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Effects of Plant-Mediated AgNPs from *Radermachera xylocarpa* on Early Growth and Chlorophyll Levels in Mustard and Mung Bean

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Abstract

Biological synthesis, characterization, and application of the nanoparticles are an important and emerging area of current research. Applications of these nanoparticles in the plant growth is another virgin field to explore. The present investigation attempts to examine the green synthesis of silver nanoparticles from rare medicinal and endemic plant *Radermachera xylocarpa* (Roxb.) K. Schum. Leaf extract was used as a reducing agent for the synthesis of silver nanoparticles. The synthesized nanoparticles were confirmed by UV-Visible spectroscopy and characterized by FTIR, XRD, and TEM analysis. The effects of these nanoparticles were tested in seed germination, seedling growth, and chlorophyll studies. It showed inhibition in root and shoot growth in the case of *Vigna radiata* and *Brassica juncea*. The seedgermination in *Brassica juncea* was potentially affected by silver nanoparticles as compared to *Vigna radiata*. Reduction in chlorophyll-a, chlorophyll-b and total chlorophyll was observed in the germinated seedlings after exposure to the silver nanoparticle.

Keywords: Silver Nanoparticles; Seed Germination; *Radermachera xylocarpa*; *Brassica juncea*; *Vigna radiata*; FTIR; TEM; XRD.

INTRODUCTION

Biomolecules in nanosize behave differently and exceptionally than their explored applications.The wide range of nanoparticles isin utilization due to completely distinguishing properties at the nano level.These properties finely alter with the size, shape, distribution, and morphology.Nanobiotechnologyhas many potentials for advancing medical science, thereby improving health care practices around the world. Many novel nanoparticles and nanodevices are in use, with an enormous positive impact on human health[1].Eventually, Nanotechnology unlocks many frontiers to improve human life. In agriculture,nanoparticles are proving valuable as compound fertilizers and nanopesticides.Most excitingly, in recent years,it is observed that the nanoparticles may act aschemical delivery agents for targeting molecules such as genes/DNA to specificcellular organelles like nuclei in plants[2].For the broad range of uses, the same principles can be applied, mainly to tackle phytopathological infections, nutrition supplements, and as growth adjuvant.[3]It is well known that nanoparticlesexhibitdistinctmechanical, optical, electrical, and magnetic properties compared to the bulk material.[4]Among metallic nanoparticles, the silver nanoparticle has gained the potential attention of the scientific community being unique in their stability, antibacterial, antifungal, and antiviral activities.[5]Some of the significant areas where silver nanoparticles are widely utilized are medicine, cosmetics, electronics, agriculture,etc.Among the full applications of silver nanoparticles, one primary application include their utilization as antimicrobials. They are emerging as the new generation of antimicrobials.[6]

Silver nanoparticle synthesis is carried out by physical, chemical, and biological methods.Both physical and chemical methods involve toxic chemicals and radiations;thus, these methods are not preferable.Instead, the biological methods are much more accessible, cost-efficient, and uses nontoxic materials for the synthesis of nanoparticles. The bacteria, fungi, and plants used to synthesize silver nanoparticles[7],[89,10]. Plants are preferable as compared to bacteria and fungi because they reduce metal ions much faster.[11]Till now, different plants and plant parts used to synthesize silver nanoparticles like extracts of*Geranium*[12], *Aloe vera*[13], *Cinnamomum camphora,*[9]similarly extracts viz.*Pinus, Persimmon, Ginkgo, Magnolia,* and *Platanus*[14].These nanoparticles were compared for theirextracellular metallic Ag-NPS synthesis from*Eucalyptus hybrid* (Safeda)[15],*Acalypha indica*[16], *Parthenium*[17], *Boswellia ovalifoliolata*[18],*Chenopodium album*[19], *Euphorbia hirta*[20], *Opuntiaficus-indica*[21], *Coriandrum Sativum*[22], *Nicotiana tobaccum*[23], *Ocimum sanctum*[24],olive leaf extract[25],*Atrocarpusaltilis*[26],*Diospyros discolor*[27],and *Datura stramonium*[28].

