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Green synthesis of copper oxide and zinc oxide nanoparticles from the leaf extract of *Nerium oleander* (L.) and *Lantana camara* (L.) and their effect on seed germination, seed vigour index and seedling growth of pigeon pea.

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ABSTRACT

We report the use of leaves extract of *Nerium oleander* (L.) and *Lantana camara* (L.) for green synthesis of CuO NPs and ZnO NPs. Phytochemicals present in plant extract the act as stabilizing and capping agents in the fabrication of CuO NPs and ZnO NPs respectively. Biosynthesized NPs were characterized by the UV-Spectroscopy, TEM, SEM with EDX and FTIR. Result revealed from the FTIR and SEM with EDX represented that CuO NPs and ZnO NPs were successfully prepared from leaves extract of *N oleander* (L.) and *L camara* (L.). UV-Visible spectroscopy peaks confirmed the formation of NPs and TEM determined the size (10-50nm) of CuO and ZnO NPs. The present study explored the impact of biosynthesized CuO NPs and ZnO NPs on the percentage (%) of seed germination, seed vigour index and seedling growth of pigeon pea. CuO NPs and ZnO NPs exhibited positive impacts in seed germination, seed vigour index and seedling growth but in dose dependent manner. CuO NPs and ZnO NPs at 20@ppm and 25@ppm significantly enhanced the seed germination, seed vigour index and seedling growth of pigeon pea. Biosynthesized NPs not always exhibit toxic effect in seed germination instead it also enhanced the seed germination.

KEYWORDS: Nanotechnology**,** Biosynthesis**,** Copper oxide nanoparticles, Zinc oxide nanoparticles, Germination

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INTRODUCTION

Cu is an important element that has a significant role in various biochemical and physiological processes of the plant like carbon and nitrogen metabolism, photosynthesis, respiration, and protection from oxidative stress[1].CuO NPs have emerged as promising agents in the crop health amelioration, garnering significant attention in plant growth and yield improvement[2]. The unique properties of CuO NPs, such as their small size, and broad surface area, contribute to their enhanced reactivity and potential applications in the maximization of plant growth and yield characters[3]. Furthermore, CuO NPs have been explored for their role in nutrient management. Studies suggest that NPs can serve as efficient carries for essential nutrients, facilitating their targeted delivery to the plants. This targeted nutrient delivery system holds promise for optimizing nutrient uptake by crops, leading to improved growth, development and ultimately enhanced yield[4]. It is important to note that while the potential benefits of CuO NPs in crop health improvement are evident, careful consideration must be given to their applications dosages and environmental implications[5]. Striking a balance between reaping the benefits of enhanced crop performances and ensuring minimal environmental impact is crucial in the responsible utilization of CuONPs in crop health enhancement[6].

Similarly, ZnO NPs have also emerged as a notable player in the domain of crop health improvement [7]. The primary advantages of ZnO NPs lies in their multifunctional role as both a nutrient source and disease management tool. ZnO NPs serving as a nanofertilizer, may enhanced the availability and uptake of zinc, an essential micronutrient by the plants. This in turn promotes various physiological processes crucial for plant growth like photosynthesis and enzymatic activities[8]. The application of ZnO NPs in crop growth enhancement required nuance approach, considering approach factors such as dosages, environmental impact and long run sustainability [9].

Plant pathogens like bacteria, fungi, and nematodes are important limiting factors in the reduction of crop production and productivity. Application of NPs have considerable role, in the management of disease caused by viruses, bacteria, fungi and nematodes. NPs with smaller size have large size/surface ratio can bind more efficiently with microbial cell surface and penetrate easily across the surface[10]. NPs such as Gold (Au), silver (Ag), cerium (Ce), copper (Cu), iron (Fe), magnesium (Mg), nickel (Ni), titanium (Ti), Zinc (Zn) are used in reducing of pathogen population[11]. NPs of metals in nature have considerable role the in the crop health amelioration and yield enhancement[12]. CuO and

ZnO NPs exhibit antimicrobial against wide spectrum of microbial pathogens[13]. CuO NPs are potentially used in the improving plant growth and yield characters, and have shown promising results[14].

Zn is required for the different metabolic reactions and boosts the synthesis of photosynthetic systems, carotenoids, and chlorophyll[15]. Cu is an important cofactor in super dioxide and phenol oxidase and is involved in redox reactions in plants, and appreciable role in the respiratory and photosynthesis chain[16]. ZnO NPs application enhanced plant growth and yield characters of *Cucumissativus* [17], and*Penicum typhoids*[18].

NPs are synthesized by physical, chemical and biological means. Physical methods have different approaches like, mechanical milling, gamma radiation, pulsed laser, and plasma[19]. Chemical methods comprises of chemical vapour[20], chemical bath deposition [21], hydrothermal approach [22], sol-gel method [23], sonochemical route[24] precipitation[25]; and electrochemical methods[26].

The objective of this section is to systemically biosynthesize and characterizes CuO and ZnO NPs employing leaf extract obtained from *Nerium oleander* (L.) and *Lantana camara* (L.). The primary focus is to evaluate the impact of these NPs as critical aspect of plant growth, including seed germination, seed vigour index and seedling growth. Through a comprehensive analysis, this section aims to contribute.

MATERIALS AND METHODS

Copper sulfate ($CuSO_4$) and Zinc sulfate ($ZnSO_4$) were purchased from Thermo Fisher Scientific Pvt. Ltd. India. Leaves of *N. oleander* (L.) and *L. camara* (L.) were collected from the department of Botany, Aligarh Muslim University, Aligarh, U.P., and India. Synthesized NPs were characterized and analyzed with different techniques like UV-VIS Spectroscopy, FTIR, (Fourier Transform Infrared Spectroscopy), TEM(Transmission Electron Microscope), and SEM (Scanning Electron Microscope) with EDX (Energy Dispersive X-ray) to gain the correct picture of newly synthesized NPs. Seeds used in this experiments GS-1 variety of pigeon pea was purchased from the Aligarh's local seed market.

