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In this work, ZnO nanoparticles (NPs) have been synthesised by hydrothermal method. This hydrothermally synthesised product has been characterised by powder X-ray diffraction and field emission scanning electron microscopy (FE-SEM) for the study of crystal structure and morphology/size. FE-SEM image revealed that ZnO NPs are spherical in shape with a diameter of 20–30 nm. The photoluminescence study of these NPs revealed that ZnO NPs consist of three emission peaks at 401, 482 and 524 nm. The UV emission peak at 401 nm is the band edge emission; however, the blue-green emission at 482 nm and green emission at 524 nm is related to defects. These ZnO NPs are used during the seed germination and root growth of *Cicer arietinum*. The effect of ZnO NPs has been observed on the seed germination and root growth of *C. arietinum* seeds. The effect of these ZnO NPs on the reactivity of phytohormones, especially indole acetic acid (IAA) involved in the phytostimulatory actions, is also carried out. Due to oxygen vacancies, the oxygen deficient, i.e. zinc-rich ZnO NPs increased the level of IAA in roots (sprouts), which in turn indicate the increase in the growth rate of plants as zinc is an essential nutrient for plants.

Keywords: indoleacetic acid; plants; nanoparticles

1. Introduction

Recently, nanoparticles (NPs) have attracted a lot of attention because of their use in a variety of areas such as electronic, cosmetic, biomedical, energy, environmental, catalytic and material applications. The large increase in the of use of NPs has stimulated researchers to know the application of NPs in plant growth. Limited studies have been reported on both the positive and negative effects of NPs on plants. Lu et al. [1] reported that a mixture of nano-SiO_2 and nano-TiO_2 could increase the nitrate reductase in soybean (*Glycine max*), enhance its abilities of absorbfing and utilising water and fertiliser, stimulate its antioxidant system and apparently hasten its germination and growth. Hong et al. [2,3] pointed out that nano-TiO_2 promotes photosynthesis and nitrogen metabolism and then greatly improves the growth of spinach at a proper concentration.

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Further, Yang and Watts [4] reported that uncoated alumina NPs inhibited the root elongation of corn, cucumber, soybean, cabbage and carrot after investigating the phytotoxicity of nano-Al_2O_3 powders with/without phenanthrene coating.

Zinc (Zn) is one of the essential nutrients required for plant growth. It is an important component of various enzymes that are responsible for driving many metabolic reactions in all crops. Growth and development would stop if specific enzymes were not present in the plant tissues [5]. Zn is, however, needed in very small amounts; therefore it is classified as a micronutrient. Its important role can be adjudged as it controls the synthesis of indole acetic acid (IAA), a phytohormone which dramatically regulates the plant growth. It is also necessary for the chlorophyll synthesis and carbohydrate formation [6]. It enables the plants to withstand lower air temperatures and helps in the biosynthesis of cytochrome, a pigment that maintains the plasma membrane integrity and the synthesis of leaf cuticle. The improvement of Zn nutritional status also reduces the uptake of harmful heavy metals, i.e. hinders their toxicity in plants, such as Cd [7]. According to Lin and Xing [8], there is a limited or no information available on the plant cell internalisation of NPs. Dissolution of metal-based NPs is a debatable mechanism for nanotoxicity. Researchers have reported both positive and negative evidences for the mechanism. Moreover, the toxicity of NPs depends on their properties, test organism species and surrounding conditions.

Bulk zinc oxide (ZnO) is very insoluble in soil; however, plant roots have the ability to solubilise ZnO in the vicinity. In this article, it is shown that the increased surface-to-volume ratio of nano-ZnO, which was prepared hydrothermally in our laboratory and characterised, was found very suitable for seed germination. This could be observed visually in *Cicer arietinum* seed germination. The experiment was repeated in triplicate and the same results were observed.

2. Experimental

2.1. *Synthesis of ZnO NPs*

The zinc acetate dihydrate $Zn(CH_3COO)_2 \cdot 2H_2O$ (98%) and sodium hydroxide (NaOH) was from E. Merck Limited, Mumbai, India. These chemicals were directly used without special treatment. ZnO NPs were prepared by the following process: zinc acetate dihydrate was put into 105 ml of distilled water under vigorous stirring. After 10 min of stirring, 10 ml of 2M aqueous NaOH solution was introduced into the above solution, resulting in a white turbid solution (pH value ~11), which was then transferred into stainless steel autoclaves, sealed and maintained at a temperature of 80°C for 5 h. The precipitate in the autoclave was taken out and washed repeatedly with distilled water and ethanol to remove any ions possibly remaining in the final product. Then, a white powder was obtained after drying at 60°C in air for 2 h. The crystal structure of ZnO NPs were characterised by X-ray diffraction (XRD, Rigaku D/MAX- 2200H/PC, Cu-Kα radiation). The photoluminescence study was carried out on a Perkin Elmer LS 55 spectrometer. The scanning electron microscopy (SEM) images were taken on Quanta 200 FEG (FEI company).

