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RESEARCH ARTICLE

EFFECT OF CHEMICALLY SYNTHESIZED NANOPARTICLES (ZNO) ON GROWTH CHARACTERISTICS OF *BRASSICA JUNCEA* GROWN UNDER CADMIUM TOXICITY

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ABSTRACT

The present work is focused on synthesis and characterization of Zinc oxide nanoparticles (ZnO NPs) to study its effects on two *Brassica juncea* cultivars stressed with different toxicity levels of Cadmium (Cd). ZnO NPs were synthesized by chemical precipitation method and characterized using X-Ray Diffraction (XRD), Field Emission Scanning Electron Microscope (FESEM) and Energy Dispersive Analysis of X-rays (EDX) techniques. Crystalline size was calculated from the XRD results using Scherer equation. In present study ZnO NPs suspension were used as priming agent for ameliorating Cadmium toxicity in *B. juncea* seeds under in vitro conditions. It was found that ZnO NPs treated seedlings displayed good growth characteristics over control and was able to overcome Cadmium toxicity in *Brassica juncea* , demonstrating a positive effect of the nanoparticles treatment.

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INTRODUCTION

Engineered Nanomaterials (ENs) have gained vast impact in agriculture over past few years. Due to their Nano size they have the potential to alter the physio-chemical properties and posses greater surface area than their bulk material. This characteristic enhances their solubility and surface reactivity (Castiglione and Cermonini, 2009). Nanoagriculture utilizes ENs to improve the growth of plants and for control of plant diseases. Zinc (Zn) and Zinc oxide (ZnO) are categorized as a commonly used metal/metal oxide Nanomaterials.

Zn is essential micronutrient for plants, animals and humans (Bai *et al.,* 2010). Since higher plants absorbs Zn as a divalent cation (Zn^{+2}), it act as a metal component or as a functional structure or a regulatory cofactor of numerous enzymes. Zn is needed for chlorophyll and biomass production, enhancing germination and fertilization (Cakmak, 2008). There are reports that Nanomaterials has both positive and negative effects on higher plants (Kikui *et al.,* 2005; Laurent *et al.,* 2008). ZnO NPs were reported to improve germination, biomass production, root and shoot length in *Vigna radiata* (Dhoke *et al.,* 2013; N.Jayarambabu *et al.,* 2014) and Peanut (Prasad *et al.,* 2012). *Brassica juncea* is an important oil seed crop being cultivated in 53 countries all over the world.

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Yield and production of *B. juncea* is negatively affected by presence of heavy metals in the soil. Among heavy metals cadmium (Cd) is a non essential and most deleterious heavy metal pollutant. Plants growing on Cd^{+2} polluted soil accumulate it, in all plant parts, resulting in growth retardation, affects nutrient uptake, cause alteration in the chloroplast structure, inactivates enzymes, reduce photosynthesis and activates enzymatic and non enzymatic antioxidant machinery (Márquez *et al.,* 2011). ZnO NPs has been reported as growth stimulant in Cd stressed plants according to (Choudhary *et al.,* 1995) Zinc coexistence with Cd in the cultivating medium results in the interactions that may be antagonistic to Cd toxicity due to physical and chemical similarity (Anjum *et al.,* 2008). Since the growth of *Brassica juncea* is adversely affected by Cd toxicity (Bohra and Sanadhya, 2015), the present study deals with the synthesis, characterization of ZnO NPs and their effects on *Brassica juncea* seedlings grown under Cd toxicity.

MATERIALS AND METHODS

Synthesis of ZnO NPs

ZnO NPs were synthesized using chemical precipitation method. Equal concentration of Zinc acetate and Potassium hydroxide were mixed in methanol separately and dissolved by constant stirring, when both the salts dissolved completely in methanol, both the solutions were mixed together to obtained a milky white solution. Stirring was continued for 30 minutes on magnetic stirrer with heating at 60°C. The solution was kept undisturbed for 24 hrs to form the precipitate. Then the solution was centrifuged at 10000 rpm for 25 minutes. The precipitate so obtained was washed thrice with deionised water at 10000 rpm for 20 minutes to remove impurities. The sample was dried at 300°C for 7 hrs. The white powder so obtained was collected and characterized.