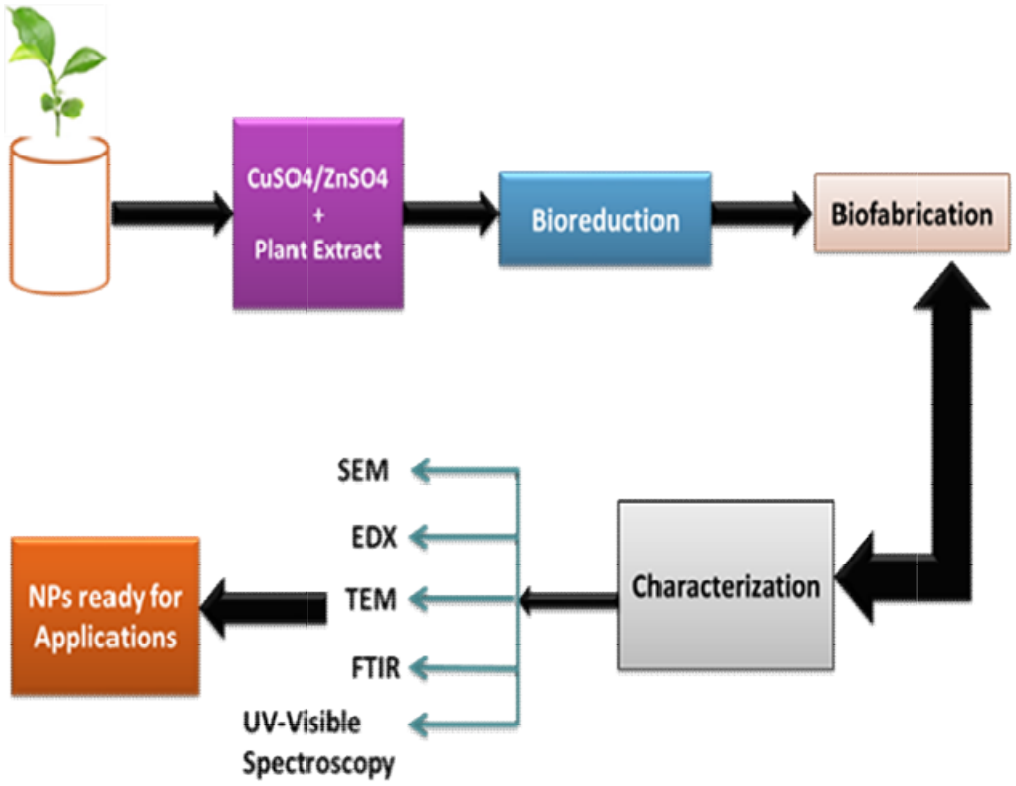


Figure (1) Biosynthesis of NPs from the plant extract.

PREPARATION OF LEAF EXTARCT

Fresh leaves of *N.oleander* (L.) and *L.camara* (L.) were collected from the department and cleaned by washing with running tap water followed by washing with double distilled water. The leaves were air-dried at room temperature until total moisture was lost. 10g leaves were boiled in 100ml water for 20 minutes and then the aqueous solution was prepared and allowed the aliquote to cooldown at room temperature (23-24°C) and then filtered with Whatman No. 1 filter paper, stored at 4°C, and used whenever needed.

BIOSYNTHESIS OF CuO AND ZnO NPs

2mM aqueous solution of $CuOSO_4$and 4mM aqueous solution of $ZnSO_4$ and aqueous leaves extract of *N. oleander* (L.) and *L.camara* (L.) were used for the biosynthesis of ZnO NPs and CuO NPs, respectively. 20ml leaf extract of *N. oleader*(L.) was added to 80 ml of 2mM$CuSO_4$ solution then the solution was incubated at room temperature. After mixing leaf extract and CuSO4 solution, this was left overnight in a shaker incubator at room temperature. After that the solution was boiled 60°C for 25

minutes. The boiled solution was allowed to cool down at room temperature. This solution was centrifuged at 2000 rpm for 20 minutes.Similarly to synthesize ZnO NPs 20ml leaf extract of *L. camara* (L.) was added to 4mM of ZnSO4 solution incubated at room temperature. After that the solution was boiled 60°C for 25 minutes. The boiled solution was allowed to cool down at room temperature. This solution was centrifuged at 2000 rpm for 20 minutes. ZnO NPs were collected at bottom of the centrifuge tube excess debris was removed and remain precipitation were dried in the oven for 5 days at 750.C temperature removing the extra moisture to get ZnO NPs.CuO NPs are found to be essential micronutrient act as plant growth promoting agent [27]. There are many metal oxide NPs TiO2 are present but copper oxide are chosen due to their significant properties antibacterial, biomedical, electrical, biomedical, catalytic, and optical implementations[28].

ZnO NPs have great role in plant growth, development, seed germination also involved in stress resistance, cell growth, synthesis of protein, and auxin[29]. CuO and ZnO NPs increased the antioxidant activities, photosynthesis and plant growth parameters in *Amaranthushybridus*[30].

CHARACTRIZATION AND ANALYSIS OF BIOSYNTHESIZED CuO AND ZnO NPs

Different techniques are utilized for the analysis, identification, determination, and characterization of NPs. Following techniques have been used in the synthesis of NPs. UV- Visible spectroscopy gives the specific wavelength which is absorbed the solution that influenced the morphology of NPs and these techniques determined the structure, size, aggregation, and stability of NPs. Morphological structure of NPs determined by scanning electron microscope (SEM). Transmission electron microscope (TEM) identified the size and crystallographic nature of NPs. Fourier transform infrared spectroscopy determines the functional group and optical characterization of NPs and Energy-dispersive X-ray determined elemental analysis and characterization of chemical composition and purity of compounds.

UV-VISIBLE SPECTROSCOPY

UV-Vis spectrophotometer (Genesys 150 UV–Vis, Thermo Fisher Scientific, and USA) was used to obtained UV-Visible spectrum of NPs. UV-Vis scanning range is 200-800nm. A quartz cuvette filled with 1ml aqueous suspension of respective NPs was analyzed while using deionized water from

Milli-Q as the blank solution for measurement. The UV-Vis spectrum of NPs is highly dependent on the particle size and shape which determined by fabrication [31].

TRANSMISSION ELECTRON MICROSCOPE (TEM)

TEM (JEOL model JEM-1010, Tokyo, Japan) was adopted to obtain the shape and size of synthesized NPs. TEM gives the information about the dispersion, aggregation, shape, and size of NPs in two dimensional images. TEM sample prepared by dispersing the sample with aid of ultrasound, in an ethanol suspension and depositing droplet of suspension on Cu grid coated with a holey amorphous carbon film. TEM employs an electrochemical lens that focuses a very fine beam of electrons to into an ultrathin section of sample. This beam passes through the specimen where the electrons either scatter or penetrate the sample and hit a florescence screen at bottom of microscope TEM is simple and convenience method for the determination of size of NPs[32].

SCANNING ELECTRON MICROSCOPE (SEM)

SEM (Hitachi SU 8230, Tokyo, Japan)is widely used to determine the microstructure and chemistry of a range of materials. Surface morphology determined by SEM analysis and is type of electron microscope that creates images of NPs by scanning the surface of particles with focused beams of electrons [33]. Protocol of SEM consists of two steps (1) spreading of particles stable suspension on the surface at low rotation speed and (2) a rapid drying at high speed. The surface density of NPs can be controlled by parameters of first step.

ENERGY DISPERSIVE X-RAY ANALYSIS (EDX)

EDX (Shimadzu DX-700HS) is used along with the SEM to analyze type and quantity of elements at NPs surface or in the vicinity of surface to provide specimen map. During EDX analysis, electron beam is transported across the sample to form the image in the samples. 1mm of specimen are generally fixed 4 paraformaldehyde and post fixed in 2% osmium tetroxide and after washing with 0.1M phosphate buffer, the sample dehydrated by a series of incubation in 30%, 50% and 70% ethanol. Before EDX analysis specimens undergo complete dehydration for maintaining elements at their physiological state so that analysis gives true meaningful results[34].

FOURIER TRANSFORM SPECTROSCOPY (FTIR)

FTIR (Termo Scientific Nicolet 6700 FT-IR spectrometer) spectroscopy is non-destructive and useful technique that does not require difficult sample preparation and using aggressive organic solvent. The benefits of this analytical technique is that it can be used for sample analysis in different such as gases, films, powder, pastes, solids and liquid. FTIR spectral measurements were carried out to evaluate efficient phytochemicals in plant extract which is responsible for capping and reducing agents. Place a small drop of compound on the KBr plates. Place the second plate on the top and make a quarter turn to obtain a nice even film. Place the plate into sample holder and run a spectrum. The KBr plates must be thoroughly cleaned after this procedure to prevent contamination of future samples. Rotation and vibration of molecules influenced by the infrared radiation at a particular wavelength is measured using FTIR. Basically FTIR is an important technique that provides a simple method to identify the presence of certain functional groups in organic molecule. These vibration frequencies fall with the infrared frequency range. As such, passing an IR signal through the organic compound causes the functional group to vibrate at specific frequency. The beam from an IR source passes through a monochromatic controlled with selector, ensuring that only specified that only specified wavelength were emitted, which may vary from 4000 to 400cm^{-1}. The sample is placed in a holder in the path of IR source. A detector read the analog signal and converts the signal to a spectrum. A computer is used to analyze the signals and identify peaks[35].

