# EFFECT OF ZINC OXIDE NANOPARTICLES ON THE GROWTH AND Zn UPTAKE IN WHEAT (*TRITICUMAESTIVUM* L.) BY SEED PRIMING METHOD

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Zinc (Zn) deficiency in crops as well as in humans is still an important health issue, especially in developing countries which is mainly due to inadequate dietary intake. The major reason behind this is the consumption of large amounts of Zn-deficient cereals worldwide. This problem can be solved by the application of Zn to cereals known as biofortification. Nanotechnology is one of the options to enhance the nutritional values of crops as some engineered nanoparticles (NPs) could be used as a fertilizer. Zinc can be used in the form of zinc oxide (ZnO) NPs. The current study used the solution evaporation method to prepare ZnO NPs, characterized it by using X-ray diffraction (XRD) and scanning electron microscope (SEM) techniques and finally evaluated the effect of ZnO NPs seed priming (0,25,50,75,100 ppm) on growth and Zn uptake in wheat (*Triticumaestivum* L.). XRD pattern confirms the formation of NPs and SEM image demonstrated the different shape and size of NPs such as bunch of irregular particles, smaller particles, round shape particles. Seed priming of ZnO NPs linearly increased the growth characteristics, photosynthesis and biomass of wheat. A significantly higher concentration of Zn were found in the roots, shoot and grains of wheat with ZnO NPs than the control which confirmed that these particles could be used as a source of Zn aiming to reduce Zn deficiency in plants. However, field studies under different conditions and plants may further enhance the mechanistic understanding of the applicability of NPs in this field.

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## 1. Introduction

Zinc (Zn) is considered an essential micronutrient and its deficiency is an important health problem around the world and is a hurdle in achieving production targets in crops [1, 2, 3]. A large portion of food is achieved through cereals-based products which contain a small amount of Zn which could not meet the human needs of Zn [1, 4]. Cereals grains such as wheat (*Triticum aestivum* L.) contain small concentrations of Zn which could not meet the human requirements. Zinc deficiency as well as its and other heavy metal toxicity also negatively affected the growth and yield of crops [1, 5, 32, 33].

Different approaches could be used to reduce Zn deficiency in plants like cereals, supplementation, dietary diversification and food as well as crop biofortification [2]. Currently, nanotechnology is being used in agriculture for different purposes and under various conditions [6, 7]. Nanoparticles (NPs) may also be used as a source of essential plant nutrients [8, 9]. Nanoparticles of Cerium Oxide ($CeO_2$) is applied on wheat and check their effect on growth and yield quality [10]. Silver nanoparticles are used for the plant diseases management. Silver nanoparticles have significant role in agriculture sector [11]. Many soluble phosphate salts such as, Phosphorus (P) fertilizers effectively used in agriculture. $TiO_2$ nanoparticles are used in wheat

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plant, but their effect on wheat plant is moderate. These nanoparticles are less effective in growth of seeds, transpiration rate, germination rate and plant biomass. However, these nanoparticles increase the root elongation on early stages of wheat plant growth [12]. Among NPs, zinc oxide (ZnO) NPs are the most widely used NPs worldwide and their potential adverse as well as positive effects on the ecosystem have been reported [8]. The soil application of ZnO NPs ($\leq$100 mg $kg^{-1}$) increased the Zn uptake by cucumber than bulk counterparts but negatively affected the growth of the plants at higher NPs (1000 mg $kg^{-1}$) [9].

Over the past few years various methods have been developed to synthesize ZnO nano crystals such as vapor phase growth [13], vapor-liquid-solid process [14], soft chemical method [15], electrophoretic deposition [16], sol-gel process [17], homogeneous precipitation [18], etc. Among these methods, solution evaporation method (soft chemical method) shows some advantages (i) Nanometer- size nanoparticles at ambient temperature (ii) The reaction is carried out under moderate condition (iii) Nanoparticles obtained with different morphologies by adjusting the reaction condition (temperature) (iv) good controllability (v) economical and cost effective method [19].

Similarly, different methods are used to apply the ZnO nano particle on wheat plant, like foliar spray, soil mixing method and seed priming method [8]. Zinc uptake by plants from NPs depends upon the soil properties [9] which may restrict the nutrient uptake by plants due to variation in dissolution, aggregation, and change in surface properties of NPs in the soil [8]. Seed priming method is simple and cost effective and primed seeds can rapidly absorb and renovate the seed metabolism, resulting in a higher germination rate and decreases intrinsic physiological non uniformity in germination [20, 21, 22]. Seed priming method can also improve the growth quality and production of crops [10, 34]. Thus, seed priming method was selected, to eliminate soil constrains, to evaluate the effect of ZnO NPs on wheat growth characteristics and Zn uptake by plants.