2.2. *Preparation of nano-ZnO suspension*

The synthesised 0.2 gm of ZnO NPs were directly dispersed in 100 ml of deionised (DI) water and it was placed under ultrasonic irradiation (100 w, 40 kHz) for 30 min.

2.3. *Germination of seeds*

Few *C. arietinum* (chick pea) seeds were immersed in 10% sodium hypochlorite solution for 10 min to ensure the surface sterility. Then, all the seeds were soaked in DI water for 1 h. After 1 h, a few seeds were taken out and dipped in nano-ZnO suspension solution. After 2 h, a few seeds from this solution were taken out and rinsed thoroughly with DI water [9] and a few seeds were kept in the ZnO suspension. These seeds were then transferred onto filter paper with 30–35 seeds per petri dish.

In total, three samples had been created:

(1) seeds in DI water only (Sample A);
(2) seeds suspended in the nano-ZnO suspension for 2 h and then thoroughly rinsed with DI water (Sample B); and
(3) seeds remaining suspended in nano-ZnO (Sample C).

The Petri dishes were covered and sealed with tape and placed in the dark for 5 days at room temperature. Almost 90% seeds were germinated and developed roots, which were photographed of as shown in Figure 1. In this study, seeds showing the emergence of radical out of the seed coat were recorded as being germinated. Each treatment was conducted with three replicates and the same results were observed. After measuring the length and thickness of the roots after 5 days, the roots from sample A, sample B and sample C were cut, crushed and extracted separately in ethanol.

2.4. *Extraction*

The roots were immersed in 250 ml ethanol (Merck) which was refluxed for 28 h at 60°C. After refluxing, the extract was filtered through Whatman filter paper. The extracts were then concentrated under vacuum to 100 ml.

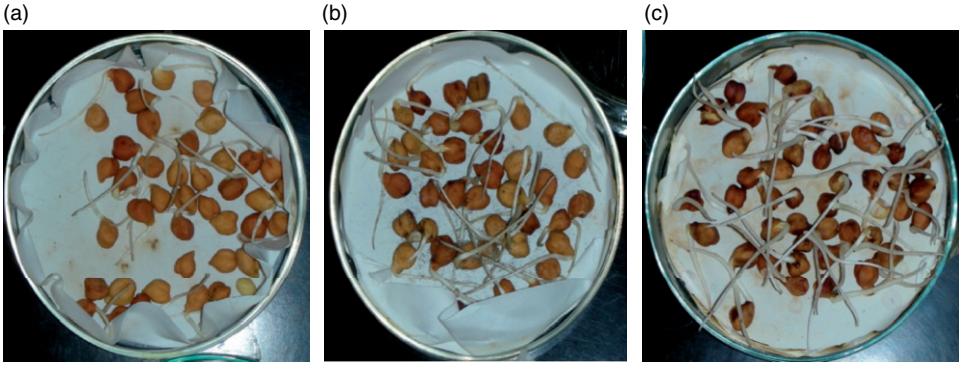

Figure 1. *Cicer arietinum* seeds: (a) suspended in DI water only for 5 days, (b) suspended in ZnO NPs suspension for 2 h only and then in DI water for 5 days, and (c) remain suspended in ZnO NPs suspension for 5 days.

2.5. *IAA identification and determination*

IAA was identified and tested by the Salkowskie $FeCl_3$ method in a which red colour was developed with nitrite and mineral acid. Further identification and determination was done by reverse phase HPLC analysis, which was performed on Metrohm HPLC consisting of 820 IC separation centre, 830 IC interface and twin 818 IC pump. For the HPLC analysis, analytical grade chemicals were used. Water, conductivity lower than 0.05 μS/cm and acetonitrile (Merck) were of HPLC grade. The standard solution (1000 ppm) of IAA (Sigma) was prepared by dissolving it in the HPLC mobile phase. The mobile phase was 20% acetonitrile/water containing 1% acetic acid at a flow rate of 1 ml/min. Ten-microlitre samples of A, B and C were injected one by one after running the column with standard.

