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Original Article

GREEN SYNTHESIS OF ZINC OXIDE NANOPARTICLES USING *SCHREBERA SWIETENIOIDES* ROXB., AQUEOUS LEAF EXTRACT AND INVESTIGATION OF ITS EFFECT ON SEED GERMINATION AND PLANT GROWTH ON PIGEON PEA (*CAJANUS CAJAN* LINN.)

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ABSTRACT

Objective: The present work intended to green and eco-friendly synthesis of Zinc oxide (ZnO) nano particles (NPs) using aqueous leaf extract of *Schrebera swietenioides* and the synthesised NPs were applied for enhancement of seed germination and plant growth in pigeon pea (*Cajanus cajan* Linn.).

Methods: The Zinc acetate was utilised as metal source and metal was reduced using aqueous leaf extract of *S. swietenioides* as green reducing agent. The synthesised NPs were characterized using various techniques such as SEM-EDS, TEM, XRD, FT-IR and UV-visible spectrophotometer. The seed germination study as well as plant growth promotion activity, was performed on pigeon pea seeds.

Results: The result achieved in characterization of NPs confirms that he NPs were hexagonal wurtzite form having a spherical shape with irregular surfaces. The average size was found to be 68 nm with the metal composition of 73.7 %. The NPs were studied for seed germination and growth promotion activity on pigeon pea seeds and the mean germination time was observed to be 38.60±0.56, 28.53±0.59 and 37.53±0.40 h whereas the final germination percentage was found as 91.33±0.58, 98.00±1.00, and 92.67±1.15 h for control, NPs treated and zinc acetate treated seeds respectively. The NPs treated plants grow more rapidly than the untreated as well as Zn acetate-treated pigeon pea plants. The pigeon pea seeds treated with ZnO NPs shows the high activity of enzyme activities such as amylases, protease, catalase than the untreated as well as Zn acetate treated seeds.

Conclusion: The aqueous leaf extract of *S. swietenioides* mediated ZnO NPs can augment the growth of pigeon pea seedlings, and the NPs treatment shows a stimulatory effect on the enzymes associated with the growth of seedlings.

Keywords: Zinc oxide nanoparticles, Nano priming, Growth promotion, Pigeon pea

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INTRODUCTION

Pigeon pea (*Cajanus cajan* (L.) Millsp.) is a protein-rich legume crop, which was cultivated in tropical and subtropical regions of the world. It is a vital grain legume crop in several countries of Asia, Africa, and Latin America. The largest share (≈75%) of global pigeon pea production comes from India. In India, Pigeon pea is cultivated in an area of 3.96 million ha and is the second-largest pulse crop in India. The dried split-seeds are consumed by most of the Indian population as a source of protein [1].

Zinc (Zn) is considered as an essential micronutrient for both plants and animals. It is absorbed by higher plants mainly as a divalent cation (Zn^{2+}). In the enzymatic system of plants, Zn acts as a cofactor, regulatory factor, and metal component and hence it is extremely essential for plant growth. Among the plant yield-enhancing micro-nutrients in India, Zn comes in forth position after nitrogen, potassium and phosphorous [2].

Zn deficiency exists in different soils of the world, including those of India. Low availability of soil zinc (Zn) adversely affects plant growth parameters such as plant height, number of branches, pod number, seed yield, and Zn concentration of seed and tissue of pigeon pea due to reduced enzyme activity influencing the plant metabolism [3].

Nanotechnology is a special branch of technology that deals with atomic or molecular aggregates that were having dimension of less than 100 nm and has gained attention of researchers for recent years [4]. The tiny dimension of the nanoparticles (NPs) provides them extraordinary surface-to-volume ratios, which permit them to confine electron motions inside boundaries associated with improving the optical properties [5]. NPs have unique thermal, optical, chemical, physical, and electrical properties that make them particularly desirable in the fields of chemistry, environment, agriculture, medicine, energy, consumer goods etc [6].

Zinc fertilizers are mostly applied as zinc oxides, sulphates and carbonates [7]. Apart from Zn fertilizers and natural source in the environment, Zn NPs were used as fertilizer for plant uptake. Adequate Zn content in seeds can ensure higher germination, boost plant development, and enhance protection against pathogens [8]. Zn NPs were the promising source of nutrients for plants. In principle, its size-dependent solubility can yield controlled-release fertilizers, or small particles can be entirely taken up by plants and slowly dissolve at the target tissues [9].

In view of the above, the present work was intended to synthesize the Zinc oxide (ZnO) NPs using aqueous leaf extract of *Schrebera swietenioides* Roxb. Further the synthesised NPs were studied for its enhanced seed germination and plant growth promotion activity on Pigeon pea.