Previous studies showed that silver nanoparticles inhibit seed growth in some plant species.Reportedly, chemically synthesized silver nanoparticles in higher concentrations (1000 µg/ml) are toxic tothe seedlings of *Oryzasativa* in Hoagland's nutrient solution.[29]The toxicity of the silver nanoparticlesfor*Arabidopsis* roots depends on size andconcentration.[30]The present investigationattempts to examine the effects of silver nanoparticles on seed germination and growth in *Brassica juncea*and *Vignaradiata*.These crops are under regular cultivation in the soil of the Vidarbha region of India.The plant material selected for the AgNPs synthesis was leaves from *Radermachera xylocarpa*.It explored as a medicinally important plant with an antivenom, antidiuretic, antioxidant potential[31,65,66,67].The optimistic view led to the formulation of the present plan of investigation to synthesize AgNPs from *Radermachera xylocarpa* and evaluate its impact on seed germination, seedling growth, and chlorophyll contents in *Brassica juncea*and *Vigna radiata*.

MATERIALS AND METHODS:

Preparation of dried biomass

The fresh, matured, and healthy leaves of*Radermachera xylocarpa* were collected from the forest nearParatwada, Taluka: Achalpur, District: Amravati, State: Maharashtrafrom India in October. Leaves were washed thoroughly in running water with Teepol followed by sterilization with 0.1% $HgCl_2$ underlaminar airflowand shed dried for 3-4 days, stored in clean polythene bags at room temperature. The dried leaves ground to a fine powder.

Silver Nanoparticles Genesis

The plant leaf broth solution was prepared by boiling 10 g dried leaf material with 100 mL of triple distilled water inthe water bath for 5 min.Theextract filtered using Whatman filter paper no. 1 and then subsequently through 0.02 µm filter paper. 0.5 ml of this leaf extract mixed with99.5 ml of 1 mM $AgNO_3$ solution.The reduction of Ag^+ ions by components of extract leads to the synthesis of silver nanoparticles, which lead to color change from colorless to dark brown.Synthesized nanoparticles stored in brown bottles at room temperature and aseptic conditions.

Spectral studies:

UV-Vis Analysis –The solution was scanned for UV Visible absorption measurements at room temperature using Shimadzu UV 1800 spectrophotometer. UV-Vis spectra of the nanoparticle solution recorded by scanning in the range between 200 nm to 1100 nm. 0.5 mM AgNO3 solution was used for baseline correction.

FTIR Analysis – Samplesfor FTIR analysis prepared by mixing 100 µl of silvernanoparticle solution with 500 mg KBr powder. The mixture was dried and used for analysis. The analysis carried out on Fourier Transform Infrared Spectrophotometer Shimadzu IR Affinity-1.

XRD Analysis – Acetone was added to nanoparticle collide in ratio 1:5 v/v. The mixture was kept at 5℃ for six hours to enhancethe rate of precipitation.After incubation, the solution turnedcolorless due to the precipitation of nanoparticles. The pelleting is done by subsequent centrifugation and washing with deionized water to remove other biomolecules. Precipitated nanoparticles were then used for XRD analysis. The structural data wereobtained from XRD studies using X-ray diffractometer (MiniFlex II, Rigaku,Japan) withCuK radiations of wavelength 1.5406 nm

TEManalysis –Following XRD analysis, synthesized nanoparticles were sent to IIT Mumbai for TEM analysis.TheSonicated sample was studied on a carbon-coated copper grid.The solvent used was evaporated by Infrared light,and TEM measurements were conducted with the PHILIPS model CM 200 instrument (voltage of 200 kV, resolution 0.23 nm).

Effect of Silver Nanoparticles on Seed Germination: Thecertified and germination frequency verifiedseeds of *Brassicajuncea,* and *Vigna radiata* acquiredfrom Shri Shivaji Agriculture College, Amravati. Washed and sterilized 120 seeds of each *B. juncea* and *V. Radiata*was placed in three separate germination trays and exposed to 0.5%, 1%, and 1.5 % silver nanoparticle solution, respectively. Seed germination was recorded daily for seven days. The speed of germination and vigorindex calculated by using the following formulae –

Speed of Germination[32] = 1g + 2g +3g + 4g + 5g + 6g + 7g + 8g + 9g + 10g

(H. E. Carley, and Watson, 1968)

Seed Vigor Index[33] = Hypocotyls Length (mm) × seed germination percent

Chlorophyll Estimation: Total Chlorophyll, Chlorophyll a and Chlorophyll were estimated spectrophotometrically in different concentrations of AgNPs[34]. The chlorophyll contents are determined in the early leaves on the seventh day.

