ABSTRACT

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

Methods: The Zinc acetate was utilised as metal source and metal was reduced using aqueous leaf extract of S. swietenoides 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 seed germination percentage was found as 91.3±0.58, 90.0±0.10, and 92.6±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 Zinc 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 as compared to the untreated seeds.

Conclusion: The aqueous leaf extract of S. swietenoides 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

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. 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 micro-nutrient for both plants and animals. It is absorbed by higher plants mainly as a divalent cation (Zn²⁺). 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 micronutrients 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 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 swietenoides 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. swietenoides 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 phosphate, 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. swietenoides* 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. swietenoides* 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 NPs

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 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].

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 Chuaaai et al. 2012 [11]. Healthy and uniform size pigeon seeds collected by visual observation 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 FT-IR spectrophotometer (Bruker, USA) in the range of 800 to 400 nm. 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 RONTECs EDX system (QuanFax 200, Germany).

Assay of hydrolytic enzymes

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 Reimderdes 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 content 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 leaf 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 Amon 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. swietenoides maybe interact with the surface of the metal and enhance the formation of 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 20 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 correlate 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.

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 (2.27±0.15 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.

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 1.34±0.004, 0.20±0.007 and 0.16±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 calculated as 6.92±0.225, 3.58±0.040 and 2.92±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 38.40±0.361, 10.29±0.106 and 8.92±0.078 units/min/g, respectively 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 agreement with the protease activity observed in the study. The water inhibition of pigeon pea seeds 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 6.92±0.225, 3.58±0.040 and 2.92±0.065 units/min/g respectively 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 38.40±0.361, 10.29±0.106 and 8.92±0.078 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 38.40±0.361, 10.29±0.106 and 8.92±0.078 units/min/g, respectively for ZnO NPs treated, Zn acetate treated and untreated pigeon pea 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/g respectively for untreated, ZnO NPs treated, Zn acetate 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. swietenoides 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. swietenoides 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 synthesised 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 pattering 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 91.35±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 catalase 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. swietenoides. 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 60 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 pigeon pea. The photosynthetic pigments, enzymatic activities such as amylase, protease, catalase etc were found to be high active in the pigeon pea plant.

FUNDING

Nil

AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

Declared none

REFERENCES

1. Shukla AK, Behera SK. All India research project on micro-and secondary nutrients and pollutant elements in soils and plants: research achievements and future thrusts. Indian J Fertil. 2019;15:522-43.
2. Prasad TNK, Sudhakar P, Sreenivasulu Y, Latha P, Munuswamy V, Reddy KR, Sreeprasad TS, Sajanlal PR, Pradeep T. Effect of nanoscale zinc oxide particles on the germination, growth and yield of peanut. J Plant Nutr. 2012;35(6):905-27. doi:10.1080/01904167.2012.663443.
3. BK, MB, SMH. Micro-nutritional studies in pigeonpea. Pak J Biol Sci. 1999;2(2):399-401. doi:10.3923/pjbs.1999.399.401.
4. Gupta M. Inorganic nanoparticles: an alternative therapy to combat drug-resistant infections. Int J Pharm Pharm Sci. 2021;13:20-31. doi: 10.22159/ijpps.2021v13i18.42643.

5. Chippa S, Suvarna V. Nanotechnology for detection of diseases caused by viruses-current overview. Int J Pharm Pharm Sci. 2021;13:1-7. doi: 10.22159/ijpps.2021v13i4.40359.

6. Panigrahi S, Kundu S, Ghosh S, Nath S, Pal T. General method of synthesis for metal nanoparticles. J Nanopart Res. 2004;6(4):411-4. doi: 10.1007/s11051-004-6575-2.

7. Montalvo D, Degryse F, Da Silva RC, Baird R, McLaughlin MJ. Agronomic effectiveness of zinc sources as micronutrient fertilizer. In: Sparks DL, editor Advances in agronomy. Vol. 139. San Diego: Elsevier; 2016. p. 215-67.

8. Cakmak I. Enrichment of cereal grains with zinc: agronomic or genetic biofortification? Plant Soil. 2008;302(1-2):1-17. doi: 10.1007/s11104-007-9466-3.

9. Das S, Chaki AK, Hossain A. Breeding and agronomic approaches for the biofortification of zinc in wheat (Triticum aestivum L.) to combat zinc deficiency in millions of a population: A Bangladesh perspective. Acta Agrobot. 2019;72(2):1770.

10. Balogun SW, James OO, Sanusi YK, Olayinka OH. Green synthesis and characterization of zinc oxide nanoparticles using bashful (Mimosa pudica) leaf extract: a precursor for organic electronics applications. SN Appl Sci. 2020;2(3):504. doi: 10.1007/s42452-020-2127-3.

11. Liu S, Yang C, Xie W, Xia C, Fan P. The effects of cadmium on germination and seedling growth of Suaeda salsa. Procedia Environ Sci. 2012;16:293-8. doi: 10.1016/j.proenv.2012.10.041.

12. Pratibha A, Guddadadarangavanahally KJ, Kevin MC, John LJ, Bhimanagouda SP. Nanoparticle mediated seed priming improves germination, growth, yield, and quality of watermelons (Citrullus lanatus) at multi-locations in texas. Sci Rep. 2020. p. 5037.

13. Tariq SR, Ashraf A. Comparative evaluation of phytoremediation of metal contaminated soil of firing range by four different plant species. Arab J Chem. 2016;9(6):806-14. doi: 10.1016/j.arabjc.2013.09.024.

14. Bernfeld O. Amylases. a and β amylase-in. Methods in enzymology Colowick SO, Kalpan NO, editors. Vol. 1(P). 149. NewYork: Academic Press; 1955.

15. Reimerdes EH, Meyer HK. Proteolytic activity assay on casein. Methods Enzymol. 1976;XIV:27.

16. Hugo A. Catalase in vitro. Methods Enzymol. 1984;105:122-6.

17. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the folin phenol reagent. J Biol Chem. 1951;193(1):265-75. doi: 10.1016/S0021-9258(19)52451-6, PMID 14907713.

18. Pratibha A, Guddadadarangavvanahally KJ, Kevin MC, John LJ, Bhimanagouda SP. Nanoparticle mediated seed priming improves germination, growth, yield, and quality of watermelons (Citrullus lanatus) at multi-locations in texas. Sci Rep. 2020. p. 5037.

19. Kahsay MH. Synthesis and characterization of ZnO nanoparticles using aqueous extract of Becium grandiflorum for antimicrobial activity and adsorption of methylene blue. Appl Water Sci. 2021;11(2):45. doi: 10.1007/s13201-021-01373-w.