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Research article

Impact of zinc oxide nanoparticles on *Cajanus Cajan* Linn. with special reference to germination, vegetative and biochemical aspects

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ABSTRACT

The synthesis of Nanoparticle using plant extracts considered as clean and environmentally accepted “green chemistry” concept. The utilization of zinc nanoparticle in the field of agriculture as growth promoting agent were not adequately studied. The present study was intended to synthesise the zinc nanoparticle using aqueous extracts of *Schrebera swietenioides* Roxb., and the synthesized nanoparticle was evaluated for its applications in the field of agriculture and removal of pollutants in water**.**The zinc oxide nanoparticles were synthesised using aqueous leaf extract of *S. swietenioides* and 0.1 M zinc acetate. The formation nanoparticle were identified by observing the colour change of the reaction mixture. The formed nanoparticle was collected by centrifugation followed by washing several times with distilled water and then drying of the particles in air oven. The nanoparticle was characterized using various characterization techniques such as UV spectra ,SEM, and FT-IR. The applicability of the synthesised nanoparticles in the agriculture field was evaluated in terms of its effect on the seed germination and growth enhancement of seedlings. The zinc nanoparticle was studied for the seed germination and plant growth promotion of Pigeon pea (*Cajanus cajan* Linn.) The influence of nano-priming on the root length, shoot length, chlorophyll content and metal uptake was evaluated.

Keywords: Nanoparticles, SEM, FT-IR, Photo catalytic reduction Seed germination.

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INTRODUCTION

Nanotechnology is the known research filed since last century and it was presented first in 1959 by Nobel laureate Richard P. Feynman, 1960 in his famous lecture “*There’s Plenty of Room at the Bottom*”. Nanotechnology produced various types of materials at nano scale level and in recent days there will be revolutionary developments in the field of nanotechnology[1] .Nanoparticles (NPs) are more preferentially referred to the materials that include particulate substances’ having 5 to ~100 nm particle size that are having feasibility to overcome various systemic barriers [2]. NPs were acts as bridge between atomic or molecular structures and bulk materials and hence are having excellent scientific interest. The size independent and size dependent were observed for bulk materials and NPs which was the key difference between bulk materials and NPs [3]. The properties of the NPs were various based on the size of the particle and the properties can change by change in the size of the NPs. The fraction or the percentage of the NPs on the surface of a material increases with decrease in the particle size and thus the properties of the NPs were varied as per continued that along with the size of the NPs, the shape of the particles also plays a crucial role that implies unique properties of the NPs. Sometimes the same particles have significantly different properties when there will be change in the shape of the NPs [4].The ZnO NPs synthesis was drawn high interest due to its wide range of applications in various fields such as optics, electronics and biomedical systems [5,6].These NPs were enlisted as “generally recognized as safe” by US FDA. These NPs have high exciton binding energy (60meV) and large band gap (3.37 eV) that implies tremendous semiconducting properties. These NPs also have anti-inflammatory, wound healing, optic, UV filtering and high catalytic activity. ZnO NPs have significant UV filtering activity and hence were widely used in preparing various cosmetics such as sunscreen lotions for protecting skin from UV light

Plants are considered as cost-efficient and eco-friendly chemical factories. Plants acts as potential heavy metal detoxicant and were used to overcome the problems caused by various environmental pollutants [7]. The synthesis of NPs using plant extracts considered as clean and environmentally accepted “green chemistry” concept [8].

Various properties of NPs such as light absorption, surface morphology, size, shape, crystalline nature etc., were characterized using various characterization techniques as per methodology [9]. The diverse properties of the NPs were greatly influenced by its shape and the NPs shape was evaluated by using microscopy techniques. The morphology, surface information and dispersion of the NPs were evaluated using scanning electron microscope (SEM). The transmittance, luminescence and reflectance futures of the NPs were carried by using optical methods. The interaction of NPs with the electromagnetic radiation during the spectroscopic analysis gives the possible information related to the size, shape and concentration of the NPs [10]. Various spectroscopic techniques such as UV-visible, infrared, UV/vis-diffuse reflectance spectrometer and magnetic resonance methods were utilized for characterization of NPs.