PREPARATION OF SEED AND GERMINATION PARAMETRES

Pigeon pea[GS-1 variety] seeds were purchased from the Aligarh's local seed market which contained 85% germination, physical purity 98%, and genetic purity 98%. Pigeon pea seeds was selected for this particular experiment because it is easily available, has capacity to tolerate environment conditions, and easy to handle. Seeds were washed with 70% ethanol for 2 minutes and washed with running tap water three times then seeds were surface sterilized with 1.5% sodium hypochlorite solution for 5 minutes then rinsing 5 times of the seed with double distilled water.

To evaluate the effectiveness of effect NPs, seeds were treated with different concentration of CuO NPs such as 10(T1), 20(T2), and 30ppm (T3) likewise, various concentration of ZnO NPs such as 10(T1), 25(T2) and 50ppm (T3) were taken into consideration. In the first of experiment 25 seeds were surface sterilized with 1.5 % sodium hypochlorite solution seeds placed in the each petriplate 10ml of NPs solution of solution of specific concentration were allowed to germinate *in–vitro* in dark state at

25°C for six days[36].The Number of germinated seeds treated with CuO and ZnO NPs was calculated after 6 days. After 6 days germinated seedling was checked for shoot length, root length and fresh weight.

$$\text{Germination percentage (\%)} = \frac{\text{Total number of germinated seeds}}{\text{Total number of seeds}} \times 100$$

For determination of effect CuO and ZnO NPs on root and shoot length, seedling was transferred from petriplate to hydrophobic Hogland nutrient media with various concentrations of CuO and ZnO NPs at 25°C temperature for one week. Plumule length (from lowest point of hypocotyle to point of growth of the cotyledon) plantlets and rootlets (from the bottom of hypocotyle to the tip of root) were measured. The vigor index was calculated by using following formula[37].

$$\text{Seed vigour index} = [\text{Average root length (mm)} + \text{Average shoot length (mm)}] \times \text{germination percentage}$$

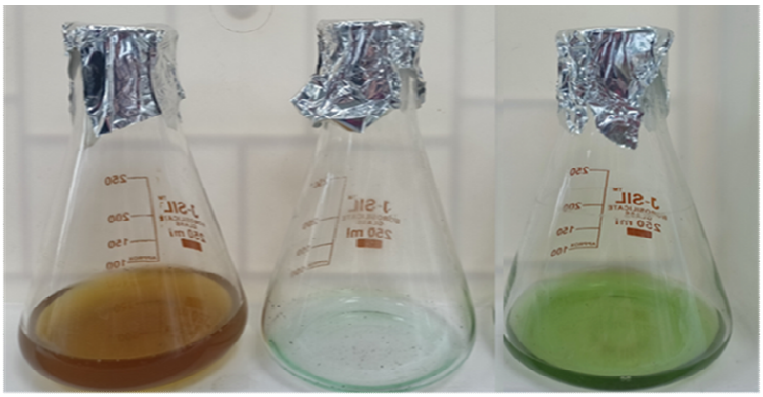
STATISTICAL ANALYSIS

The obtained value was expressed as mean value ± standard deviation. The data were statistically analyzed by the one-way analysis of variance (ANOVA) software and Multiple-range Duncan test (DMRT) the P≤0.05% level difference was calculated as a significant difference.

RSESULTS

UV-Visible Analysis of Biosynthesized Nps

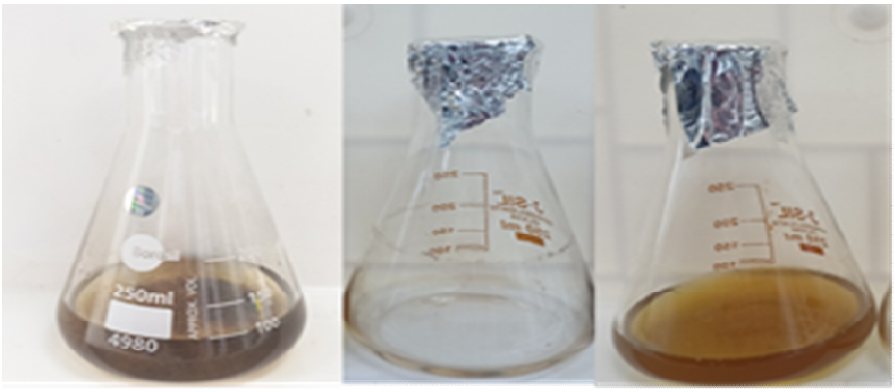
The biosynthesis of CuO NPs and through the leaf extract, changing in coloration from bluish to dark green is an indication of the formation of CuO NPs(Figure 2).



A B C

Figure (2) Visual observation of biosynthesis CuO NPs (A) Leaves extract of *N. oleander* (L.) (B) CuSO4 salt solution (C) Biosynthesized CuO NPs

Likewise biosynthesis of ZnO NPs by leaf extract mixed with $ZnSO_4$ salt solution it turned into dark brown color (Figure 3)



A B C

Figure(3) Visual observation of biosynthesis ZnO NPs (A) Leaves extract of *L. camara* (L.) (B) ZnSO4 salt solution (C) Biosynthesized ZnO NPs

CuO NPs showing optical absorption peak at wavelength at 280nm (Figure 4A)

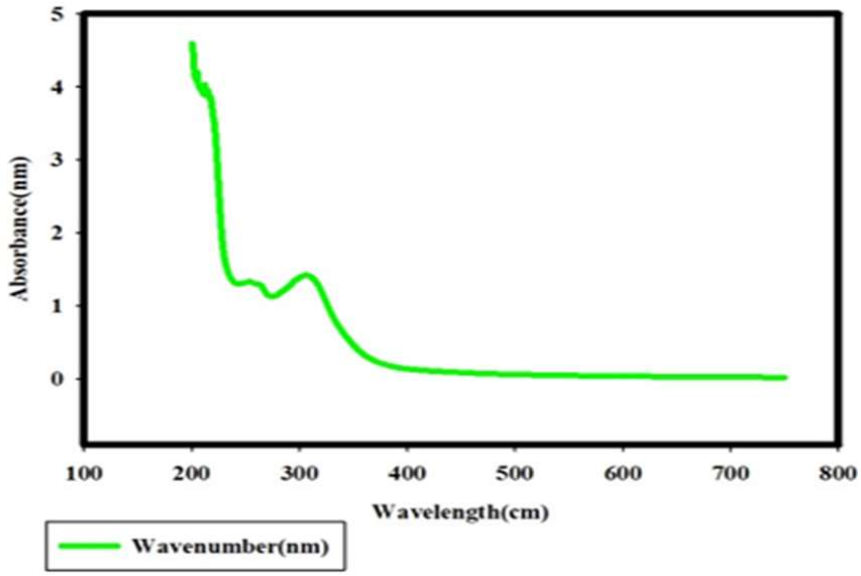


Figure (4A) UV-Vis Spectra of biosynthesized CuO NPs

Dark color of mixture implying the reduction Zn^{+} to ZnO NPs. Biosynthesis of ZnO NPs from the leaf extract of *L. camara* (L.) was characterized by UV-VIS Spectroscopy. Optical absorption of NPs is analyzed with UV-VIS spectroscopy which is widely used in the determination of structural organization of NPs. The biosynthesis of ZnO NPs was ranged of 300-400nm and the sample exhibited a peak at this wavelength which is a good indication of the synthesis of ZnO NPs (Figure 4B).