Characterization of ZnO NPs

The synthesized sample was characterized by using following techniques, such as XRD, FESEM, and EDX X-Ray Diffraction analysis of synthesized sample was obtained by X-pert pro diffractometer having an X'celerator detector. The measurements were taken at 25°C and value of 2θ ranges from 20° to 80°. The scan rate was noted per 2 sec to determine the size of crystal. Surface morphology was studied using FESEM (Field Emission Scanning Electron Microscope) model (NOVA NANOSEM 450) operated at 18KV and elemental analysis of sample was carried out using EDX (Energy Dispersive Analysis of X-Rays) model (Bruker X Flash 6130)

Preparation of ZnO NPs suspension

The Nano ZnO with concentration 10 ppm was suspended in deionised water by using ultrasonicator (115W, 60 kHz) for 60 minutes.

Seeds

Two Cultivars of *Brassica juncea* were procured from CCSHAU Hisar. Uniformly selected seeds were surface sterilized with 5% NaOCl for 5 minutes and then washed repeatedly for two to three times with distilled water to prevent fungal/bacterial contamination. Filter papers were also sterilized in autoclave to reduce any chances of microbial growth. The seeds were primed for 4 hrs using prepared nanoparticles suspension by keeping in orbital shaker at constant temperature. Heavy metal test solutions were prepared using four different concentrations of Cadmium (Cd^{+2}) viz. 0.5mM, 1mM, 1.5mM and 2 mM. The chemicals used were of analytical reagent grade. Distilled water was used as control. Seeds were germinated in glass Petri dishes of 15 cm diameter lined with filter paper circles moistened with control and various concentrations of Cadmium to impose four levels of stress. Nearly 10 seeds were sown in each Petri dish and incubated in growth chamber set at 25 ± 2°C for 7 days and each treatment was replicated thrice. Occurrence of Germination was considered when roots were 2mm long; Germination percentage was recorded in every 24h, till the end of experiment. The following parameters were analyzed for the study.

Germination percentage

A mean of 10 seeds were taken and expressed as percentage.

$$\text{G.P}\ (\%) = \frac{\text{Total number of seeds germinated}}{\text{Total number of seeds}} \times 100$$

Root length and Shoot length

The length of root and shoot of seedlings were recorded at 7^{th} day of germination. Mean values were calculated for both root and shoot length and values were expressed in cm.

Seedling Height

Heights of seedling were recorded at 7^{th} day of germination. Mean values were calculated and expressed in cm.

Wet Biomass

The Wet Biomass was recorded from the 7^{th} day old seedling. The whole seedling was surface dried with the blotting paper and their Wet Biomass were recorded

Dry Biomass

The same seedlings were used and dried in oven for 24 h at 80°C and weighed again. This represented the dry matter

RESULTS AND DISCUSSION

X-Ray diffraction analysis

The crystalline structure of ZnO NPs was determined by XRD analysis. Fig 1 shows the XRD pattern of synthesized ZnO NPS. The peak and relative intensities obtained for the ZnO match with the literature values (Maguire, 1982).The presence of strong and sharp diffraction peaks located at the 2θ value of 31.82°, 34.42° and 36.29° corresponding to (100), (002) and (101) planes respectively indicating the formation of ZnO NPs (Figure-1) .The average particle size of the nanomaterial was determined using the Scherer equation and it was found to be 25.9 nm.

FESEM Analysis

FESEM was used to determine the surface morphology and size distribution of ZnO NPs. FESEM images of ZnO NPs with different magnification are shown in Figure- 2. The average particle size of ZnO NPs ranges from 19- 45 nm. It is observed that FESEM results are in good agreement with size distribution of ZnO NPs measured by XRD.

EDX Analysis

Energy dispersive X-ray spectroscopy (EDX or EDS) reveals the presence of Zn and O elements in synthesized sample of ZnO. Fig 3 and Table 1 shows elemental composition along with their weight percentage of synthesized sample of ZnO

Root length, Shoot length and Seedling size

In present study ZnO NPs (10ppm) primed seeds showed increased in root, shoot length and seedling size at all four concentrations of Cd (0.5mM, 1 mM, 1.5 mM, and 2mM) as compared to unprimed seeds Figure-4, 5, 6.