The nanotechnology shows potential benefit in agriculture sector by providing eco-friendly solutions to various environmental problems, increase food productivity. The NPs have significant effect on the stimulation of seed germination and its growth. due to smaller size with large surface area of NPs, they can easily seep in the pores of the seeds and can able to activate the phytohormones that are involved in the seed germination [11]. The NPs were also proved to be having significant enhancement of the growth plants by stimulating the growth hormones and enhancing the enzymatic activities. Due to the non-toxic nature of the green synthesised NPs, they can utilise as nano fertilizers and nano pesticides [12-14].

MATERIALS AND METHODS

Chemicals and reagents

The chemicals used in the study such as zinc acetate dihydrate, sodium hydroxide, hydrogen peroxide, dibasic disodium phosphate, monobasic sodium dihydrogen phosphate, Methyl orange, crystal violet, potassium dichromate, lead nitrate was of analytical reagent grade and were purchased from Merck chemicals and SD fisher scientific, Mumbai.

Preparation of solutions

0.1 M Zinc acetate solution

Zinc acetate dihydrate was used for the preparation of required concentration of zinc metal solution for the synthesis of zinc nanoparticles. 2.195 grams of Zinc acetate dihydrate was weighed accurately in a 100 mL volumetric flask containing 75 mL of double distilled water. Then it was sonicated for 5 min to dissolve the Zinc acetate dihydrate completely in water and then the final volume in the volumetric flask was made up to the mark using the double distilled water. Then the obtained solution was filtered and preserved in an amber colour bottle for use.

Collection of plant material

The leaves and roots of the plant *Schrebera swietenioides* Roxb., was collected in the hill area located in Tirumala, Tirupati, Andhra Pradesh. The collected plant sample was authenticated by Dr. Ch. Srinivasa Reddy, Assistant Professor, Department of Botany, SRR & CVR Government Degree College (A) Vijayawada with plant authentication number SRR-CVR/ 2019-20/Bot/31. The collected leaves were surface cleaned to remove the sand and dirt on it using sterile cotton followed by distilled water.

Preparation plant extracts

The plant aqueous extract was prepared as per the procedure reported by Mithun *et al*., 2020 and the same procedure was utilised for the preparation of leaf extracts separately. An accurately weighed 10 grams of plant powder was taken in a 250 mL beaker containing 100 mL of double distilled water. The plant material with water was boiled on a hot plant at 40 ^{0}C for 80 min. The phytochemical constituents present in the plant material were extracted to the water solvent. Then the extract was filtered, the plant material obtained as sediment was discarded and the clear filtrate was made up to 100 mL with distilled water. The clear filtrate obtained was used for the synthesis of nanoparticles.

Green synthesis of nanoparticles

Leaf extract mediated zinc oxide nanoparticles:

The zinc oxide nanoparticles were synthesised by utilizing aqueous leaf extract of *S swietenioides* and zinc acetate solution the aqueous leaf extract and zinc acetate solution in 10:90 (v/v) was mixed and the homogenous mixture was prepared by stirring the solution in a magnetic stirrer for 4 h without heating. Then 20 mL of ethanolic sodium hydroxide solution was added and stirred for 5 min. The Zn $(OH)_2$ was precipitated at the bottom of the flask and was collected by removing the excess mother liquid. The Zn $(OH)_2$ precipitated at the bottom of the flask was collected by evaporating the liquid and then washed with excess distilled water to remove the extract particles on the precipitate. Then the Zn $(OH)_2$ was converted in to powdered ZnO nanoparticles by heating the purified precipitate at 300 0C for 45 min in a muffle furnace.