3. Results and discussion

3.1. *Structural study of ZnO NPs*

Figure 2 shows the XRD pattern of ZnO NPs synthesised by hydrothermal process. All the diffraction peaks in the pattern can be indexed as the hexagonal ZnO with lattice constants $a = 3.249$ and $c = 5.206$ Å, which are consistent with the values in the standard card (JCPDS 36-1451). Furthermore, it can be seen that the diffraction peaks are higher and narrower, implying that the ZnO crystallises well. No characteristic peak of impurities such as $Zn(OH)_2$ were observed.

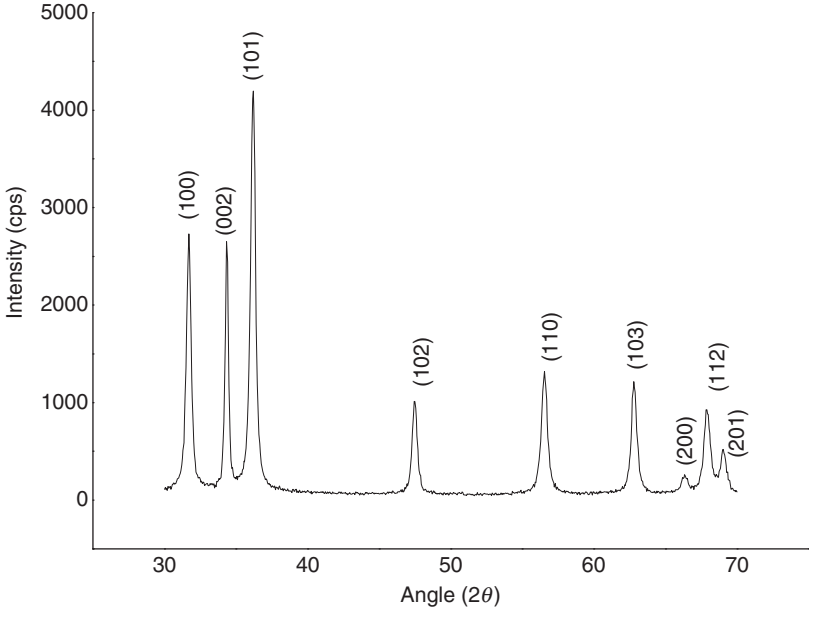

Figure 2. XRD pattern of ZnO NPs synthesised by hydrothermal method.

3.2. *Morphology study of ZnO NPs*

Figure 3 shows the FE-SEM image of ZnO NPs synthesised by the hydrothermal method. From the FE-SEM image of ZnO NPs, it is clear that the particles are spherical in shape with a diameter of 20–30 nm.

3.3. *Photoluminescence study of ZnO NPs*

Figure 4 shows the photoluminescence spectrum of ZnO NPs at an excitation wavelength of 225 nm. The photoluminescence spectrum of ZnO NPs consists of three emission peaks at 401, 482 and 524 nm. The UV emission peak at 401 nm is the band edge emission. However, the blue-green emission at 482 nm and the green emission at 524 nm are related to defects. It is commonly considered that the band edge emission at 401 nm should be attributed to the recombination of excitons [10] and the green emission corresponds to the singly ionised oxygen vacancy in ZnO, resulting from the recombination of a photon-generated hole with the single ionised charge state of this defect [11]. The blue-green emission may result from the electron transition from the level of the ionised oxygen vacancies to the valence band [12].

3.4. *Effect of ZnO NPs on the growth of plants*

The length and the thickness of the roots after experiments were as follows:

Sample A: 30.0 mm long and 1.0 mm thick.
Sample B: 35.0 mm long and 1.5 mm thick.
Sample C: 45.0 mm long and 2.5 mm thick.