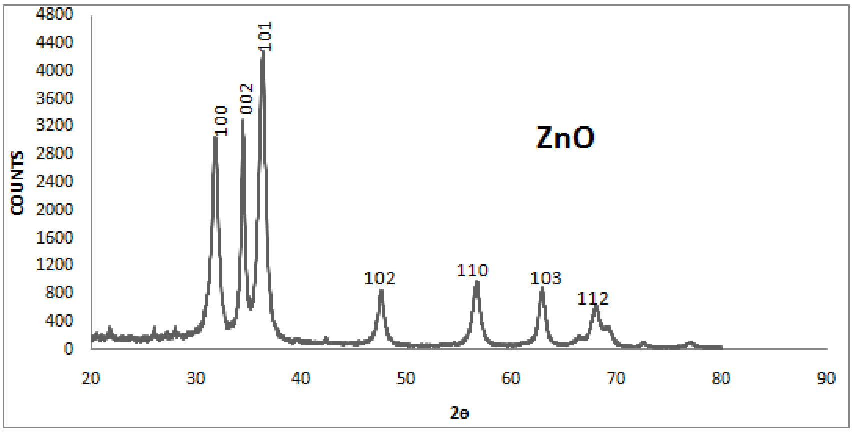

Figure 1. XRD pattern of ZnO Nanoparticles

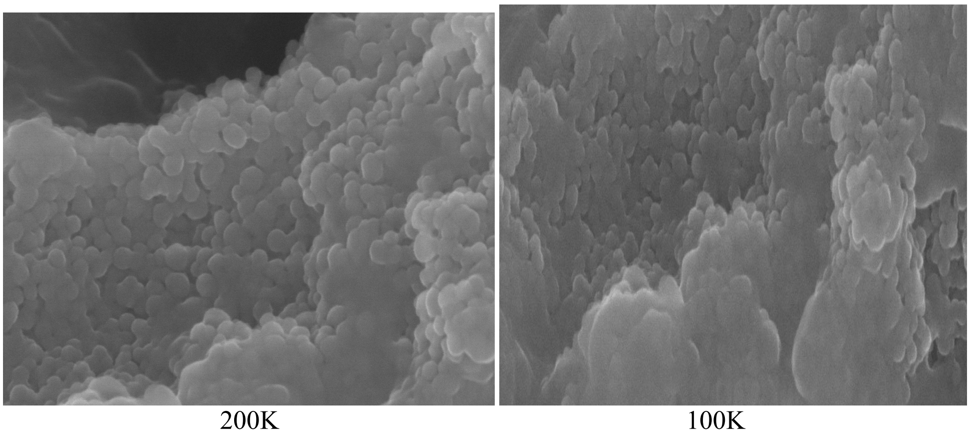

Figure 2. FESEM images of ZnO Nanoparticles at different magnifications

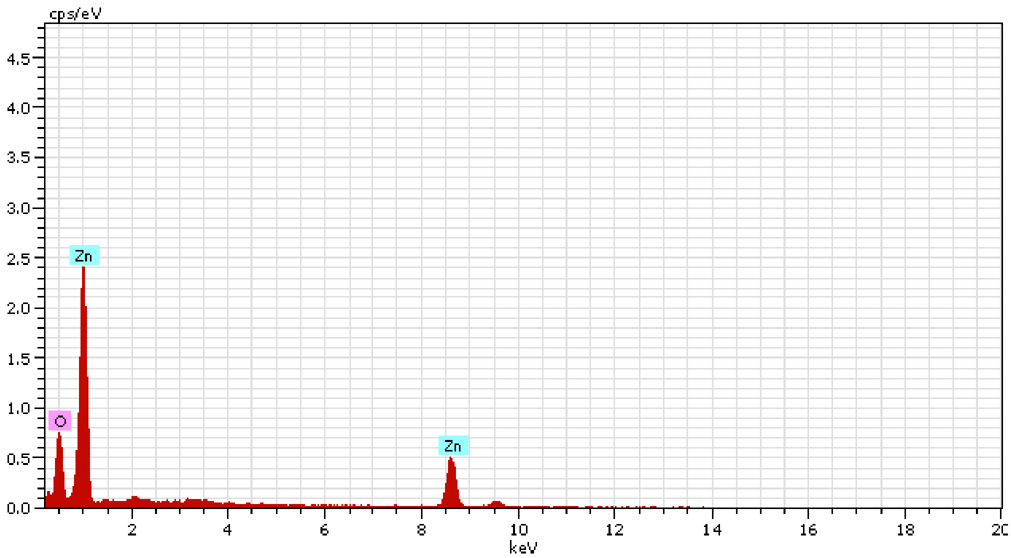

Figure 3. EDX of ZnO Nanoparticles

The present study was carried out with two cultivars of *Brassica juncea* (RB-50 and RH-0406). ZnO NPs primed seeds of both the cultivars showed increased in root-shoot length and seedling size at all four concentration of Cd as compared to unprimed seeds. Between two cultivars, RB-50 showed better results with ZnO NPs. This observation is in similarity with the previous findings of N.Jayarambabu *et al.,* 2014 who reported that ZnO NPs showed increased in root-shoot length in *Vigna radiata.* Roots were found to be more sensitive to Cd toxicity than shoots, as roots are the first organ to come in contact with toxic heavy metal which results in its higher accumulation (Bauddh and Singh, 2010), presence of