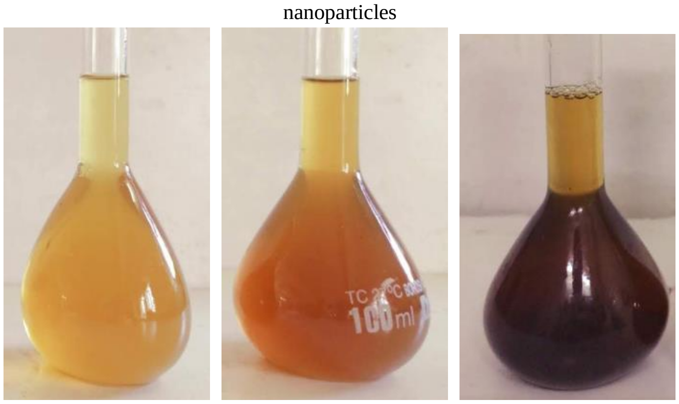
Characterization of nanoparticles

The synthesised zinc oxide was characterized using various characterization techniques such as UV - visible spectrophotometer, FT IR, SEM, the size, shape, surface morphology, functional groups and chemical composition of the *S swietenioides* aqueous extracts mediated zinc nanoparticles was evaluated using various characterization techniques.

Zinc oxide nanoparticles synthesis

The zinc oxide nanoparticles were synthesised using leaf aqueous leaf extract and 0.1 M zinc acetate solution. The formation of nanoparticles was initiated while the addition of the aqueous leaf extracts of *S. swietenioides* and zinc metal solution. The presence of various secondary metabolites in the aqueous leaf extract of *S. swietenioides* can reduce the zinc ions in the zinc acetate solution and hence nanoparticles were formed. The addition of the plant aqueous extract and the zinc acetate solution, the formation of nanoparticles was initiated and the reaction mixture was light yellow in colour. During the process of the formation of the nanoparticles, the colour of the reaction mixture was changed from light yellow to dark brown and the colour change in the reaction mixture indicates the formation of nanoparticles. The change in colour of the reaction mixture during the process of formation of nanoparticles was represented in figure-1.

Figure 1: Colour changes observed during the synthesis of zinc oxide nanoparticles



RESULTS AND DISCUSSION

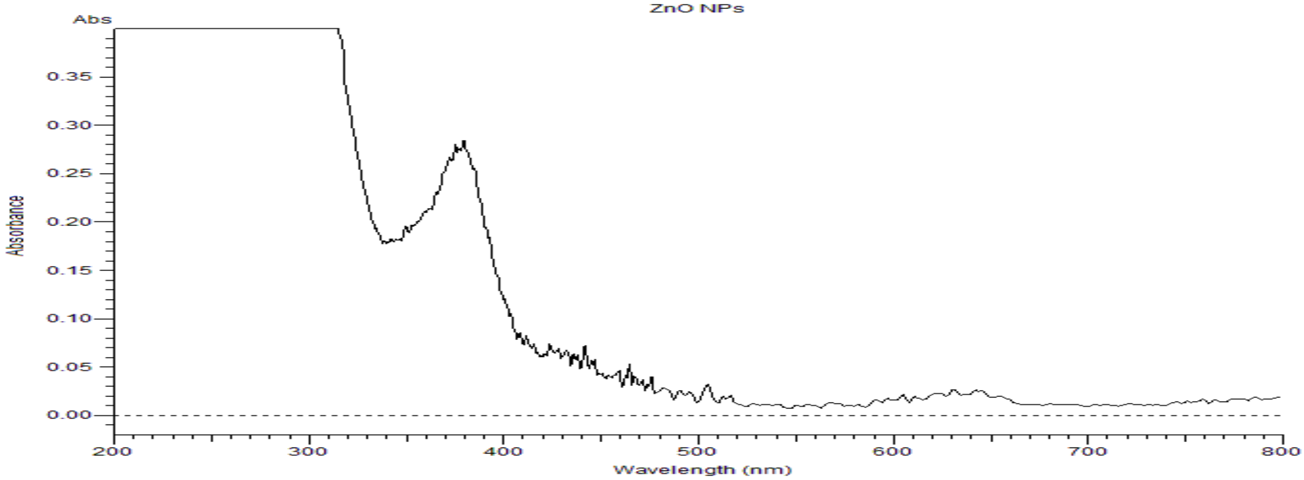
Characterization of nanoparticles

The synthesised zinc oxide nanoparticles were characterized using various characterization techniques such as UV-visible spectrophotometer, FT-IR, SEM, studies.