MATERIALS AND METHODS

Collection of plant material

The fresh leaves of *S. swietenioides* were collected from Tirumala hills, located in Tirupati, Andhra Pradesh. The plant material was identified by Dr. Ch. Srinivasa Reddy, Assistant Professor, Department of Botany, SRR and CVR Government Degree College (A) Vijayawada and a dried specimen was stored in the department with specimen number SRR-CVR/2019-20/Bot/31. The leaves were cleaned using sterile cotton till the complete removal of dirt particles on it and dried in less than 50 °C temperature in shade, powdered using mechanical blender and preserved powder in an airtight amber bottle.

Chemicals and reagents

Analytical reagent-grade Zinc acetate, sodium hydroxide, potassium hydrogen phoasphate, phosphoric acid, tyrosin, pholin phenol reagent etc., were purchased from Merck chemicals, Mumbai.

Preparation of aqueous leaf extract

The shade dried leaf powder of *S. swietenioides* was used for the preparation of extract for the synthesis of ZnO NPs. The aqueous leaf extract was prepared by boiling 100 g of dried leaf powder in 100 ml distilled water at 70 °C for 1 H. After completion of boiling, cool the extract and filtered using Whatman filter paper. The filtrate was used in ZnO NPs synthesis.

Green synthesis of zinc oxide nanoparticles

Zinc acetate solution at a concentration of 0.1 M and leaf extract of *S. swietenioides* were mixed in the ratio of 9:1 (v/v) and was stirred without heat for 4 h using magnetic stirrer for homogenous mixture. Sodium hydroxide (NaOH) solution was then prepared by mixing it with 0.8 M aqueous ethanol and stirred without heat for 4 h. The two solutions were added together and stirred for 6 h for homogenous mixture and chemical reaction. $Zn(OH)_2$ precipitate that settled at the bottom of the sealed beaker was obtained by removal of excess mother liquor. The precipitate $Zn(OH)_2$ by-products was removed by washing with deionized water and acetone. Heating process or baking was carried out at the temperature of 300 °C for 45 min to evaporate the solvent in a carbolite in muffle furnace and to convert $Zn(OH)_2$ into ZnO NPs particle powder [10].

Characterization of Ka-ZnO_2NPs

The UV-visible spectrophotometer (JASCO, Japan) was used to evaluate the optical characterization of ZnO NPs by scanning the aqueous solution of NPs in the range of 800 to 400 nm. The functional group analysis of synthesized nanoparticles was carried out using an FT-IR spectrophotometer (Bruker, USA) in the range of 500–4000 cm^{-1} that confirms the plant biomolecules involved in the bio-reduction of Zn to form ZnO NPs. The FE-SEM (Field emission scanning electron microscope-NOVA NANOSEM 450, FEI, USA) analysis of synthesized NPs was carried to determine morphology and size. The lattice structure and crystalline nature of the ZnO NPs were confirmed by X-ray diffraction (XRD) analysis. The XRD analysis was performed on x-ray diffractometer (Rigaku Corporation) at a scan speed of 2°/min in the diffraction angles (2θ) from 20° to 80°. The % metal content and the elemental composition in the synthesized ZnO NPs were confirmed by EDS (Energy-dispersive X-ray spectroscopy) studies carried on RONTEC's EDX system (QuanTax 200, Germany).

Assessment of germination of pigeon pea seeds

The effect of synthesized ZnO NPs on the seed germination of pigeon pea seeds was carried as per the procedure described by *Chuanhai et al.*, 2012 [11]. Healthy and uniform size pigeon seeds collected by visual observation and were stored in a refrigerator at 4 °C to simulate seed dormancy. The surface of the seeds was sterilized using 0.5% by weight mercury (II) chloride for 10 min. Then the seeds were washed several times with sterile double distilled water. The germination study of pigeon seeds was carried using an incubator which was conducted in darkness at 30 °C using three treatments: control (untreated seeds), metal treated (manganese acetate) and ZnO NPs treated seeds. In all three treatment conditions, healthy chickpea seeds were cultured in sterile Petri dishes (100 ×15 mm) with 15 seeds per dish on Whatman filter paper in the culture container. The filter paper was moistened with 5 ml of sterile distilled water (control), 10 mg/l zinc acetate (metal treated), and 10 mg/l of ZnO NPs. Each treatment was carried in three replicates and the same volume of the treatment solution was added every day to prevent drying. In all the treatment studies, the emergence of radical to 2 mm or more was considered as germination and the number of seeds germinated was recorded in every 24 H of incubation.