Statistical analysis: Statisticalanalysiswas conducted,andthe results presented as an average with ± SE (Standard Error). Each of the experimental values compared to its corresponding control. The results were also analyzedfor Standard Deviation using StatisticalPackage for Social Sciences (SPSS) Version 11.5.

RESULT AND DISCUSSION

Terrestrial crops are directly exposed to silver nanoparticles (Ag-NPs) and their environmentally transformed analog silver sulfide nanoparticles.Its carried out using wastewater treatment biosolids as fertilizer to agricultural soils. Such provision of the supplement revealed its bioavailability to plants[35]. In consonance with this, silver nanoparticle synthesis is considered a remarkabletool to achieve a new biological system to improve crop yield. An exposure of $AgNO_3$ to leaf extract for nanoparticle synthesis, turning a solution from yellow to brown[12].An extensive survey of literature revealed that the present investigation might be the first effort to synthesize silver nanoparticles fromthe rare medicinal plant *Radermachera xylocarpa* (Roxb.) K Schum.

Spectrophotometric Analysis of Silver Nanoparticles

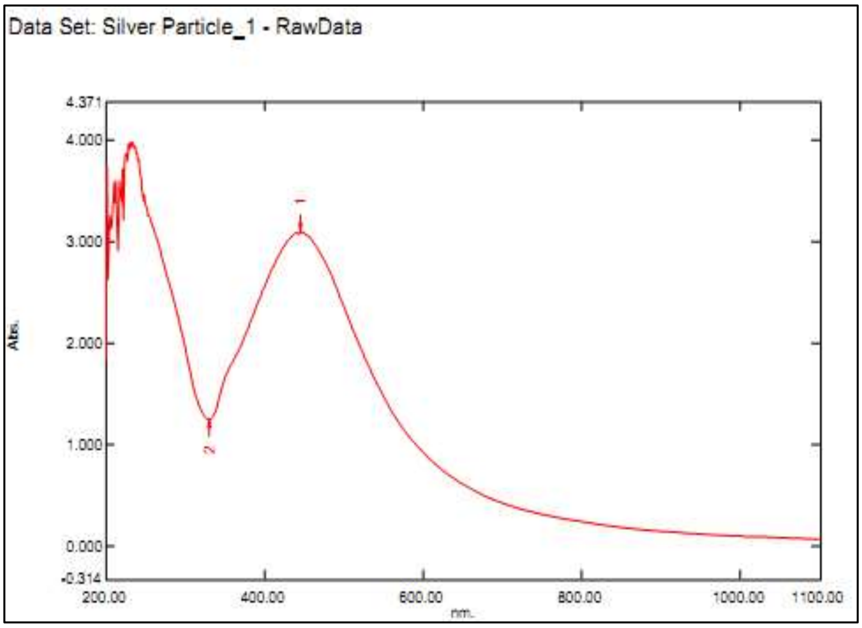

Figure 1: UV-Vis absorbance spectra ofthe solution containing aqueous silver nitrate (1 mM) andleaf broth.

Absorption of UV-Visible wavelength is the characteristic property of the molecule, and it is dependent on the nature of the absorbent molecule, absorption maxima change with the availability of free electronin the absorbent molecules[36].The silver nanoparticle absorbs UV-Visible wavelengths around 440 nm due to the surface plasmon resonance exhibited by silver nanoparticles[12,37].After exposure to the leaf extract,the bio-reduction of silver nitrate was observed. In the present study, the biologically synthesized nanoparticles detected spectrophotometrically, figure – 1 shows the UV-Vis absorbance spectra of the solution containing aqueous silver nitrate (1 mM) andleaf broth.The absorption maxima observed at 445 nm. The synthesis of silver nanoparticles was confirmed by the change in color from yellowish-green to brown. A broad absorption peak that appeared at around λmax = 435 nm represents the characteristic SPR of spherical and aggregated AgNPs[38]. The synthesized nanoparticles remained stable for around 12 weeks.

Figure 2: Synthesized silver nanoparticles exhibit a characteristic dark brown color.

As the reaction progressed with time, the color becomes dark brown(Fig. 2).Thecolor changeattributed to the surface plasmon phenomenon, it was dipole oscillation resulting from anelectromagnetic field in the visible range, coupled with the collective oscillations of conduction electrons[12].This phenomenon strongly depends on the particle size, dielectric medium, and chemical surroundings[39].Mie's theorysuggests thata single SPR band in the absorption spectra reveals spherical nanoparticles, and anisotropic particles indicatetwo ormore SPR bands based on the shape of the particles[40].