UV-visible spectrophotometer

The preliminary confirmation of the formation of the nanoparticles was confirmed by UV – visible spectrophotometer analysis. The colour change in the reaction mixture was monitored for the evaluation of the formation of nanoparticles. The UV – visible absorption spectra shows the characteristic maximum absorption wavelength peak at a wavelength of 379 nm confirms its narrow size distributed and mono-dispersed nanoparticles (Shraddha *et al*., 2019). The similar type of absorption peak was not identified in the absorption spectra of plant leaf extract as well as the metal solution. Hence it can be confirmed that the characteristic wavelength maximum is due to the formation of zinc oxide nanoparticles. Figure-2 shows the absorption spectra of synthesized zinc oxide nanoparticles

Figure 2: UV-visible absorption spectra of zinc oxide nanoparticles



FT-IR analysis of synthesized nanoparticles

The FT-IR analysis was performed to evaluate the functional groups corresponds to the bioactive chemical compounds of the aqueous leaf extract of *S. swietenioides* that involved in the binding of the metal particles and causes the reduction of the metal in to its nanosized particles. The FT-IR spectrum was given in figure-3 and the functional groups that are identified in the FT-IR spectrum and its corresponding functional groups were given in table-1.

Figure 3: FT-IR spectrum of zinc oxide nanoparticles synthesized using aqueous leaf extract of *S. swietenioides*

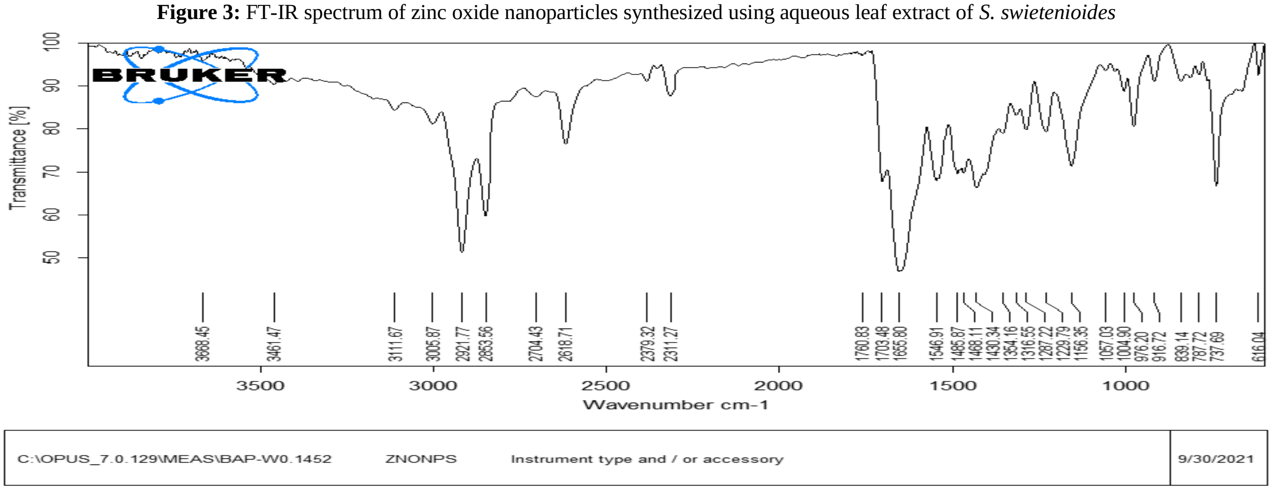


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Table 1: Bioactive functional groups identified in the FT-IR spectrum

Absorption	Functional group
3668 cm^{-1}	free -OH in alcohols
3461 cm^{-1}	O-H stretching in intramolecular bonded alcohols
2800-3000 cm^{-1}	N-H stretching in amine salts
3005 cm^{-1}	C-H stretching in alkanes
1655 cm^{-1}	C-H bending in aromatic compounds
1314 cm^{-1}	C-N stretching
1287 cm^{-1}	C-O stretching in aromatic esters

SEM analysis

The SEM analysis was performed to evaluate particle size and shape of the zinc oxide nanoparticles synthesised using aqueous leaf and root extract of *S swietenioides*.