To ascertain the nano-priming on the germination of pigeon seeds, the germination rate, and final germination percentage, the mean germination time was calculated as per the procedure described by *Arturo et al.*, 2016[12].

$$FGP = (Ae \times M)/100 \text{ ---------- (1)}$$

Where FGP = final germination percentage is the germination capacity of pigeon pea germinated completely at a given time

Ae = Germination accumulated until the last evaluation;

M = Total of sowed chickpea

$$MGT = \sum(nt)/(\sum n) \text{ ---------- (2)}$$

WhereMGT = Average time for a pigeon pea seed to germinate

n = Number of pigeon peas newly germinated at time t,

t = Number of days from sowing.

$$\text{Germination rate} = \sum n/t \text{ ---------- (3)}$$

Water uptake of pigeon pea seeds

The water uptake (WUT) by seeds during imbibition was determined in triplicate; each replicate having 25 seeds. Weighed seeds were placed between water-saturated cotton in a plastic box and incubated at 25 °C. At intervals of 40, 80 and 120 min, all the seeds were removed, blotted dry and weighed. Changes in weight due to imbibition were expressed as the amount of water absorbed per seed dry weight which was calculated by the following formula.

$$WUT = [(\text{Fresh weight of seed} - \text{Dry weight of seed}) \times 100]/\text{Dry weight of seed} \text{ ----- (4)}$$

Assessment of plant growth of pigeon pea

A greenhouse study was conducted for the evaluation of the growth promotion activity of synthesized ZnO NPs on pigeon pea. The study was carried in three treatments as per the procedure described by *Pratibha et al.*, 2020 [13]. Twenty-five germinated seedlings in the seed germination study conducted previously were individually planted in 25 cells plastic seedling trays containing soil. All the experimental trays were thoroughly moistened using distilled water for control, zinc acetate, and ZnO NPs solutions. The seedlings were grown for up to 7 d. The effect of nanoparticles on chickpea's growth promotion was evaluated by determining the numbers of plants grown in the treatment study, shoot length, root length, several leaflets observed in each plant. In all the treatment studies, the Zn metal content in the roots, stem, and leaves was determined using atomic absorption spectroscopy (Shimadzu, Japan) as per the procedure described by *Saadia and Azka*, 2016 [14].

Assay of hydrolytic enzymes

Preparation of crude enzyme extract from germinating seeds

One gram of germinating seeds was collected each time at different intervals of the growth period (0 to 7th day of germination) and weighed after removing the seed coat. The sample was then homogenised in a mortar with the help of pestle to a very fine paste by adding 10 ml of ice-cold phosphate buffer (0.1 M; pH 7.6). The buffer extract was filtered and centrifuged at 10,000 rpm and 4 °C for 15 min. Later the supernatant was saved, and the pellet was discarded. The supernatant was considered as crude enzyme extract and was used for the determination of enzymatic studies as well as the protein content analysis.

Amylase activity

Five ml of crude enzyme extract was subjected to direct estimation of β-amylase activity while another 5 ml of cell extract was subjected to temperature treatment (70 °C for 15 min) to denature β-amylase and the activity of α-amylase determined from the β-amylase free cell extract following the method of Bernfeld 1955 [15]. The unit of amylase (both α and β) activity was expressed as mg maltose produced mg^{-1} protein.

Protease activity

The crude enzyme extract was used to determine the protease activity and the analysis was carried as per the procedure reported by Reimerdes and Meyer 1976 [16] using casein as substrate. The measurement was carried out by estimating the release of tyrosine calculated from the standard curve prepared with tyrosine. One unit of protease activity was defined as the amount of enzyme required for liberating 1 mg of tyrosine in 30 min at 45 °C.

Catalase activity

Catalase activity of germinated seeds was evaluated based on the procedure reported by Aebi 1983 [17]. The reaction mixture

contained 50 µl of enzyme extract 50 mmol phosphate buffer (pH 7.0) to make the final volume 0.9 ml. The reaction was started by the addition of 200 µl of 45 mmol hydrogen peroxide in the above buffer and the decrease of absorbance was recorded at a wavelength of 240 nm using UV-visible spectrophotometer and the catalase activity was expressed as mol/min/mg protein.

Determination of protein content

The protein contest in the germinating seed extract was evaluated based on the method described by Lowry *et al.*, 1951 [18].

Determination of chlorophyl content in plant leaves

The chlorophyl A and B content in the fresh leave of pigeon pea grown in the greenhouse study was determined based on protocol given by Surbhi *et al.*, 2020 [19]. The chlorophyl A and B content in the samples was calculated as per the equation given by Arnon 1949 [20].

Determination of metal uptake by plant

The metal uptake by the plants and the accumulation of Zinc in the leaves and roots of the plant was evaluated using atomic absorption spectrophotometer and the assay was performed as per the procedure described by Surbhi *et al.*, 2020 [19].