## 2. Experimental Procedure

### 2.1. Soil sampling and analysis

Soil samples were collected with the help of stainless steel spade from an agricultural field at a depth of 20 cm at different locations and all the samples were mixed and air-dried and finally passed through 2 mm sieve. Initial properties of the soil (Table 1) were estimated with standard protocols such as particle size fractions were determined by hydrometer [27] and soil pH, electrical conductivity (EC), as well as other soluble ions were estimated by using procedures developed by US Salinity Lab. Staff, [28] or Page et al. [29]. Bioavailable Cu, Ni and Zn were estimated after extracting the soil with ammonium bicarbonate diethylene triaminepentaacetic acid (AB-DTPA) at pH 7.6 [30].

*Table 1. Properties of soil used in the pot experiment*

| Texture | Clay loam |
|---|---|
| Sand (%) | 64.5 |
| Silt (%) | 13.3 |
| Clay (%) | 22.2 |
| pHs | 7.54 |
| ECe (dS $m^{-1}$) | 4.85 |
| Cation exchange capacity (CEC) ($cmol_c$ $kg^{-1}$) | 12.9 |
| Soluble $CO_3^{2-}$ ($mmolL^{-1}$) | 0.91 |
| Soluble $HCO^{3-}$ ($mmolL^{-1}$) | 3.96 |
| Soluble $Cl^-$ ($mmolL^{-1}$) | 6.73 |
| Soluble $Ca^{2+}$ + $Mg^{2+}$ ($mmolL^{-1}$) | 15.43 |
| Organic matter (%) | 0.78 |
| Available Ni ( mg $kg^{-1}$) | 0.26 |
| Available Cu ( mg $kg^{-1}$) | 0.53 |
| Available Zn ( mg $kg^{-1}$) | 0.82 |

### 2.2. Synthesis method of ZnO NPs

In order to synthesize the ZnO NPs, firstly the stock solution of Zn $(CH_3COO)_2.2H_2O$ of (0.1M) in 50ml methanol was prepared with the help of magnetic stirrer and then 25ml of NaOH (0.2 M) solution prepared in methanol was added under continuous stirring. These solutions were transferred into oven and temperature was maintained at 150 °C for 6h then allowed to cool it at room temperature. After the completion of reaction white powder was obtained.The structural and morphological characteristics of ZnO have been investigated by XRD and SEM.
The average crystallite size of prepared ZnO NPs is calculated using Scherer's relation

$$L=\frac{0.9\lambda}{\beta\cos\theta} \quad (1)$$

Where L is the crystal size, β is the line width (FWHM), θ is the diffraction angle and λ is the wavelength of X-ray radiation.
The following reaction occurred to synthesis ZnO nano-particles.

$$Zn(CH_3COO)_2.2H_2O + 2NaOH \rightarrow Zn(OH)_2 + 2CH_3COONa + 2H_2O$$
$$Zn(OH)_2 + 2H_2O \rightarrow Zn(OH)_4^{2-} + 2H+$$
$$Zn(OH)_4^{2-} \rightarrow ZnO + H_2O + 2OH$$

### 2.3. Pot experiment

Before sowing, wheat seeds were primed under different concentrations of Zn NPs (0, 25, 50, 75, 100 ppm) with continuous aeration for about 24 h. After this, seeds were washed with distilled water and air-dried under shade and stored for further use. During wheat season, primed seed were sown in plastic pots having 3.0 kg of soil under ambient conditions using completely randomized design (CRD) with four replicates of each treatment.Initially six seeds were sown in each pot and thinned to 4 seedlings per pot after 7 days of germination. Each pot was fertilized with urea (N), diammonium phosphate (DAP), and potassium sulfate at a rate of 120-50-25 kg $ha^{-1}$ respectively with full DAP and K whereas half N at the time of sowing and other half N was applied after 35 days of sowing.Random rotation on regular basis was done mainly to minimize environmental effects in the experiment.

### 2.4. Plant harvesting

Plants were harvested after 126 days of sowing when the plants were at physiological maturity. Data regarding plant height, number of tillers per plant, spike length were recorded and then samples were washed with distilled water and oven-dried at 70 °C and dry weight of shoots, roots and grains was recorded and then samples were ground to small size with the help of grinder and stored for further analysis.