The SEM analysis result of the green synthesised zinc oxide nanoparticles confirms that the nanoparticles were more or less spherical in shape. The observation of the individual particles of the nanoparticles confirms that the surface of the particles were rough surfaces. The zinc oxide nanoparticles were in the range of 54 nm to 97 nm and the average size of the nanoparticles was observed to be 68 nm. Figure-4 gives the SEM micrograph of the aqueous leaf extract of *S swietenioides* mediated zinc oxide nanoparticles.

Figure 4: SEM micrograph of the aqueous leaf extract of *S swietenioides* mediated zinc oxide nanoparticles.

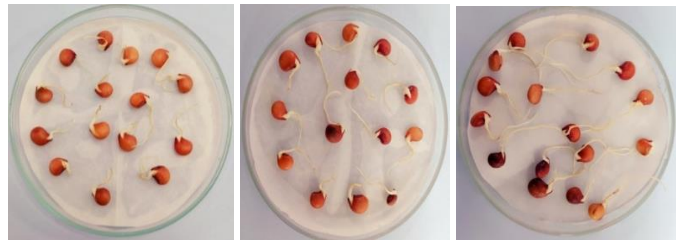
Application of nanoparticles on seed germination and plant growth promotion

The aqueous *S swietenioides* leaf extract mediated zinc oxide nanoparticles were studied for its effect on the germination of seeds and growth enhancement on pigeon pea (*Cajanus cajan* Linn.)

Seed germination study

The effect of aqueous *S swietenioides* leaf extract mediated zinc oxide nanoparticles on the enhancement of seed germination was studied on pigeon pea seeds. The seed germination study was conducted on the pigeon pea seeds with three treatment conditions such as treated with water, zinc acetate metal solution and aqueous leaf extract of *S. swietenioides* mediated zinc oxide nanoparticles. The MGT was observed to be 38.60±0.56, 37.53±0.40 and 28.53±0.59 hours respectively for untreated, zinc metal solution treated and the synthesized zinc oxide nanoparticle treated pigeon pea seeds. This confirms that the nano priming enhances the germination of the seeds significantly when compared with metal solution treatment as well as untreated pigeon pea seeds. The FGP was observed to be 91.33±0.58, 92.67±1.15 and 98.00±1.00 % respectively proves that the nano priming enhances the germination of the seeds. Figure 5 shows the results observed in the seed germination study on pigeon pea seeds. The comparative graph of FGP and MGT results were given in figure-6 and 9 respectively.

Figure 5: Results observed in germination study on pigeon pea seeds using zinc oxide nanoparticles



A B C

A) Treatment with distilled water; B) treatment with zinc acetate solution; C) Treatment with zinc oxide nanoparticles

Figure 6: Final germination percentage results of pigeon pea seeds in the seed germination study