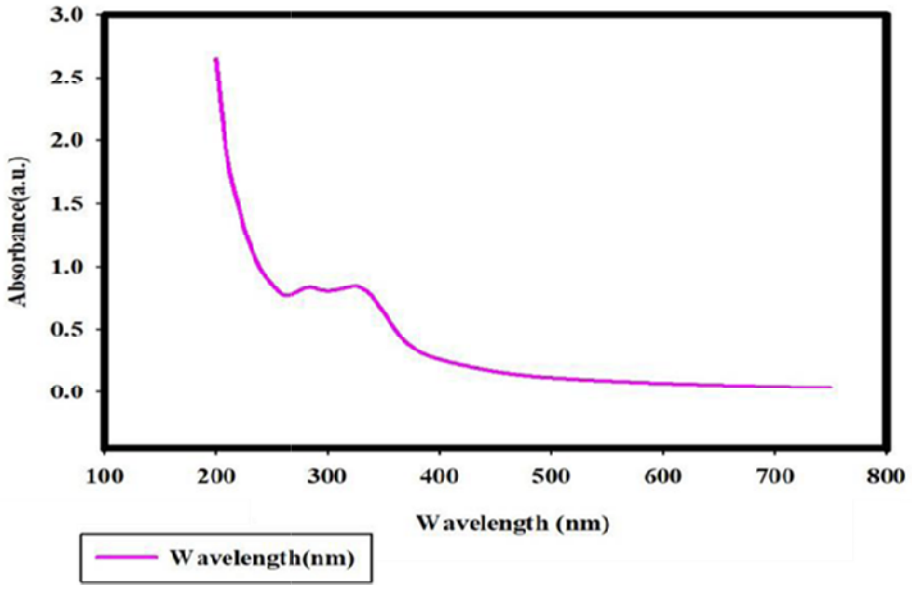


Figure (4B) UV-Vis Spectra of biosynthesized ZnO NPs

FTIR ANALYSIS OF NPs

FTIR was used for the identification of the interaction between CuO NPs and plant extract because there are many phytochemicals present in the extract which act as reducing and capping agents. FTIR is used for the determination of functional groups which remain bound with the CuO NPs surface (Figure 5A).

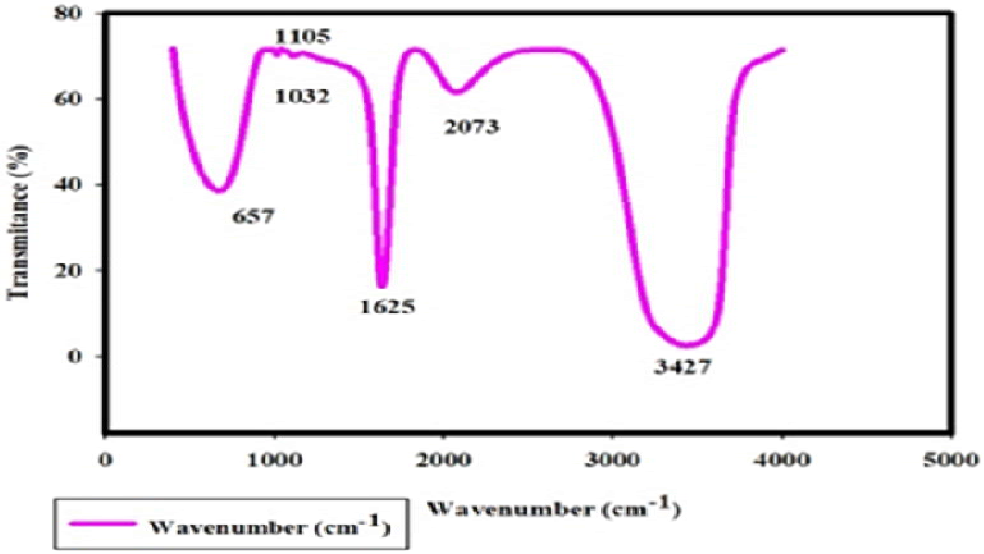


Figure (5A) FTIR Spectra of biosynthesized CuO NPs

Spectra of FTIR biosynthesized CuO NPs by the leaf extract of *N. oleander* (L.) were exhibited in Figure (5A). Broad peak at 3427cm-1 determined the hydrogen bond –OH group of alcohol, phenol, and amine of –N-H- amide. The band at 2013 cm-1 is assigned to C-H and –CH2 alkanes group. Band at 1643 cm-1 identified the amide and carbonyl group in amide I and II. The band 657 cm-1 is determined the aromatic group. FTIR used in the characterization and analysis of functional group. It is also gives the information about the interaction between metal atoms and phytochemicals, and phytochemicals act as a stabilizing and reducing agents. FTIR is type of spectroscopy which can detect changes in the total composition of phytochemicals by the characterizing change in functional group. The vibration and rotation of biomolecules influenced by the infrared radiation at particular wavelength in measured using FTIR. The synthesized ZnO NPs from leaf extract that has many phytochemicals and secondary metabolites identified by the peak of FTIR (Figure 4B). FT-IR radiation from 3417-3906cm^{-1} exhibited the –OH functional group, and by the stretching of C=C, C-H, C=C-C aromatic compound confirmed by

peak 1636cm-1. Carboxylic group –C=O- found at the peak 1136-1636cm^{-1}. Peak at 2042cm-1 was showed –C-C=C and C=C group (Figure5B).

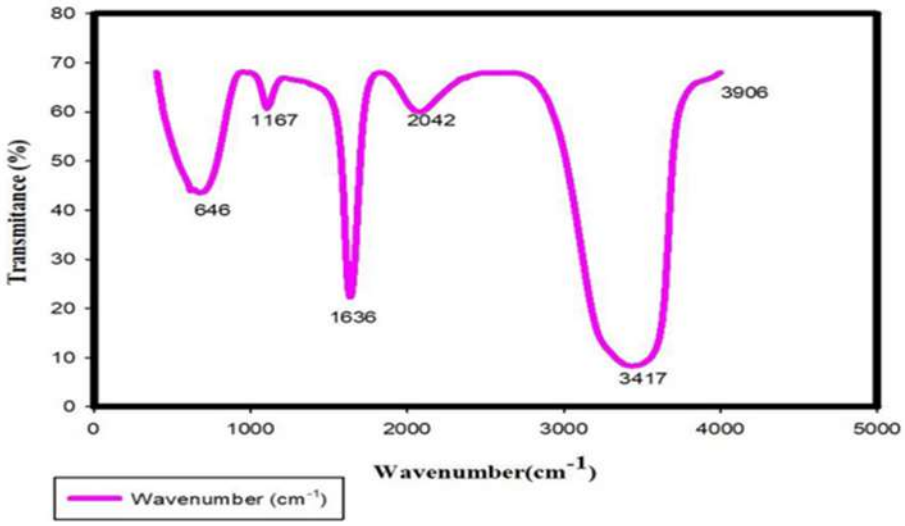


Figure (5B) FTIR Spectra of biosynthesized ZnO NPs

TEM AND SEM WITH EDX OF NPs

TEM is mainly employed for the identification of the size of nanoparticles.Size of NPs is determined by the TEM. The biosynthesized CuO NPs exhibited 10-100nm in size (Figure 6A).