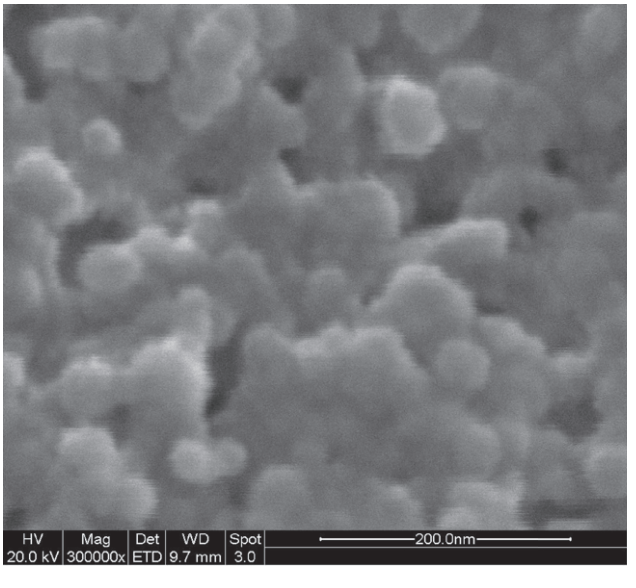

Figure 3. SEM image of ZnO NPs synthesised by hydrothermal method.

Figure 1 clearly shows the difference observed with the nano-ZnO treatment. Germination is normally known as a physiological process beginning with water imbibitions by seeds and culminating in the emergence of the rootlet. This can be understood as – with the imbibition of water – the hormone signal whereby IAA is carried out from the embryo to the layers of endosperm. IAA activates the DNA for gene encoding α-amylase in endospermic cells and breaks down the starch into sugar maltose which is transported to the embryo. The sugar fuels respiration in the embryo so that it can grow. The radical protrudes from the seed coat and the germination is accomplished [13]. The seed coat plays a very important role in protecting the embryo from harmful external factors. Seed coats can have selective permeability. Pollutants, though having obviously inhibitory effect on root growth, may not affect the germination if they cannot pass through the seed coats. This explains why germination cannot be toxicated if NPs intend to promote germination. Since Zn is a constituent of an enzyme which influences the secretion of IAA, nano-ZnO gives a very positive response in the seed germination.

3.5. *Characterisation of root extracts*

The increase in the IAA level in the extract of seeds remaining suspended in nano-ZnO for 5 days, is shown in HPLC chromatogram (Figure 5), showing a sharp rise in the IAA peak. This is due to the fact that the level of free IAA increased as a result of soaking the seeds in water [14–18]. The IAA further increased when its secretion is catalysed by nano-ZnO. However, the IAA level remained at the lower side when seeds remained in DI water only. The observed result in Figure 1 is thus supported by a HPLC analysis.

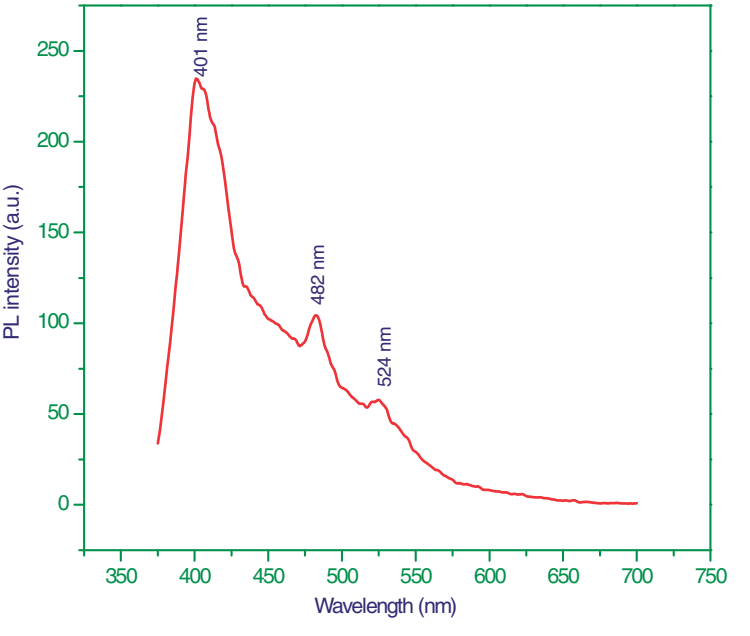

Figure 4. Photoluminescence spectrum of ZnO NPs synthesised by the hydrothermal method.