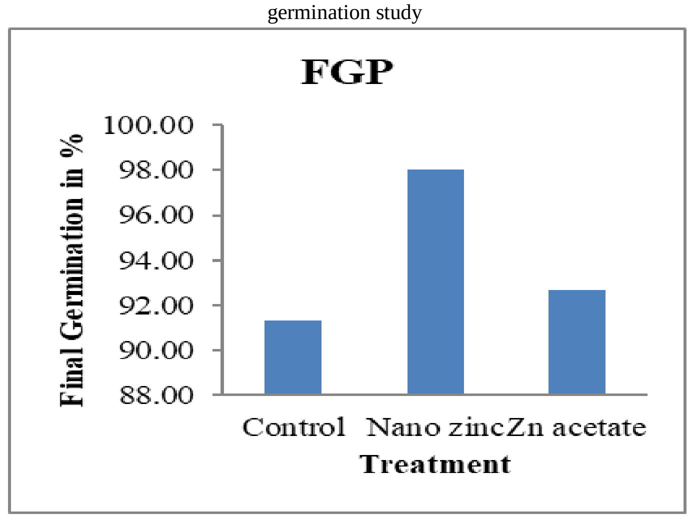
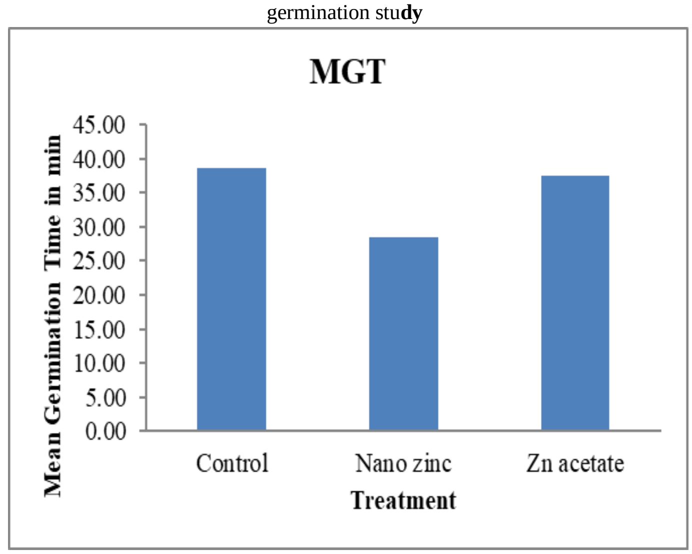


Figure 7: Mean germination time results of pigeon pea seeds in the seed germination stu**dy**



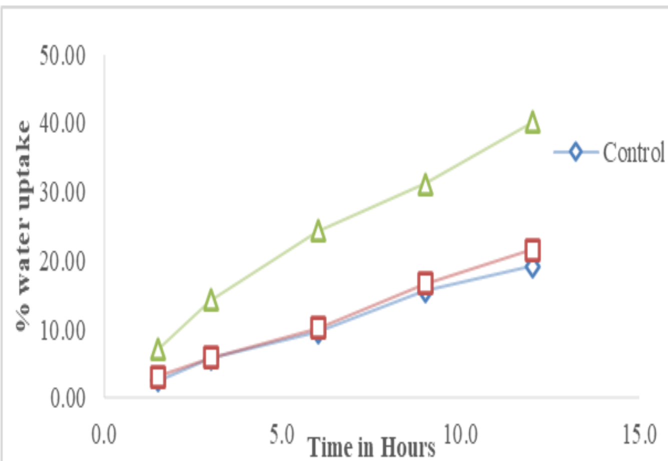
Water uptake study of pigeon pea seeds in various treatment conditions

The effect of the zinc oxide nanoparticles synthesised using aqueous leaf extract of *S. swietenioides* on the water absorption was studied. The results achieved in the study confirm that the zinc oxide nanoparticle treatment significantly enhances the water absorption capacity of the pigeon pea seeds. Within 12 hours of the water uptake study, the % water uptake of the nanoparticles treated pigeon pea seeds was observed to be 40.18±0.070 % whereas the untreated and metal solution treated pigeon pea seeds was observed to be 19.16±0.068 % and 21.51±0.067 % respectively. Hence the nano treatment enhances the water uptake significantly than the untreated and metal solution treated pigeon pea seeds. The results in the study were given in the form of table as table 2 and were represented in comparison graph as shown in figure-8.