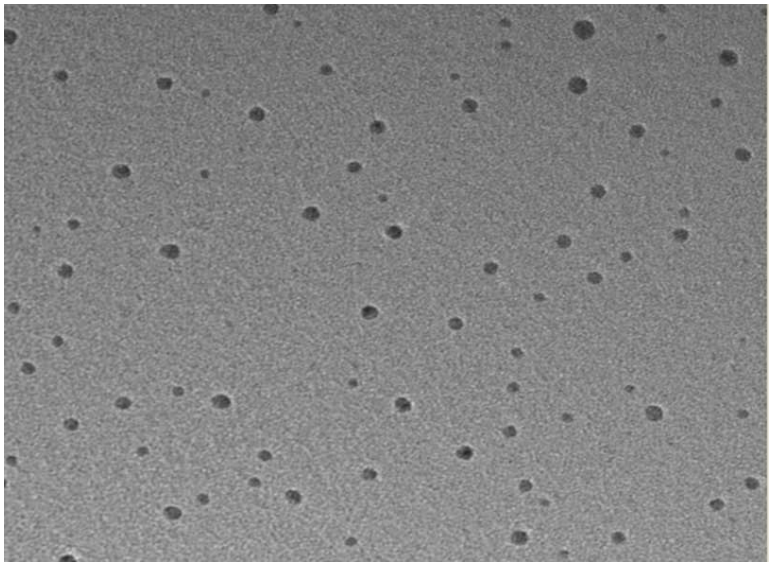


Figure (6A) TEM image biosynthesized synthesized of CuO NPs

The size of biosynthesized ZnO NPs reflected from 11-12nm (Figure 6B).

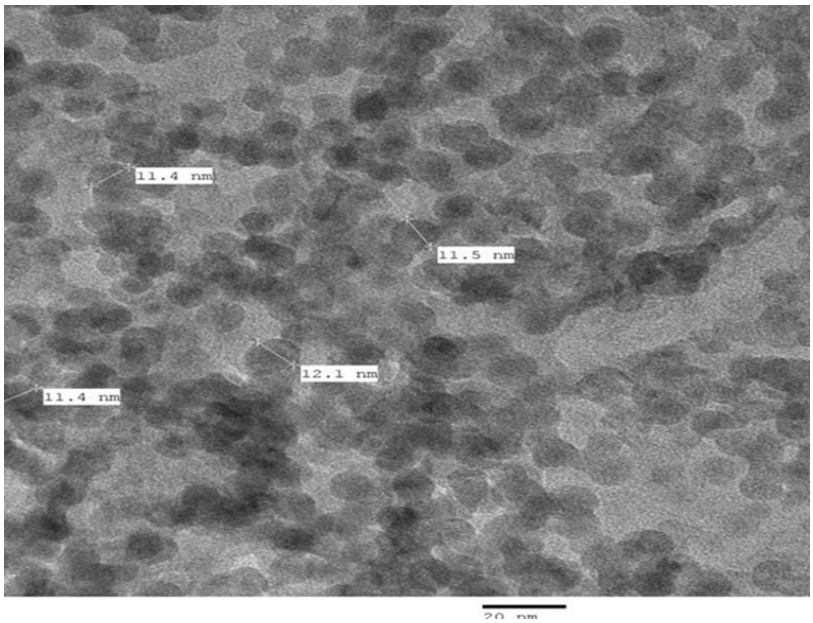


Figure (6B) TEM image biosynthesized synthesized of ZnO NPs

The CuO NPs showed the stable surface morphology that confirms the formation of CuO NPs. SEM has been utilized to examine the surface morphology and in the determination of structural, rectangle, radial, hexagonal, spherical, and rod shape (Figure7A).

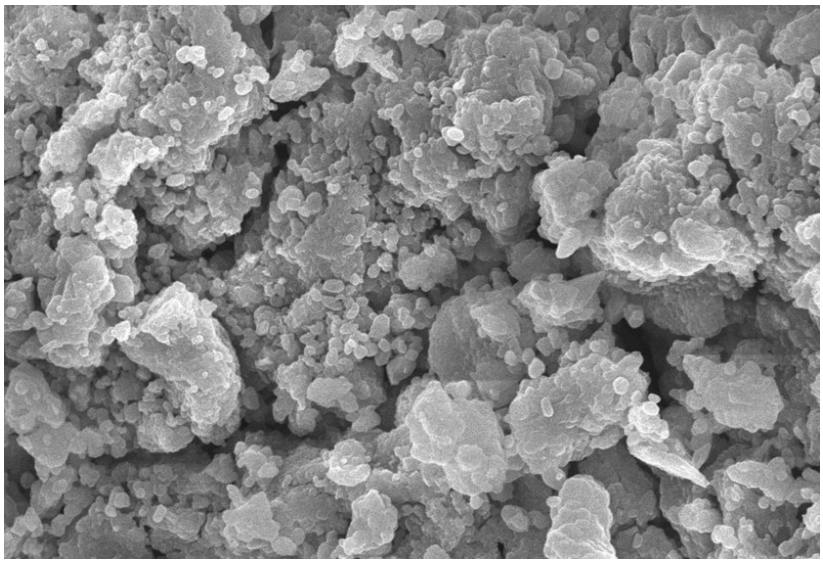
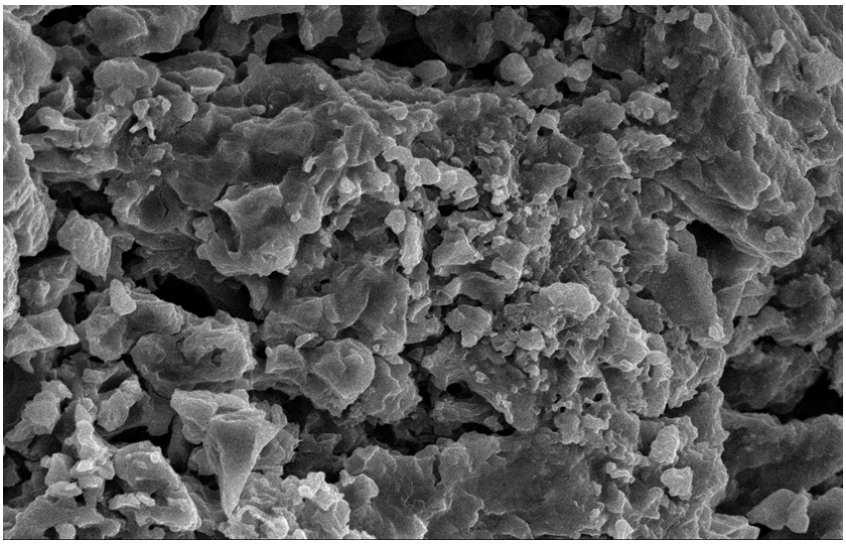


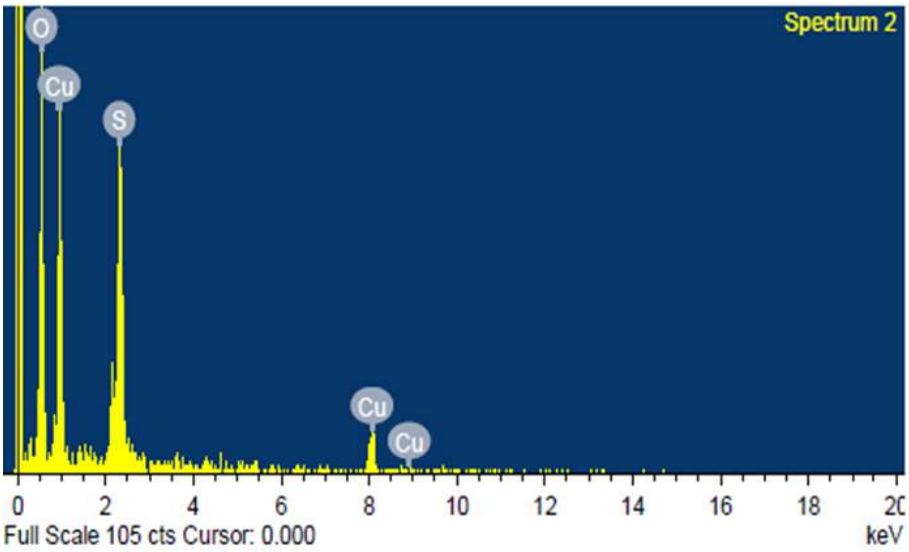
Figure (7A) SEM image of biosynthesized CuO NPs

ZnO NPs synthesized from the leaf extract of *L.camara* (L.) characterized from the SEM showedhexagonal, crystalline, spherical and rod shape (Figure 7B).