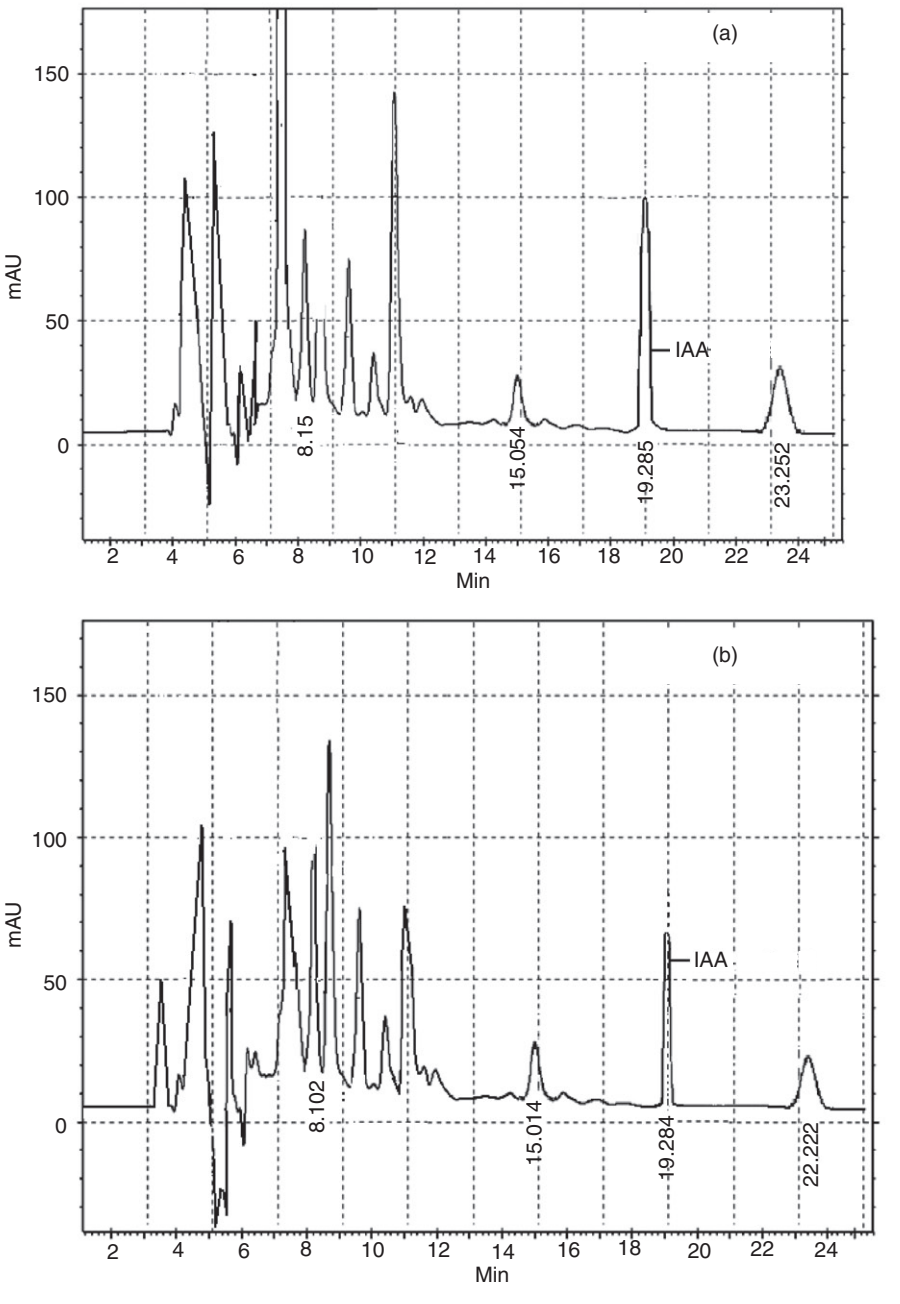

Figure. 5. HPLC chromatogram of root extracts of: (a) seeds remaining suspended in nano-ZnO for 5 days, (b) seeds suspended in nano-ZnO suspension for the limited time of 2 h only and then thoroughly rinsed with DI water, (c) standard IAA solution and (d) seeds in DI water only.