Table 2: Results achieved in water uptake study of pigeon pea seeds

Time in hours	% water uptake of pigeon pea seeds treated with ± SD		
	Control	Zn acetate	Nano zinc
1.5	2.47±0.085	3.09±0.085	7.11±0.046
3.0	5.69±0.085	5.86±0.076	14.33±0.072
6.0	9.60±0.181	10.19±0.065	24.46±0.111
9.0	15.55±0.146	16.67±0.110	31.20±0.062
12.0	19.16±0.068	21.51±0.067	40.18±0.070

Figure 8: Comparative results of water uptake study of pigeon pea seeds in various treatment conditions



Green house study for plant growth promotion activity

A greenhouse experiment was conducted for the evaluation of the impact on synthesised nanoparticles on the growth enhancement of plants and the applicability of the nanoparticles as growth promoters in agriculture field. The seeds grown for the seed germination study were planted on plastic seedling trays and the same treatment conditions were applied for the growth of the plants.

The synthesised zinc nanoparticles were applied for the plant growth of pigeon pea plants. The zinc nanoparticle treated plants grow significantly higher than the plants treated with zinc acetate and distilled water. The root length of the zinc oxide nanoparticles treated pigeon pea plants was observed to be 7.13±0.25 cm which is observed to be very higher than the plants treated with distilled water (2.27±0.15 cm) and zinc acetate solution (2.67±0.15). The shoots of the plants treated with nanoparticles was observed to be grown higher than the plants treated with distilled water and zinc acetate. The photosynthetic pigments such as chlorophyll A and B on the leaves of the pigeon pea plants were estimated and results confirm that the quantity of both chlorophyll A and B was observed to be very high on the plants treated with zinc nanoparticles. The uptake of the metal by the plant roots and accumulation of the metal on the leaves of the plant was also analysed using the atomic absorption spectrophotometer. The AAS analysis confirms that the zinc content in the plant roots was observed to be very higher for the plants treated with zinc nanoparticles than the distilled water treated and zinc acetate treated plants. Hence based on the results it can be confirm that the zinc oxide nanoparticles can easily absorbed in to the plant and can stimulate the growth of the plant. Hence the zinc nanoparticles can be applicable as nano priming and growth promoting agent. The results observed in the study were given in summarized in table -3. The plant growth promotion activity study results were represented in figure-9.

Table 3: Results observed in plant growth promotion activity of pigeon pea

Parameters	Results observed for pigeon pea plants treating with ± SD		
	Control	Zinc acetate	Nano zinc
Root length in cm	2.27±0.15	2.67±0.15	7.13±0.25
Shoot length in cm	6.57±0.21	7.13±0.26	15.60±0.21
Chlorophyll A in mg/g fresh weight	13.83±0.87	15.30±0.40	22.07±0.67
Chlorophyll B in mg/g fresh weight	16.23±0.61	19.17±0.50	36.27±0.47
Zinc metal content on roots in mg/kg	47.75±0.181	51.27±0.076	87.24±0.130
Zinc metal content on leaves in mg/kg	29.33±0.101	31.24±0.095	59.39±0.146

Figure 9: Pictorial representation of pigeon pea plants in growth promotion activity study.

9 (A) treated with distilled water

9 (B) treated with zinc acetate sol

9(C) treated with zinc oxide nano particle

9 (D) Comparison of pigeon pea plant growth in the treatment condition studied

CONCLUSION

Various characterization techniques proved that the zinc oxide nanoparticles synthesised. The applicability of the synthesised nanoparticles in the agriculture field was evaluated in terms of its effect on the seed germination and growth enhancement of seedlings. The zinc nanoparticles were studied for the seed germination and plant growth promotion of Pigeon pea (*Cajanus cajan* Linn.) respectively. The nanoparticle treatment increases the rate of germination and decrease the mean germination time and shows the positive effect during the seed germination. Based on the findings achieved in the study, it can be conclude that the green synthesized zinc oxide nanoparticles synthesised using aqueous extracts of *Schrebera swietenioides* Roxb The nanoparticles were also utilized as nano fertilizer and growth promoting agents in the enhancement of plant growth in modern agriculture. This green method of synthesis was found to be eco-friendly and cost-effective.

DECLARATIONS

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Conflict of interest: No conflict of interest to declare

Ethical approval: No animals were used during the research work

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