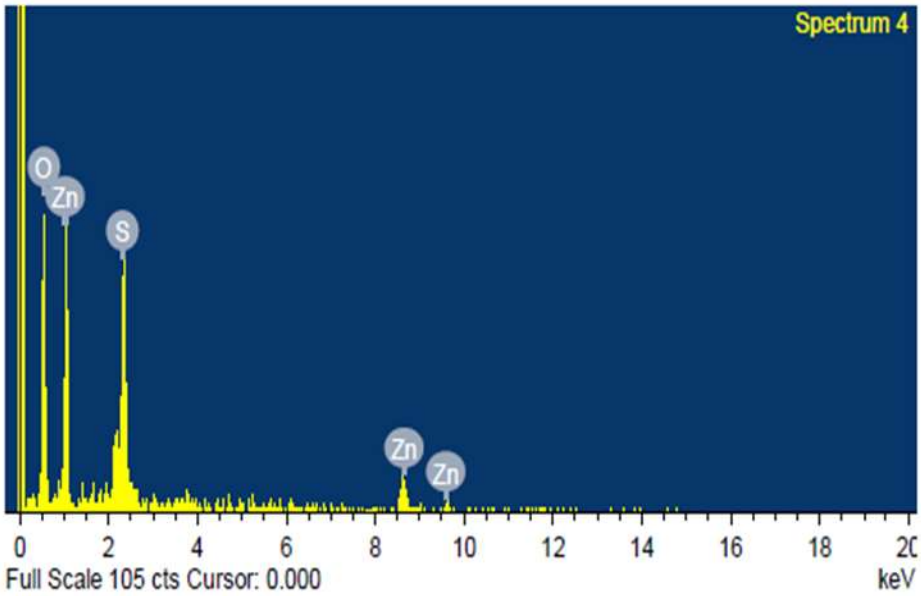


Figure(7B) SEM image biosynthesized of ZnO NPs

EDX used along with the SEM, which determined elemental analysis and composition of NPs. EDX exhibited rough homogeneities and rough agglomeration and agglomeration property increases surface catalytic activity. Results revealed that cooper (Cu) and O (oxygen) elements in the CuO NPs and spectra presented (Figure. 8) and zinc (Zn) and O (oxygen) elements in ZnO NPs and spectra presented (Figure.9).



Figure(8) EDX Spectra of biosynthesized CuO NPs

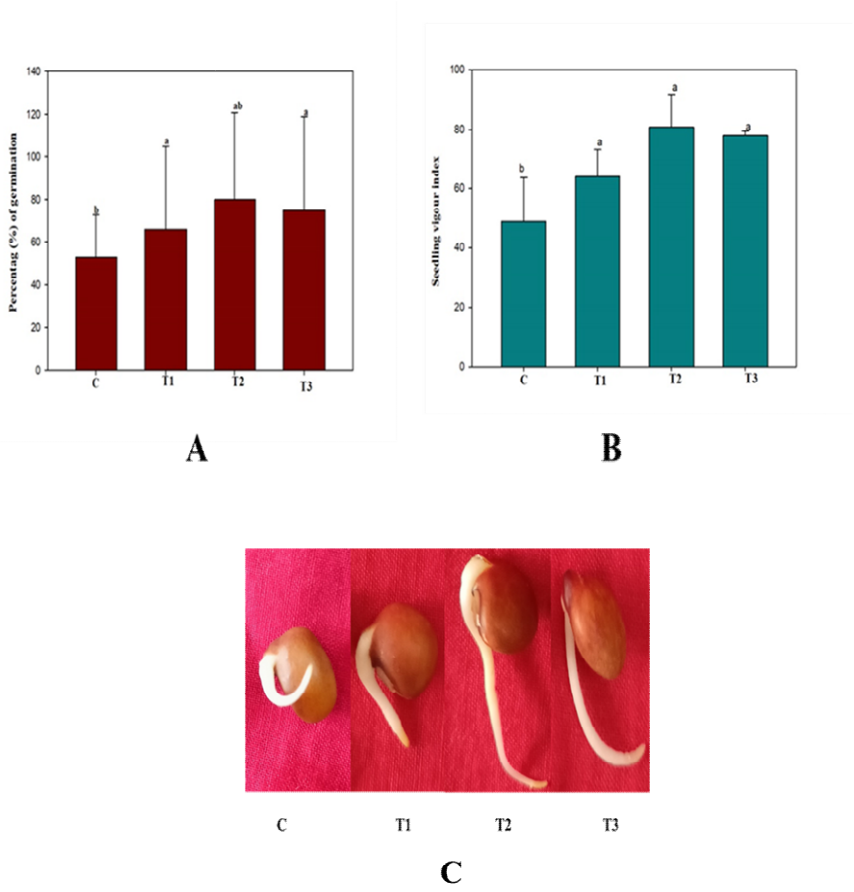


Figure(9) EDX spectra biosynthesized of ZnO NPs

EFFECT OF CuO and ZnO NPs ON SEED GERMINATION TEST

Effect of CuO and ZnO NPs were observed on percentage of seed germination, seed vigour index and seedling growth. Percentage of seed germination, seed vigour index and seedling growth increased when increased the concentrations of CuO and ZnO NPs as compared to control (untreated seeds).

The CuO NPs treated pigeon pea seeds exhibited considerable enhancement in percentage of seedling, seedling vigour index and seedling as compared to control. The seed quality measured in percentage of seed germination, seed vigour index and seedling at 10 (T1), 20 (T2) and 30ppm (T3) (Figure 10ABC). Percentage of seed germination, seed vigour index and seedling length were more increased at 20 (T2) and 30ppm (T3) (Figure 10ABC). But significant growth was observed at 20ppm (T2) as compared to control (Figure 10ABC).



Figure(10) Effect of CuO NPs on seed germination and seedling vigour index and Seedling Grwoth Fig. (A) Germination(%), C=Control (untreated);T1=10ppm; T2=20@ppm; T3=30@ppm; Fig. (B) Seedling vigour index C=Control (untreated); T1=10@ppm; T2=20@ppm; T3=30@ppm; Fig. (C) Seedling growth C=Control (untreated);T1= 10@ppm; T2=20@ppm; T3=30@ppm

Pigeon pea seeds were treated with different concentrations of ZnO NPs 10 (T1), 25 (T2) and 50ppm (T3). Results revealed that ZnO NPs was increased the percentage of seed germination, seedling vigour index, and seedling growth at 25(T2) and 50ppm (T3) (Figure 11ABC). But significant seed germination parameters were found increased at 25ppm (T2) (Figure 11ABC) as compared to control.