Fourier Transform Infrared Spectroscopy (FTIR)

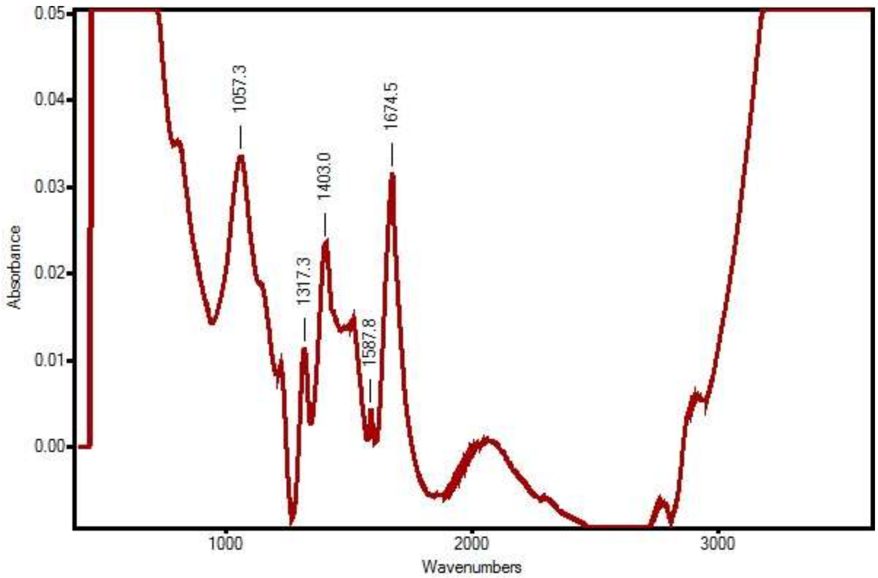

Figure 3: FTIR spectra of AgNPs

FTIR analysiswas carried out for the synthesized nanoparticles.Five absorption peaks, near 1057.3 cm^{-1}, 1317.3 cm^{-1}, 1403.0 cm^{-1}, 1587.8 cm^{-1} and 1674.5 cm^{-1} (Figure 3) were resulted. The peak at 1057 cm^{-1} may be due to the stretching vibration of C–O, and C–N bonds ofphenol, alcohols, amines, carboxylic acid, and their derivatives present in the leaf[41], whereas the band at 1317.3 cm^{-1} may be due to bending mode of -CH_3 in aldehydes and ketones[42].The band at around 1403.0 cm^{-1}may originate from the O-H bend of polyphenol and confirms the presence of an aromatic group[43-45].Otherpeaks at 1587.8 cm^{-1}confirming the presence of amine groups[46] and peak at 1674.5 cm^{-1} correspond for C=O stretching vibrations[47,48]. The synthesized nanoparticles may interact with proteins through the carboxylic groups and free amine groups[49].

X-Ray Diffraction (XRD)