Zn may be results in reduction of Cd uptake due to physical and chemical similarity. Both the varieties of *Brassica juncea* had been evaluated earlier by us (Bohra and Sanadhya, 2015) for their responses to Cd toxicity and it was observed that RB-50 was more tolerant than RH-0406.

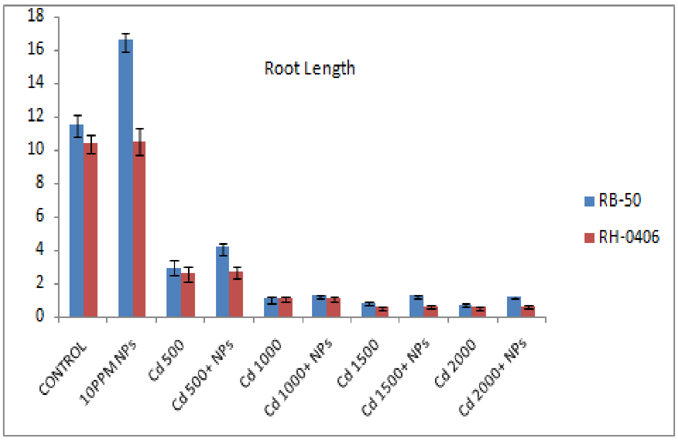

Figure 4. Effect of different concentration of Cadmium on root length of ZnO Nanoparticles primed and unprimed seeds of two *Brassica juncea* cultivars. Data are the means of three replicates ± s.e.

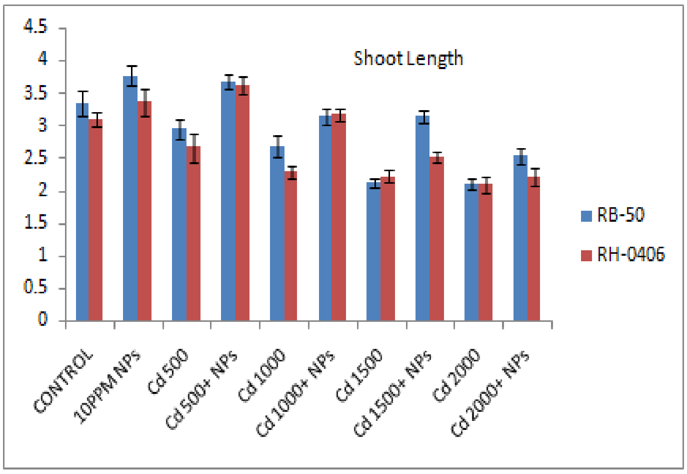

Figure 5. Effect of different concentration of Cadmium on shoot length of ZnO Nanoparticles primed and unprimed seeds of two *Brassica juncea* cultivars. Data are the means of three replicates ± s.e.