RESULTS

The UV-visible absorption spectra of the synthesised ZnO NPs shows sharp absorption peak at 379 nm (fig. 1A) which was confirmed as the characteristic absorption maximum for the ZnO NPs [21]. The sharpened nature of the absorption peak proved that the NPs are constructed in the form of mono-dispersed narrow size distribution of particles [21]. The type of bioactive molecules or functional groups that are actively involved to bind the metal and formation of NPs was evaluated by shifting in wavenumber in FT-IR spectrum. The FT-IR spectrum (fig. 1A) shows the signal at 3668 cm^{-1} and 3461 cm^{-1}associated with free-OH in alcohols and O-H stretching of intramolecular bonded alcohols, respectively. Strong peak in the range of 2800-3000 cm^{-1}corresponds to N-H stretching in amine salts. The medium peak observed at 3005 cm^{-1}represents the presence of C-H stretching in alkanes. Strong peak at 1655 cm^{-1} corresponds to C-H bending in aromatic compounds. The peak corresponds to C-N stretching in aromatic amines and C-O stretching in aromatic esters was identified at 1314 $cm^{-1}$and 1287 cm^{-1} respectively. The π-electrons present in various bioactive compounds in the aqueous leaf extract of *S. swietenioides* maybe interact with the surface of the metal and enhance the formation of NPs.

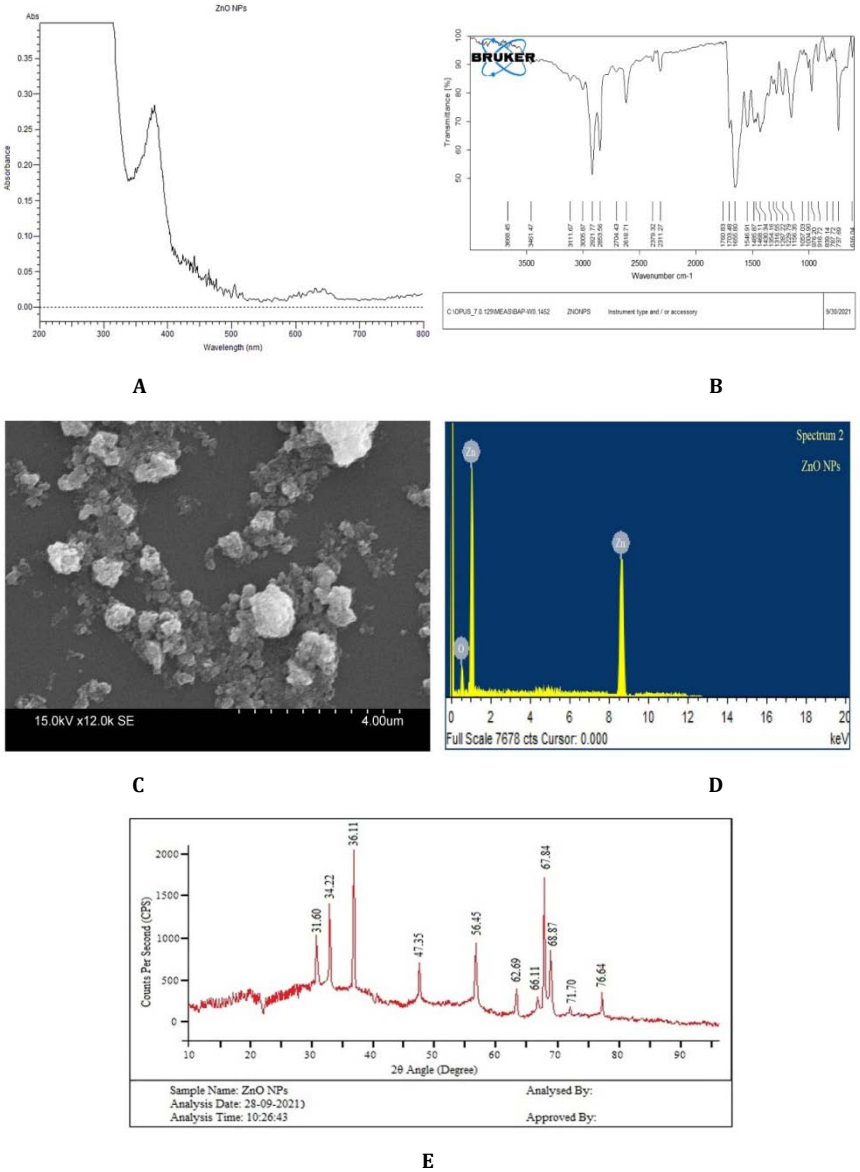
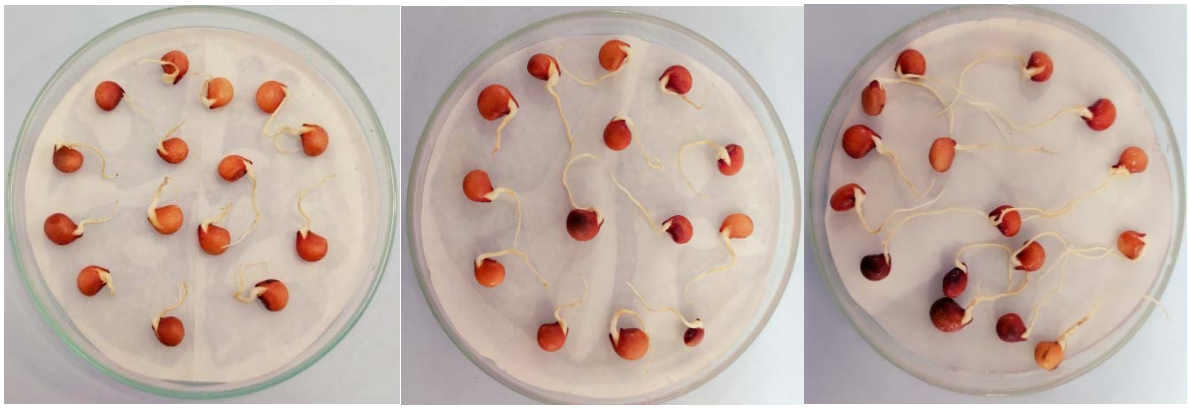