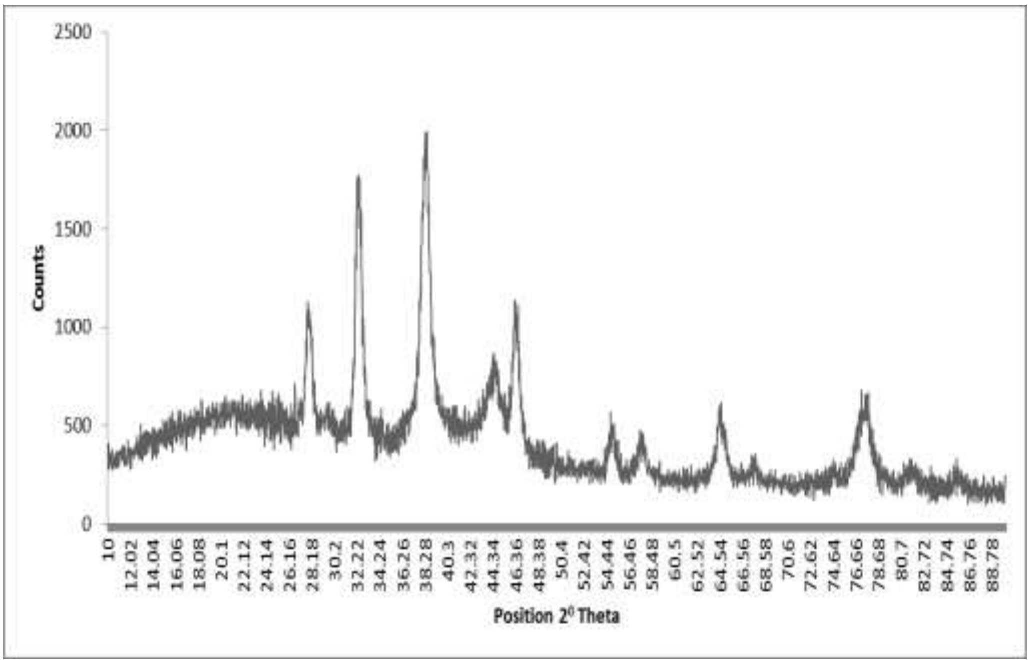

Figure 4: XRD pattern of AgNPsshowing the facets of crystalline silver after bioreduction.

The XRD patterns of the AgNPs synthesized from *Radermachera*to establish the metallic nature of particlesare shown in Fig. 4;the presence of intense 2θ peaks of AgNPsat38.28, 44.44, 64.44, and 77.78 corresponds to the 111, 200, 220, and 311reflection planes of anfcc lattice of silver (ICSD No. 98-018-0878), respectively[39,50]. Literature datajustifiedthatthe silver nanoparticles formed by the reduction of Ag^+ ions by the *Radermachara xylocarpa* leaf extract are crystalline and are present in a silver face-centered cubic (fcc) phase[51,52].

TEM Measurements

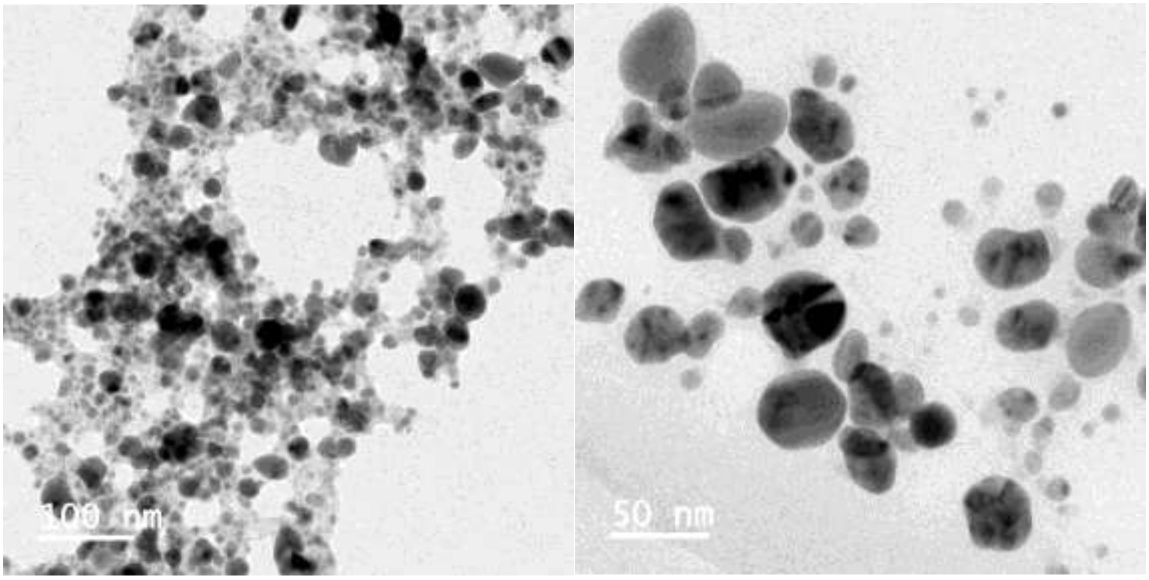

Fig 5 A. AgNO3 with an average size of 100 nm,B. Spherical AgNPs with an average size of 50 nm