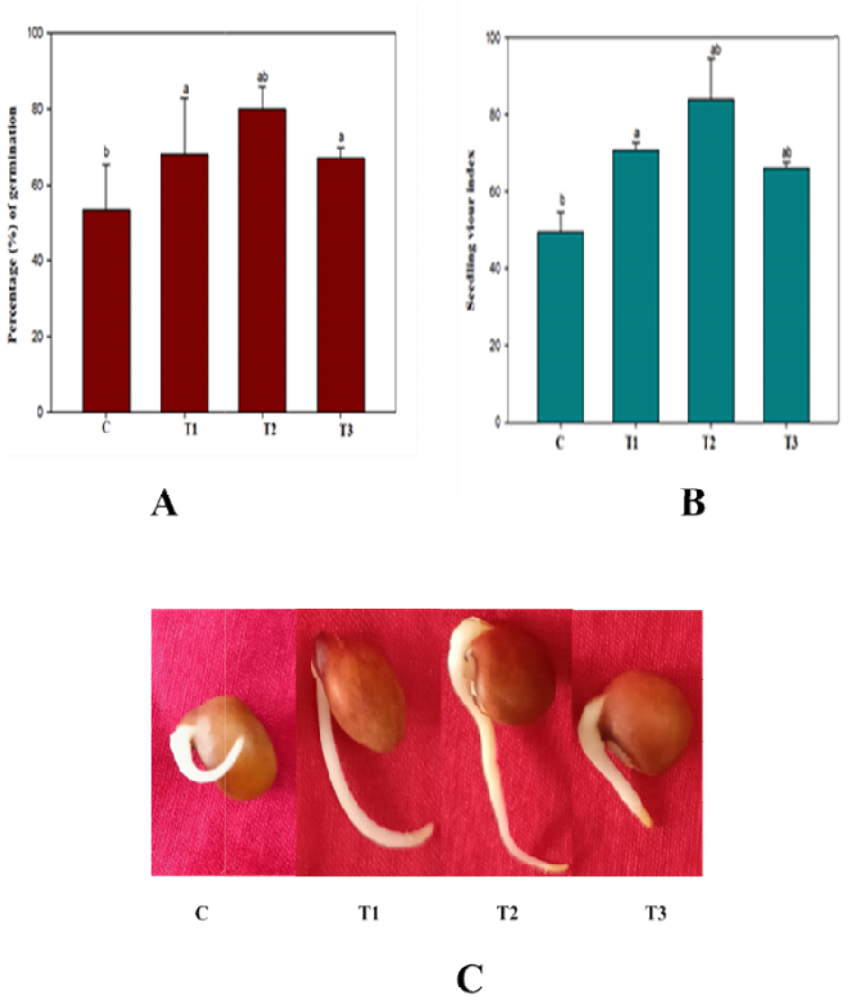


Figure (11) Effect of ZnO NPs on seed germination and seedling vigour index and Seedling Grwoth Fig. (A) Germination(%) C=Control (untreated); T1=10@ppm; T2=25@ppm; T3=50@ppm Fig. (B) Seedling vigour index C=Control (untreated); T1=10@ppm; T2=25@ppm; T3=50@ppm Fig. (C) Seedling growth C=Control (untreated); T1=10@ppm; T2=25@ppm; T3=50@ppm

The observation have shown that percentage of seed germination, seed vigour index and seedling length of pigeon pea was significantly increased at different concentrations of CuO (10, 20 and 30ppm) and ZnO NPs (10, 25 and 50ppm) respectively. CuO NPs at various concentrations such as 10, 20 and 30ppm enhanced the pigeon pea seed germination parameters (Table1). But CuO NPs significantly increased the seed germination 50%, seed vigour index 65% and seedling length 62% at 20ppm as compared to control (Table 1).

Table (1) Effect of biosynthesized CuO NPs on seed germination percentage (%), Seedling vigour index and seedling growth

Treatments	Seed germination (%)	Seedling Vigour Index	Seedling growth (cm)
C	53.33^{b} ±12.01	49.00^{b} ±14.64	1.6^{b} ±0.33
T1	66.33^{a} ±7.63	64.33^{a} ±9.02	2.3^{ab} ±0.61
T2	80.00^{ab} ±0.33	81.33^{a} ±10.80	4.3^{ab} ±1.20
T3	75.00^{a} ±7.88	78.00^{a} ±1.52	3.0^{ab} ±0.57
LSD (P≤0.05)	**1.2**	**2.3**	**1.5**

C=Control (untreated);T1=10ppm; T2=20@ppm; T3=30@ppm; Seedling vigour index C=Control (untreated); T1=10@ppm; T2=20@ppm; T3=30@ppm; Seedling growth C=Control (untreated);T1= 10@ppm; T2=20@ppm; T3=30@ppm

ZnO NPs also at 10, 25 and 50ppm significantly enhanced the pigeon pea seed germination parameters (Table 2). But ZnO NPs significantly enhanced the seed germination 50%, seed vigour index 71% and seedling length at 62% as compared to control (Table 2).

Table (2) Effect of biosynthesized ZnO NPs on seed germination percentage (%), Seedling vigour index and seedling growth

Treatments	Seed germination (%)	Seedling Vigour Index	Seedling growth (cm)
C	53.33^{b} ±12.01	49.55^{b} ±5.21	1.6^{b} ±0.33
T1	68.00^{a} ±14.81	70.66^{a} ±2.06	3.6^{ab} ±0.57
T2	80.00^{ab} ±5.77	84.00^{ab} ±10.80	4.3^{a} ±1.20
T3	67.33^{a} ±6.53	66.21^{ab} ±1.45	2.3^{ab} ±0.33
LSD (P≤0.05)	**1.6**	**2.3**	**1.9**

C=Control (untreated); T1=10@ppm; T2=25@ppm; T3=50@ppm; Seedling vigour index C=Control (untreated);T1=10@ppm; T2=25@ppm; T3=50@ppm; Seedling growth C=Control (untreated);T1=10@ppm; T2=25@ppm; T3=50@ppm

These results confirmed that CuO and ZnO NPs enhanced seed germination parameters (seed germination, seed vigour index and seedling length) as compared to control, but in concentration dependent manner.

DISCUSSION

The Biosynthesis of NPs was confirmed by the changing colour under dark conditions. The morphological and structural organization of biosynthesized CuO and ZnO NPs were analyzed by employing the UV-Visible spectroscopy, FTIR, TEM, and SEM with EDX. A strong peak of UV-visible was observed at 250-350nm, depicting the formation of CuO NPs[38] and a peak at 380nm depicted the formation of ZnO NPs[39]. Note the differential presence of peaks in the maximum absorbance CuO and ZnO NPs 200-400 nm respectively [40]. The FTIR technique was employed to know the range 400-4000cm^{-1} in order to understand the role of played by plant extract as a capping and reducing of new functional group and biomolecules attached to surface of CuO and ZnO NPs. The spectrum at 657cm-1 depicted the M-O stretching of CuO and ZnO NPs. The vigorous and broad absorption bands were 2073 cm^{-1} and 3427 cm^{-1} identified the O-H, H bond, alcohols, phenols, aldehyde, and C-H stretching[41]. Bands at 1032 cm^{-1} and 1105 cm^{-1} showed the C-O stretching, at 1625 cm^{-1}. C=C stretched depicts a wide range of alkanes in capping and also exhibited C=O, N-H, and O-H- stretching[42]. The data revealed that the functional group also participated in the formation of CuO and ZnO NPs. The size variation of CuO and ZnO NPs was confirmed by using TEMwhich ranges from 10-100nm size[43]. SEM with EDX determined the shape and purity of NPs.The synthesized CuOand ZnO NPs were found to be spherical shape as per analysis [44]. Surface morphology of CuO and ZnO NPs determined by SEM which is spherical, hexagonal and rod shaped (Figure 7A andB).