Fig. 1: Characterization of ZnO NPs synthesised using *S. swietenioides* leaf extract, A) UV-visible scanning spectra; B) FT-IR spectra; C) SEM analysis; D) EDS spectra; E) XRD analysis spectra of the synthesised ZnO NPs

In the SEM analysis (fig. 1C), the topographical view of show more or less spherical-shaped particles confirms that the NPs were spherical in shape. The surface of the individual particles was observed to be rough in nature. The EDS analysis (fig. 1D) proved that Zn and Oxygen are the key elements involved in the NPs. The peak corresponds to Carbon also identified in the spectra that is maybe due to the bio-active compounds of the plant. The characteristic peak corresponds to Zn was identified at 8.61 (Kα) and 1.09 keV (Lα), whereas the peak corresponds to Oxygen was observed at 0.5 keV. The metal content in the synthesised NPs was found to be 73.7 %. The XRD analysis spectra (fig. 1E) shows 2θ characteristic peaks corresponding to planes of the crystal lattice structure were identified at 31.60 (100), 34.22 (002), 36.11 (101), 47.35 (102), 56.45 (110), 62.69 (103), 66.11 (200), 67.84 (112), 68.87 (201), 71.70 (004) and 76.64 (202). The peaks are in a good argument with the hexagonal Wurtzite form of the NPs and were corelate with standard JCPDS Card No. 89-0510. The diffraction peaks were observed to be narrow and robust peaks confirms that the formed NPs were in uniform size. The Debye–Scherrer's equation was applied for the calculation of size of the NPs and the average size of the ZnO NPs was calculated as 68 nm.

The germination of pigeon pea seeds and emergence of seedling by nano priming with ZnO NPs was studied. The results (table 1) confirm that the nano treatment significantly increases the percentage germination as well as the speed of germination of pigeon pea seeds. The MGT of the pigeon pea seeds was observed to be decreased with nano treatment when compared with the metallic zinc treatment as well as untreated pigeon pea seeds. The MGT of the nano zinc treated seeds was decreased but there was a very less significant different observed in FGP due to all the seeds in the study are viable. This confirms that the nano treatment enhances seed germination by decreasing the MGT of pigeon pea seeds. Fig. 2 shows the pigeon pea seed germination study photographs showing the seed germination enhancement with nano treatment.



A **B** **C**

Fig. 2: Seed germination study results, A) Control with no treatet; B) Metal treatment that treated with Zinc acetate solution; C) Nano treatment that treated with *S. swietenioides* leaf extract mediated Zno NPs

The effect of ZnO NPs treatment on the growth enhancement on pigeon pea seeds was evaluated in greenhouse study and the seeds were planted for growth in 25 cells plastic seedling trays. The results obtained in this study confirms that the ZnO NPs treated pigeon pea seeds shows enhanced growth with a greater number of leaves with high shoot length. The root length of ZnO NPs treated plants was observed to be 7.13±0.25 cm which was significantly more than the untreated (2.27±0.15 cm) as well as metallic Zn treated (2.67±0.15 cm) plants. The shoot length of 15.60±0.21 cm was observed for nano-treated plants and was greatly enhanced than the untreated (6.57±0.21 cm) and Zn metal treated (7.13±0.26 cm) plants. The chlorophyll A and B content of the plants were estimated, and the results proved that both chlorophyll A and B in the nano-treated plants was observed to be very high than the untreated and metal-treated plants (table 1). The photosynthetic potential and primary production of the plants greatly affects its chlorophyll content. It was also related to the stress in plants. The enhanced chlorophyll content in the ZnO NPs treated plants shows high photosynthesis capacity that reflects the morphological as well as physiological characteristics of pigeon pea plants. Hence the nano treatment enhances the plant chlorophyll content as well as the growth of the plant. The growth promotion activity study results were given in fig 3.