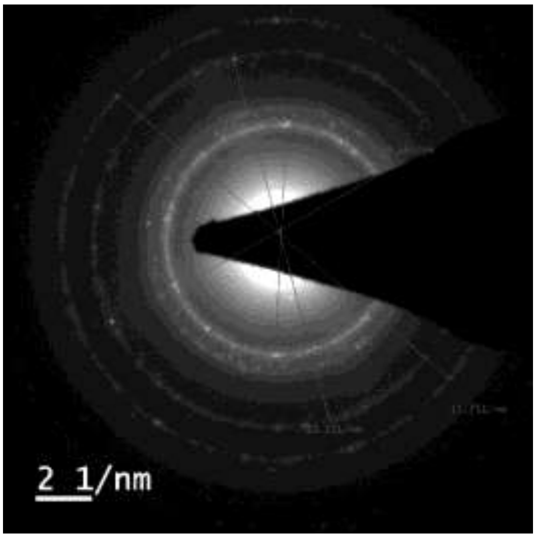

Fig 5. C. TEM-SAED pattern

Fig. 5A and 5B show the TEM images loaded withAgNPs. Nanoparticles show polydisperse nature concerning size, andhigher magnification showed the average size roughly ranges from 5 nm 40 nm in size. It is also evident that most of the AgNPs arecrystalline, predominantly spherical,and rarely triangular. The TEM-SAED patternshowed the bright silver diffraction ring, which is the indication ofthe cubic centered (FCC) crystalline nature of the AgNPs (Fig. 5C).

Most of the synthesized nanoparticles were free, but some show adherence with each other results in the formation of aggregates. Thediffraction rings of theAgNPs indexed as 111,200, 220, and 311,consistent with the face-centered cubic (fcc)structure of Ag[53], also confirmed the polycrystalline AgNPs structure[54,55].

Effect on Seed Germination and early growth

The widespread use of AgNPsinconsumer, agricultural, and medical products has led toincreasing concerns about their intentional or unintentionalrelease into the environment[56].Owing to the variety of properties of AgNPs, their prospects need to explore plant growth.In the present investigation,seed germination and seedling growth studies were conducted to evaluate the Speed of Germination (SG) and Seed VigorIndex (SVI) as an essential parameter to assess the impact of AgNPs. Almost 80 to 100 % germination in both the experimental plants recorded.The SG and SVIfound significantly reduced as the nanoparticle treatment was applied (Table 1).

Table 1. Effect of Silver Nanoparticles Germination and Seed Vigor Index

Treatment	% Germination		Speed of Germination		Seed Vigor Index	
AgNP's conc.	***Vigna***	***Brassica***	***Vigna***	***Brassica***	***Vigna***	***Brassica***
Control	100%	80%	26	46	1581	507
0.50%	100%	90%	20	43	1087	252
1.00%	100%	100%	24	35	617	145
1.50%	100%	80%	22	51	1140	184

These adverse effects of the AgNPsearlier demonstrated in the seedling growth studies of the *Phaseolus radiatus* and *Sorghum bicolour* exposed to AgNPs expressed as a percentage of the growth of the plants in the control treatment group. It was observed that the growths in both the plants were adversely affected by increasing the AgNPs exposure concentrations. As compared to the controlled growth, the percent seedling growth of the *P. radiatus* and *S. bicolor* exposed to AgNPs of 40 mg /l were 20% and 47%, respectively[57].Analogous outcomes were also published[58] in the study of 11 species of wetland plants[59].

Fig. 6A, B, C, D:-Effects of different concentrations on Seed Germination in *Vigna radiata*.

Table 2:Effect of Silver Nanoparticles on seedling growth in *Vigna radiata*

Parameters	AgNP's conc.	AVR	SDEV	SE	% Change
Root Length	Control	8.121	1.588	3.500	
	0.5	7.571	1.693	0.379	-6.773
	1	6.324	2.561	0.573	-22.128
	1.5	4.088	1.166	0.261	-49.668
Shoot Length	C	14.967	5.948	1.330	
	0.5	6.173	5.199	1.163	-58.757
	1	12.215	4.730	1.058	-18.384
	1.5	11.407	5.098	1.140	-23.786
RL/SL	C	0.543	0.280	0.063	
	0.5	0.399	0.279	0.062	-93.539
	1	1.417	3.098	0.693	-87.580
	1.5	1.079	0.355	0.079	98.808

The seedling growth in root and shoot growth recorded on the seventh day. In the case of *Vignaradiata, a* decrease in root length, shoot length,and rootlength:shoot length ratio reduction was observed in 0.5%, 1%, and 1.5 % silver nanoparticle concentrations over the control, respectively (Table 2, Fig 6. A, B, C and D).