Germination is a normal phenomenon; it started with water imbibition through the seeds and end up with the emergence of rootlet[45]. In this study, the seeds are showing germination through the emergence of cotyledons or radicals identified by the germination percentage, seedling vigor index and seedling growth. According to Jayarambabu et al., (2014)[46]ZnO NPs increased the seed germination, seed vigor index at 20ppm concentration in *Vignaradiata*. Similar results have been observed in *Camelina* and *Brassica napus* so that 10ppm of ZnO NPs increased the seedling vigor index, germination percentage, and seedling growth[47]. Another is that treatment of seed with high concentration of CuO and ZnO NPs may interacted with the cell and reached at plasma membrane, cytoplasmic organelles and proteins, which caused alternation in cellular signaling, and metabolism that stimulate the cellular response created the oxidative stress in the plant[48].

Wheat seeds treated with different concentrations of CuO NPs (1, 2, 4, and 6mg/L) improved the seed germination, seedling vigour index, and seedling length[49]. According to Adhikari et al., (2012)[50]CuO NPs increased the 100% germination of chickpea and soybean. Shende et al., (2017)[51] reported that biosynthesized CuO NPs at 20ppm (T2)significantly increased seed germination of pigeon pea.My results revealed that CuO NPs improved the percentage of seed germination, seed vigour index, and seedling length at 10 (T1), 20 (T2), and 30ppm (T3) however, at 20ppm (T2), CuO NPs exhibited showed 50% more significant growthon seed germination as compared to control.

Similarly, biosynthesized ZnO NPs increased seed vigour index, and seedling growth at 500 mg/L concentration in *Eleusine coracana*[52]. According to Sowjanya et al., (2023)[53] biosynthesized ZnO NPs exhibited positive growth at 1250ppm concentration in seed germination, seed vigour index, and seedling growth of pigeon pea.ZnO exhibited a positive impact on seed germination, shoot, and root and leaf growth of wheat [54].Rajuand Rai,(2017)[55]reported that ZnO NPs significantly enhanced the seed germination, seed vigour index and seedling growth at 25ppm as compared to control in pigeon pea. My results also revealed that biosynthesized ZnO NPs at 25ppm significantly increased the seed germination (50%), seed vigour index (71%) and seedling length (62%) as compared to control. ZnONPs exhibited a positive impact on seed germination, shoot, and root and leaf growth of wheat reported by Sharma et al., (2022)[56].ZnO NPs enhanced the plant growth and seed germination at very low concentration reported by Raskar et al., (2014)[57].ZnO NPs enhanced the different enzymes like, CAT (Catalase) and SOD (Super oxide dismutase) which is very helpful in seed germination and improved the water uptake during seed germination which was very helpful in healthy seedling growth[58].

CuO and ZnO NPs at very low concentration boost SOD activity which increased the plant capability to cope harmful metal oxide radical[58]. These findings suggest that the CuO NPs and ZnO NPs showed significant improvement in the percentage of seed germination, seed vigour index, and seedling growth of pigeon pea and functioned as a nutrient for plant growth promotion.

CONCLUSION

In this work, the leaves extract of *N. oleander* (L.) and *L.camara* (L.) were utilized for the biosynthesis of CuO and ZnO NPs, and their effect was observed on seed germination (seed germination, seed vigour index, and seedling growth) of pigeon of pea, respectively. Different techniques such as UV-Visible spectroscopy, Scanning Electron Microscope (SEM) with Energy

Dispersive X-ray (EDX), Transmission Electron Microscope (TEM) Fourier Transmission Infrared spectroscopy (FTIR) were utilized to understand fully the nature of newly synthesized NPs. Moreover the effects of both NPs were also observed on seed germination (seedling growth, seed vigour and percent of seed germination). It was found that both NPs if applied as seed priming @10, 20, 25 and 50ppm, evaluated under field condition, can enhance the seed germination. Biosynthesized CuO NPs and ZnO NPs increased the seed germination and seedling growth of pigeon pea enhanced the plant growth protected plant from biotic and abiotic stress such as oxidative stress, reactive oxygen species (ROS), protected plant organells from damage, increased the protein synthesisand the photosynthetic efficiency (Figure12). CuO NPs and ZnO NPs could be utilized as Nano fertilizers.

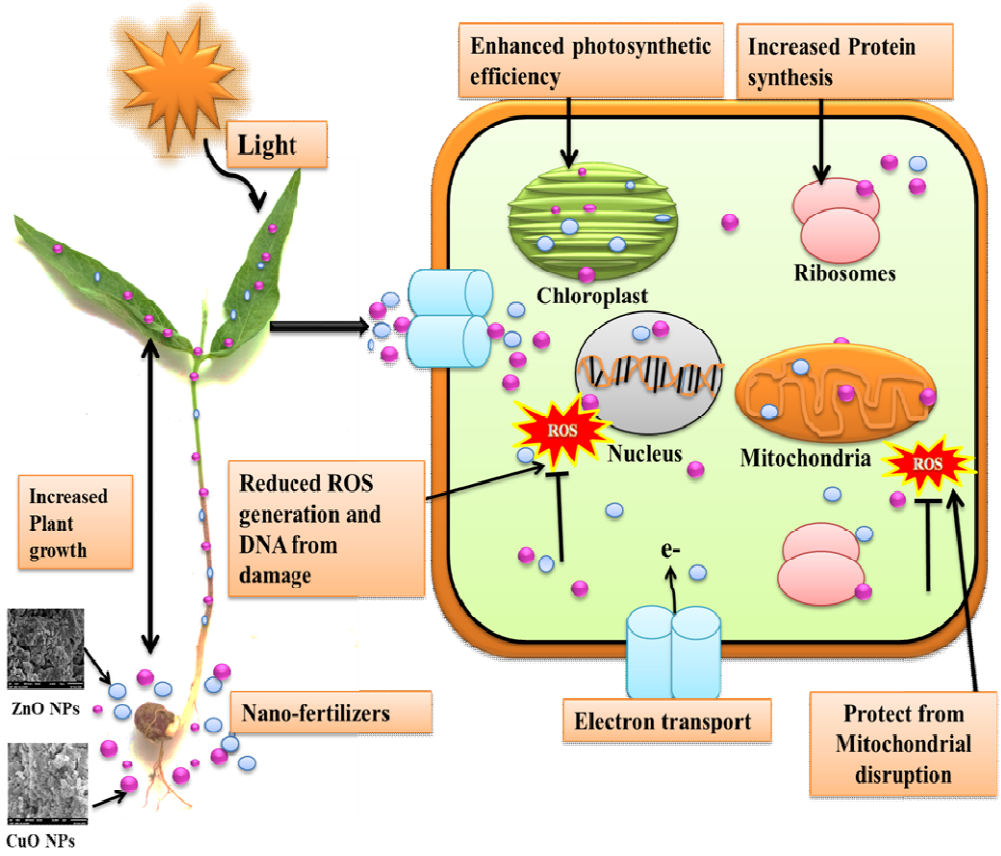


Figure (12)Role of Biosynthesized CuO NPs and ZnO NPs in plant growth

It was further found that *N. oleander*(L.) and *L.camara* (L.) may be utilized for the biofabrication of CuO and ZnO NPs in a sustainable and ecofriendly ways. However before, commercializing them further rigorous studies are needed to validate this study.

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