Table 1: Seed germination and plant growth enhancement activity study results

S. No.	Treatment	FGP	MGT in H	Root length in cm	Shoot length in cm	Chlorophyll in mg/g fresh weight	
						A	B
1	Control	91.33±0.58	38.60±0.56	2.27±0.15	6.57±0.21	13.83±0.87	16.23±0.61
2	ZnO NPs	98.00±1.00	28.53±0.59	7.13±0.25	15.60±0.21	22.07±0.67	36.27±0.47
3	Zn acetate	92.67±1.15	37.53±0.40	2.67±0.15	7.13±0.26	15.30±0.40	19.17±0.50

Results given in table are the mean±standard deviation for three repeated measurements

The amylase activities (both α and β) were identified in the cotyledons of the seeds only confirms that the amylases were situated in the cotyledons of the seeds only. The α and β amylase enzyme activity of the pigeon pea seeds during the seed germination study was determined and the results were represented in fig 4A and 4B, respectively for α and β amylase activity. The results observed in the study confirms that the α–amylase activity was decreased in the study whereas the β–amylase activity was increased on increase in the germination time. The change in the amylase activity was very less in the early days the germination confirms and the activity was significantly changed after three days of the germination study. The activity of the seeds treated with ZnO NPs was observed to be very high than the seeds treated with Zn acetate as well as untreated seeds. Based on the results the enzyme activity of the ZnO NPs treated seeds was observed to be enhanced than the Zn acetate treated as well as untreated pigeon pea seeds.

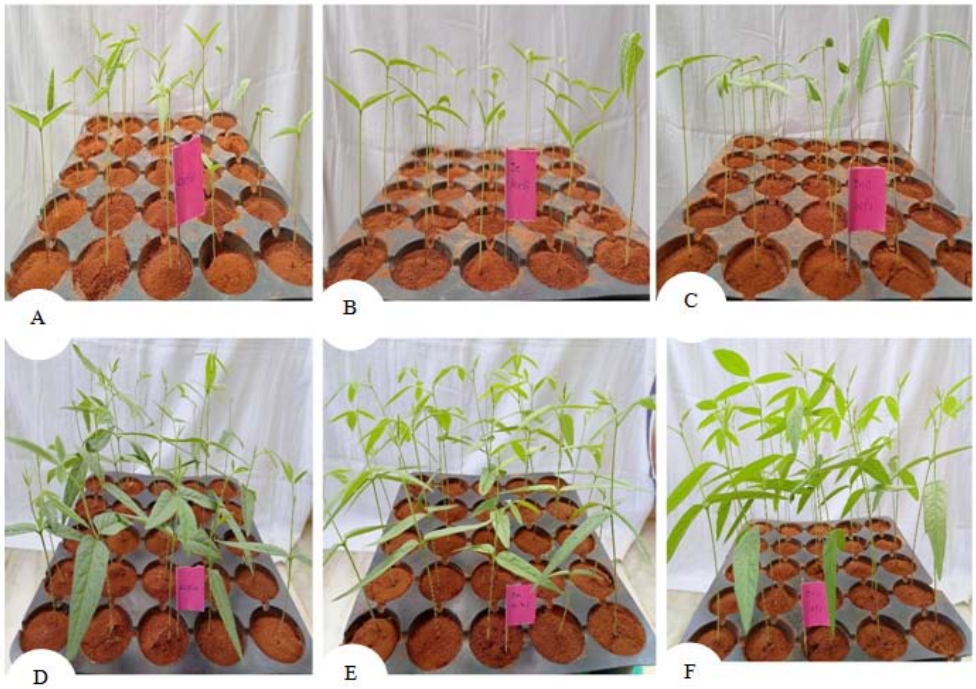


Fig. 3: Pictorial view of pigeon pea plants in growth promotion activity study, plant growth observed for untreated (A), Zn acetate treated (B) and ZnO NPs treated (C) pigeon pea seeds 3rd day of germination study. Plant growth observed for untreated (D), Zn acetate treated (E) and ZnO NPs treated (F) pigeon pea seeds 8th day of germination study