Fig. 6E,F,G,H :-Effects of different concentrations on Seed Germination in *Brassica juncea.*

Table 3:Effect of Silver Nanoparticles on seedling growth in *Brassica juncea.*

Parameters	AgNP's conc.	AVR	SDEV	SE	% Change
Root Length	Control	3.693	1.331	0.384	
	0.5	1.299	0.671	0.194	-64.839

	1	0.612	0.484	0.140	-83.436
	1.5	0.987	0.709	0.205	-73.287
Shoot Length	C	6.560	1.285	0.371	
	0.5	3.046	0.586	0.169	-53.567
	1	1.256	0.418	0.121	-80.849
	1.5	2.099	0.589	0.170	-68.001
RL/SL	C	0.588	0.265	0.077	
	0.5	0.442	0.250	0.072	-24.880
	1	0.544	0.470	0.136	-7.412
	1.5	0.505	0.417	0.120	-14.050

The seedling growth studies in *Brassica juncea* also adversely affected by silver nanoparticle over control (Table 3, Fig6.E,F,G and H).The most significant reduction was in root and shoot length was observed in 1.5% AgNPsfrom*Brassica juncea*. Previous studies on silver nanoparticles show growth inhibitory effect on the *Oryzasativa,*where both root and shoot growth was inhibited by them[60]. Similarly, a recent study on *Solanum lycopersicum* revealed the positive influence of sodiumto allow amphopolycarboxyglycinate-stabilized AgNPs on the yield and quality of fruits with the further effectiveness of the preparation against late blight and *Alternaria* blight[61]. The present investigation reported similar results in *Brassica juncea*and *Vigna radiata,* where both root and shoot growth inhibited by silver nanoparticle treatment.

Effect on chlorophyll content

Table 4:Effect of Silver Nanoparticles on Chlorophyll contents

Treatment	Chl a		Chl b		TChl	
AgNP'sconc	*Vigna*	*Brassica*	*Vigna*	*Brassica*	*Vigna*	*Brassica*
Control	3.96	3.77	10.48	7.29	14.43	11.05
0.50%	2.83	0.96	4.87	4.14	7.70	3.10
1.00%	2.88	1.38	6.52	3.76	9.40	5.14
1.50%	2.15	0.56	4.51	1.04	9.09	1.60

Chlorophyll is a significant biomarker that reflects the status of plant growth. The AgNPswere administered in different concentrations (0.5, 1.0, and 1.5%) for seedling growth till the seventh day after sowing and found retarding effects on chlorophyll contents of *Brassica juncea*and *Vigna radiate* (Table 4). The decrease in chlorophyll content reported to the greatest extent in higher concentrations of 1.5% AgNPs. The reduction in the chlorophyll contents in *Brassica* was higher than *Vigna.*The results conform with the studies of pure AgNPs supplied by King Abd Alla Institute on *Phaseolus vulgaris* and *Zea mays*, reportedly 80 and 100 ppm concentration of AgNPs reduced the chlorophyll contents[62].Similar reports were also published in the studies of the AgNP toxicity mechanism in terrestrial plants. The results of TEM and metal content analysis in *Arabidopsis thaliana*showed that AgNPs were accumulated in leavesand disrupted the thylakoid membrane structure with an eventual decrease in chlorophyll contents, which may inhibit plant growth[63].

The critical review on the effects of AgNPsrevealed that AgNPsaretoxic to the plants, and both vegetative growth and root elongation inhibited. The AgNPs exhibited their toxicity in a concentration-dependent manner, being more toxic in the high concentration than in the low concentration. This phenomenon also reported in aquatic (*Lemna minor*) and terrestrial (*Loliummultiflorum*) plants, algae, fungi, vertebrates (zebrafish), invertebrates (*Caenorhabditis elegans*), microorganisms (*Escherichia coli*, *Pseudomonas putida*), and human cells[64].Similarly,the size-dependent toxicity of silver nanoparticlesreported in*Allium cepa* where toxic effects silver nanoparticles increases as the size of nanoparticles decreases[68].

CONCLUSION

The present study has successfully used as an aqueous extract of *Radermachera* leaf to synthesize silver nanoparticles.Being a promising candidate for many applications, employing genesis of nanoparticles from it may bring its multiple utilities. TheseAgNPs have good reducing potential and are found to be stable at room temperature, which may be due to natural products present in the extract. X-ray diffraction studiesshowed the face-centered cubic lattice nature of AgNPs.Germination and seedling growth studies showed retarding effects on germination, early growth, and chlorophyll contents. Hence, it may be safely concluded that AgNPs havethe potential property of herbicide.The further extension of the research may be giving promising results in the formulation of potent herbicide.

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