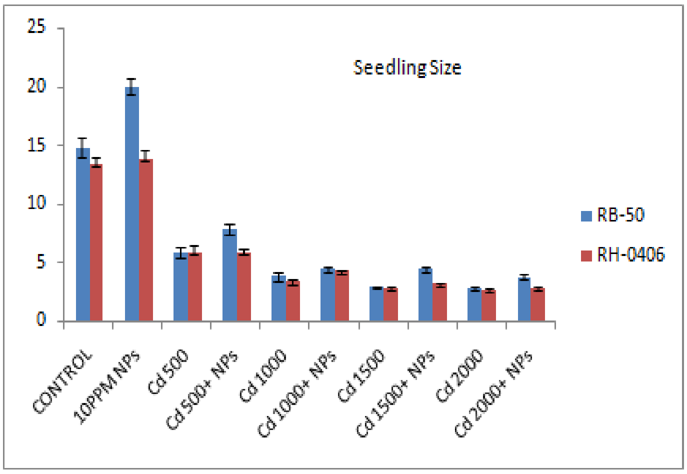

Figure 6. Effect of different concentration of Cadmium on seedling size of ZnO Nanoparticles primed and unprimed seeds of two *Brassica juncea* cultivars. Data are the means of three replicates ± s.e.

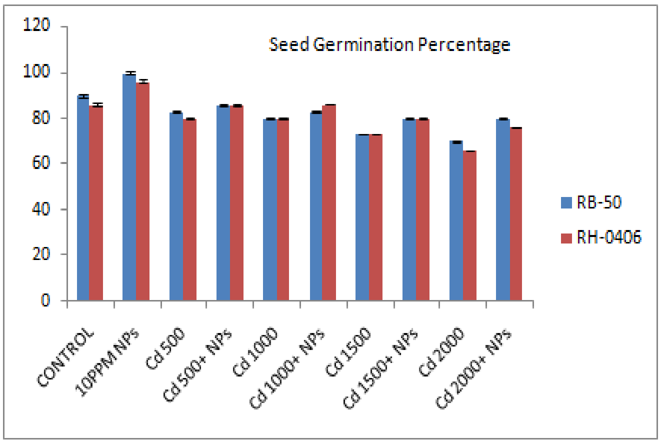

Figure 7. Effect of different concentration of Cadmium on seed germination percentage of ZnO Nanoparticles primed and unprimed seeds of two *Brassica juncea* cultivars. Data are the means of three replicates ± s.e.

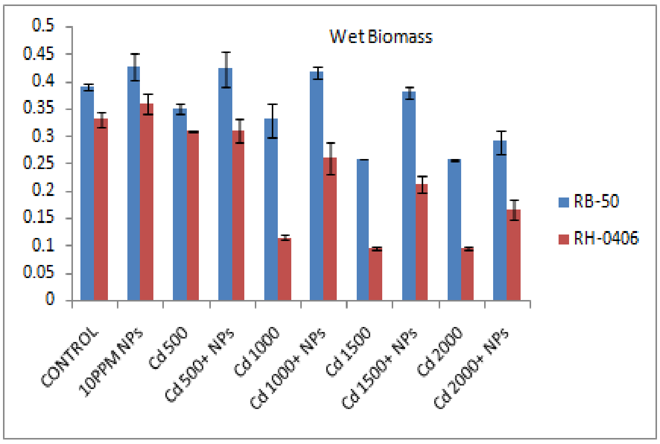

Figure 8. Effect of different concentration of Cadmium on wet biomass of ZnO Nanoparticles primed and unprimed seeds of two *Brassica juncea* cultivars. Data are the means of three replicates ± s.e.