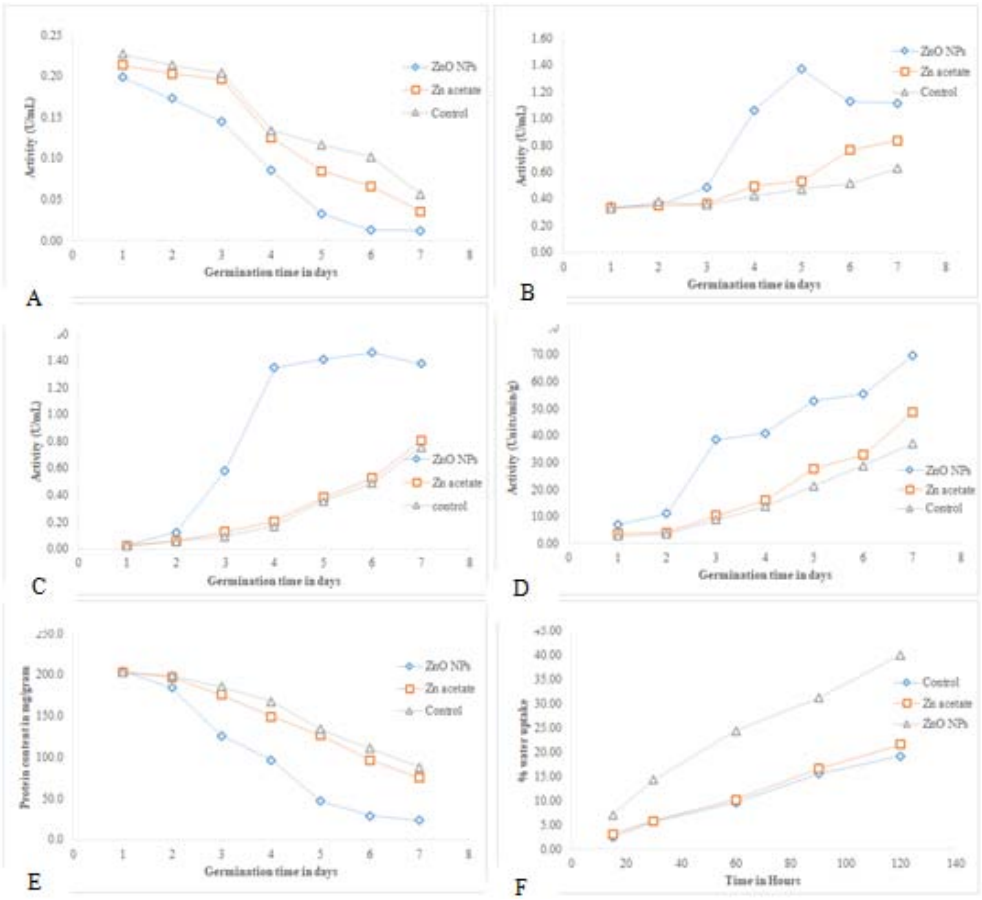


Fig. 4: Mean enzymatic assays, protein content and germinating seeds water uptake study graphs for results observed during the germination and plant growth enhancement study of NPs on pigeon pea, A) α-amylase enzyme activity; B) β-amylase enzyme assay; C) Protease activity assay; D) Catalase activity; E) Protein content; F) Water uptake study results of germinating seeds

In the germination process of seeds, the storage proteins present in the seeds are hydrolysed by proteolytic enzymes and thus, the nutrients required for the development and growth of seedlings was obtained. The protease activity of the germinating pigeon pea seeds was observed to be very high on 4th day of germination for ZnO NPs treated seeds, whereas the protease activity of the Zn metal treated and untreated pigeon pea seeds was observed to be very less (ure 4C). The protease activity on day 4 of the germination study was found to be 1.348±0.004, 0.204±0.007 and 0.167±0.001 units/ml for ZnO NPs treated, Zn acetate treated and untreated pigeon pea seeds. This proved that the ZnO NPs treatment enhances the protease enzyme activity that subsequently enhances the seed germination process.

Catalase is an antioxidant enzyme that allows the tolerance of plant under stress conditions, and it is also considered an effective component in the seed germination physiology. Catalase acts as preservation of viability during storage and is essential for seed germination and early seedling growth. The catalase activity of the germinated pigeon pea seeds, when treated with ZnO NPs, was observed to be very high than the untreated and Zn acetate treated seeds. On first day of the germination the catalase activity was calculated as 6.926±0.225, 3.587±0.040 and 2.927±0.065 units/min/g respectively for ZnO NPs treated, Zn acetate treated and untreated pigeon pea seeds. Whereas on 3rd day of germination the activity was observed to be 38.407±0.361, 10.290±0.106 and 8.927±0.078 units/min/g, respectively for ZnO NPs treated, Zn acetate treated, and untreated pigeon pea seeds proved that nano treated seeds showing enhanced activity (fig. 4D).