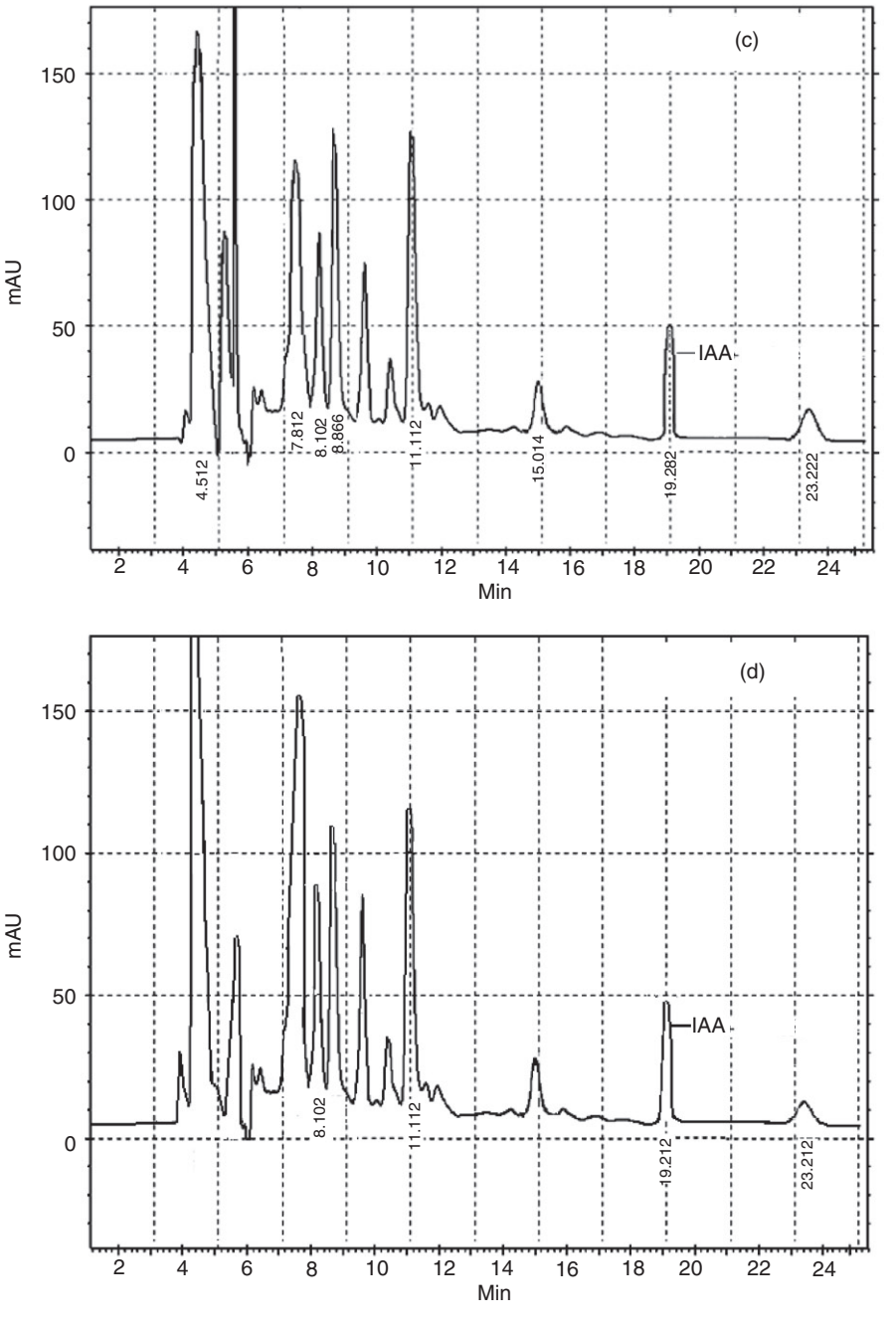

Figure. 5. Continued.

The most probable reason for the root growth may be accomplished because of oxygen vacancies in ZnO NPs (Figure 4). The ratio of zinc and oxygen in ZnO is greater than 1. Again, since Zn is required in only trace amounts, the amount of Zn that a plant can take is only very little, i.e. the delivery of Zn to the plant is in a controlled fashion due to which the excess of Zn that may toxicate the plant is avoided. Moreover, ZnO is an eco-friendly and bio-friendly material, therefore its excess amount would least spoil the soil quality and can be used as a green reagent.

4. Conclusion

In this study, the effect of ZnO NPs on the germination of *C. arietinum* seeds has been observed. For this purpose, ZnO NPs consisting of oxygen vacancies have been synthesised by hydrothermal method. The nano nature of ZnO is confirmed by XRD analysis and SEM image. The presence of oxygen vacancies in ZnO NPs has been observed by the photoluminescence study of these ZnO NPs. The presence of IAA in seeds and also the fact that the content of IAA increases with the response to a change of conditions favouring germination indicated that IAA is of importance in the germination process. In our study, a positive response is observed with ZnO NPs. Due to oxygen vacancies, the oxygen deficient, i.e. zinc-rich ZnO NPs increased the level of IAA in the roots (sprouts), which in turn indicate the increase in the growth rate of plants. HPLC analysis supported and confirmed the increased level of IAA.

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