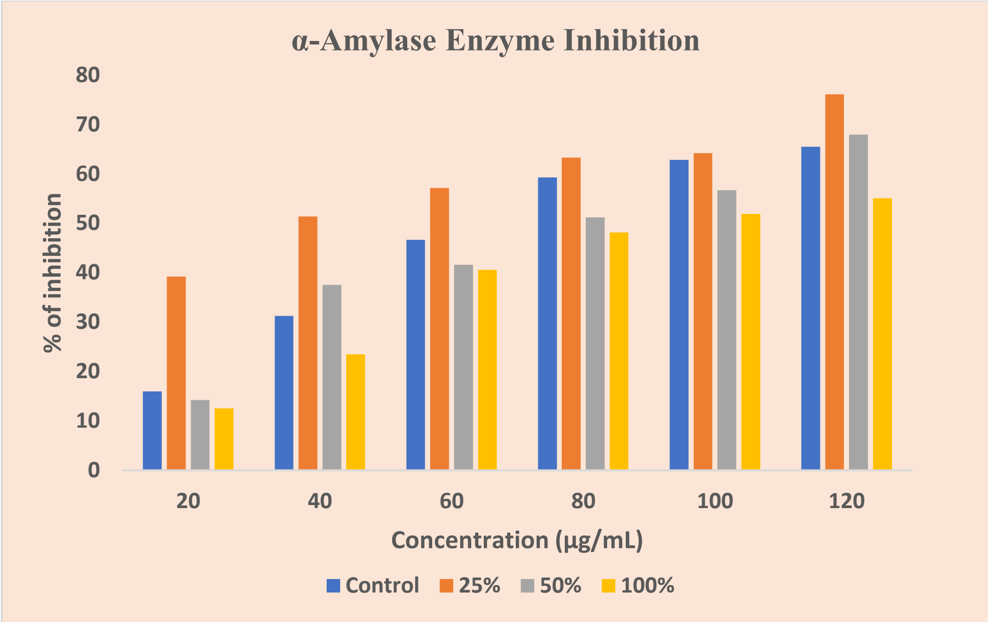


Figure 8: Effect of different concentration of AgNPs on antidiabetic activity of radish plant leaves

Conclusion

The present study has shown the biosynthesis route combination of silver nanoparticles from seed coat waste of peanut has received increasing attention due to the development of eco-friendly technologies in material science. The characterization with UV-Visible spectroscopy, FT-IR and SEM is the evidence for the formation of nanoparticles. The effect of silver nanoparticles on the radish plant has been shown to improve the growth of radish plant at lower concentration but higher concentration is toxic to the radish plant. Different concentrations (25%,50%) of AgNPs increased plant growth, photosynthetic pigments, folic acid, vitamin C contents, and invitro antidiabetic activity of radish plant leaves, when compared to the control. Among various concentration used in the study, spraying of 25% AgNPs emulsion was found to be the most effective treatment for the improvement in growth, biochemical activity, and antidiabetic activity against alpha-amylase enzyme inhibition of radish leaves.

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