The protein content in the germinating seeds was observed to be decreased with increase in germination time. The decrease in protein content of the germinating seeds represents the increase in protease activity. The protein content was significantly decreased in fourth day of germination and the decline continues till the 7th day of germination. The protein content in first day of germination was observed to be 204.52±0.292, 202.20±0.361 and 202.93±0.961 mg/gram for ZnO NPs treated, Zn acetate treated and untreated pigeon pea seeds. Whereas the protein content in fourth day of germination was found to be 96.65±0.771, 148.87±0.379 and 167.80±0.700 mg/gram for ZnO NPs treated, Zn acetate treated and untreated pigeon pea seeds (fig. 4E). The results are in good argument with the protease activity observed in the study. The water imbibition of pigeon pea seeds treated with ZnO NPs was observed to be very high than the untreated and zinc acetate treated seeds (fig. 4F) proved that the nano zinc treatment enhances the water uptake by the seeds.

The chlorophyll content in the leaves of the grown pigeon pea plants was studied. The chlorophyll A was found to be 13.83±0.87, 22.07±0.67 and 15.30±0.40 mg/g, respectively whereas the chlorophyll B content was found to be 16.23±0.61, 36.27±0.47 and 19.17±0.50 mg/gram respectively for untreated, ZnO NPs treated, and Zn acetate treated pigeon pea plants. The results confirm that the pigeon pea plants treated with ZnO NPs is significantly enhances the biosynthesis of main photosynthetic pigments.

DISCUSSION

In the current study, the bio-active compounds present in the leaf extract of *S. swietenioides* was utilised as biological reducing agent for the reduction and formation of ZnO NPs. The initial signature for the formation of ZnO NPs is to change the color of the reaction mixture from light green to dark brown with in few minutes. The bio-active chemical-constituents present in the aqueous leaf extract of *S. swietenioides* maybe acts as biological reducing agent for the formation of ZnO NPs.

The initial conformation for the formation of NPs was done by observing characteristic UV-visible absorption maxima at 379 nm which was in correlation with reported results [22, 23]. The XRD analysis of synthesized NPs shows characteristic peaks at 31.60 (100), 34.22 (002), 36.11 (101), 47.35 (102), 56.45 (110), 62.69 (103), 66.11 (200), 67.84 (112), 68.87 (201), 71.70 (004) and 76.64 (202) which confirms its hexagonal Wurtzite form of the NPs and were correlate with standard JCPDS Card No. 89-0510. The XRD patter observed in the present study was in a good argument with the previous findings [22, 24]. The average size of the NPs was observed to be 68 nm and the % metal content in the synthesized NPs was observed to be 73.7 % which is very higher than the few findings reported [23], which confirms that the metal content was very high in the present study.

The effect of synthesised NPs on the germination of pigeon pea seeds was evaluated in laboratory study and the results confirms that the NPs treatment enhances the seed germination with reduced mean germination time. The FGP was achieved at 91.33±0.58, 98.00±1.00 and 92.67±1.15 % whereas the MGT was found to be 38.60±0.56, 28.53±0.59 and 37.53±0.40 H respectively for the pigeon pea seeds treated with distilled water (control), synthesized ZnO NPs and zinc acetate, respectively. Further the NPs were studied for its effect on the growth of pigeon pea plants. The enhanced root length of 7.13±0.25 cm was achieved for the plants treated with ZnO NPs whereas the root length for untreated and zinc acetate treatments was found to be 2.27±0.15 cm and 2.67±0.15 cm respectively. The photosynthetic pigments such as chlorophyll A and B was observed to be 22.07±0.67 mg/g and 36.27±0.47 mg/g, respectively which was significantly higher than the untreated and zinc acetate treated plants confirms that the NPs treatment enhances the pigeon pea plant growth. The enzymatic studies such as amylase, protease and catalyse activities of the NPs treated seeds also significantly higher for the pigeon pea seeds during the germination proves the enhanced activity due to the NPs treatment. The findings in the present study were compared with the literature available and proved that the ZnO NPs were found to be improved activity then the findings available in literature. Hence the synthesised NPs were proved to be having enhanced activity on the pigeon pea seed germination and growth of pigeon pea plant.

CONCLUSION

The present work demonstrated the green synthesis and characterization of ZnO NPs using aqueous leaf extract of *S. swietenioides*. The formation of ZnO NPs was initially confirm by its characteristic absorption maxima at 379 nm and the NPs were hexagonal wurtzite form crystals having spherical shape with rough surfaces with an average size of 68 nm and having 73.7 % of zinc content. The seed germination activity on pigeon pea seeds shows mean germination time of 28.53±0.59 H which was significantly less than the untreated (38.60±0.56 H) and zinc acetate treated (37.53±0.40 H) seeds confirms its enhanced seed germination. The results suggested that the synthesised NPs enhances the water intake and seed germination as well potentially improves the plant growth when compared with Zn acetate treated and untreated pigeon pea. The photosynthetic pigments, enzymatic activities such as amylase, protease, catalase etc were found to be high active in the seeds treated with ZnO NPs. Hence it can be concluded that the treatment of ZnO NPs shows remarkable enhancement on germination of seeds as well as the growth of the pigeon pea plants.

FUNDING

Nil

AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

Declared none

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