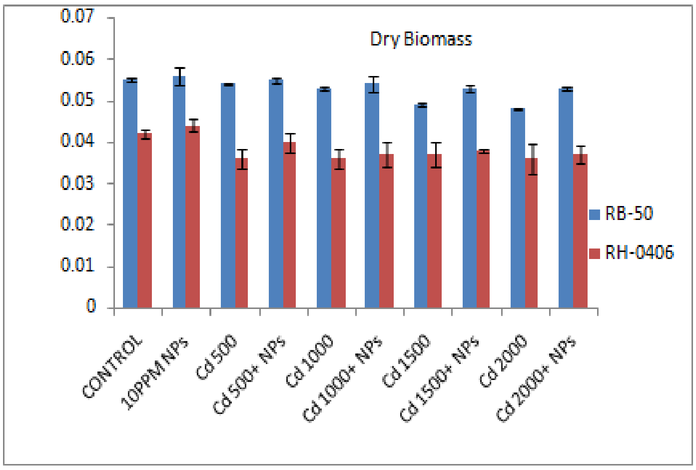

Figure 9. Effect of different concentration of Cadmium on dry biomass of ZnO Nanoparticles primed and unprimed seeds of two *Brassica juncea* cultivars. Data are the means of three replicates ± s.e.

Table 1. Elemental composition of ZnO Nanoparticles

Element	Weight %
Zn	69.67
O	30.33
Total	100.00

Seed Germination Percentage

Seed germination percentage of ZnO NPs primed seeds was significantly higher for both cultivars (RB-50 and RH-0406) at all four concentration of Cd, as compared to unprimed seeds Figure 7. Cultivar RB-50 showed higher germination percentage than Cultivar RH-0406. ZnO (10ppm) primed seeds of RB-50 showed 100% germination, whereas control (unprimed seeds) showed 90% germination. ZnO (10ppm) primed seeds of RB-50 at Cd concentration 0.5mM, 1 mM, 1.5 mM, and 2mM showed 86%, 83%, 80% and 80% germination respectively. Whereas unprimed seeds Of RB-50 at Cd concentration 0.5mM, 1 mM, 1.5 mM, and 2mM showed 83%, 80%, 73% and 70% germination respectively.

ZnO (10ppm) primed seeds of RH-0406 showed 96% germination, whereas control (unprimed seeds) showed 86% germination. ZnO (10ppm) primed seeds of RH-0406 at Cd concentration 0.5mM, 1 mM, 1.5 mM, and 2mM showed 86%, 83%, 80% and 76% germination respectively. Whereas unprimed seeds Of RH-0406 at Cd concentration 0.5mM, 1 mM, 1.5 mM, and 2mM showed 80%, 80%, 73% and 66% germination respectively. These results may indicate that presence of ZnO NPs during seed germination and early seedling growth has very important physiological role. The results of present investigation are in similarity with the work of Prasad *et al.,* 2012 who reported that ZnO NPs enhance the rate of germination in peanut.

Wet and Dry biomass

Wet and Dry biomass of ZnO NPs primed seeds were significantly higher for both cultivars (RB-50 and RH-0406) at all four concentration of Cd, as compared to unprimed seeds Figure 8,9. Cultivar RB-50 showed higher biomass accumulation than Cultivar RH-0406. T he results are in conformity with the results of Dhoke *et al.,* 2013 who reported the increased in total biomass on application of Nano ZnO on *Vigna radiata.* Increase in total biomass on application of ZnO NPs could be the result of more protein synthesis, elongation of cells and tolerance to environmental stresses in presence of Zn (Cakmak 2000).

Conclusion

In present study ZnO NPs were synthesized and characterized by different techniques for calculation of particle size, analysis of surface morphology and elemental composition. It has been observed that suspension of ZnO NPs were able to overcome Cd toxicity in *Brassica juncea*. An increase in root and shoot length, seedling size, Seed germination percentage as well as accumulation of total biomass was recorded for nanoparticles primed seeds as compared to the control (unprimed seeds) for both the cultivars. Between two cultivars RB-50 showed better results for all the tested parameters. Current findings may be suggested that the Cd bioavailability can be reduced and the resistance to Cd toxicity can be increased in Cd polluted soils by the application of ZnO NPs. Hence this approach could be subjected to field trials to analyze its beneficial effects. Further the most efficient method of application has to be evaluated.

Conflict of Interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

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