### 2.5. Measurement of photosynthetic parameters, electrolyte leakage and Zn concentration

Total chlorophyll contents in leaves were determined by taking the fresh leaves after 65 days of growth. Chlorophyll contents were extracted by using 85% acetone (v/v) in a dark and centrifuging the samples (4000g at 4 °C) for 10 min. Finally, reading were taken at different wavelengths (470, 647, and 664.5 nm) by using spectrophotometer (Halo DB-20/ DB-20S, Dynamica Company, London, UK) and chlorophyll contents were determined by using coefficients (Lichtenthaler, 1987). Leaf gas exchnage parameters were recorded by using a portable Infra-Red Gas Analyzer (IRGA), (Analytical Development Company, Hoddesdon, England) during sunny day (11:00 to 12:00 am) assuming that plants were fully functional at that time.

Electrolyte leakage (EL) was estimated by using standard procedure [31]. Fresh samples were cut into different pieces of appropriate sizes and put vertically in the tubes having distilled water and samples were autoclaved for a specific time period (2h at 32 °C) and EC of the samples were recorded termed as $EC_1$ and then again the samples were autoclaved (20 min at 121 °C) and final EC was recorded termed as $EC_2$. An equation given below was used to calculate the EL of the samples

$$EL = (EC_1/EC_2) \times 100 \tag{2}$$

Plant samples were digested by using 10 ml consisting of $HNO_3$-$HClO_4$ (3:1, v:v) and atomic absorption spectrophotometer (Thermo electron S series) was used for the determination of Zn.

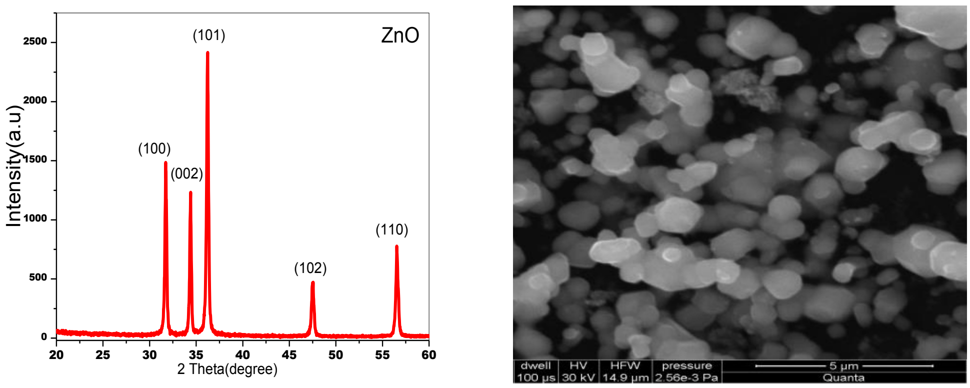
#### 2.6. Statistical analyses

One-way ANOVA was used to analyse the data statistically at 5.0% of probability by using IBM SPSS software (Version 21.0. Armonk, NY: IBM Corp). Where significant, Tukey's HSD post hoc test was employed for the multiple comparisons of means.

## 3. Results

#### 3.1. X-ray diffraction (XRD) and Scanning Electron Microscopy (SEM) analysis

Both XRD pattern and SEM image of ZnO NPs have been shown in Fig. 1. The XRD pattern confirms the formation of NPs and these particles are observed to be highly crystalline. Peaks at 2θ of 31.75°, 34.37°, 36.49°, 47.49°, 56.47° were referred to XRD planes (100), (002), (101), (102), (110).The XRD results showed that ZnO has the hexagonal warutzite structure. The SEM image demonstrates different shape and size of NPs. This SEM image also contains different kinds of particles such as bunch of irregular particles, smaller particles, round shape particles. Complex nanostructure is formed due to different size and shape of NPs. This complex nanostructure image is distributed over the whole scanned area.The average crystalline size of ZnO NPs is 34.4 nm (Table 2).




*(a)* *(b)*

*Fig. 1. XRD pattern (a) and SEM image (b) of ZnO NPs.*

*Table 2.The crystalline size of single peak.*

| Temperature | Size from (100)Peak | Size From (002) Peak | Size From (101) Peak | Size From (102) Peak | Size From (110) Peak | Average crystallite size (nm) |
|---|---|---|---|---|---|---|
| 150°C | 38.39 nm | 41.1 nm | 36.8 nm | 27.3 nm | 28.8 nm | 34.4 nm |

#### 3.2. Plant growth and biomass

Studied growth parameters such as plant height, number of tillers per plant, spike length, and dry weights of shoot, root and grains linearly increased with increasing ZnO NPs concentrations in the priming solution (Figure 2, 3). The highest increase in these growth attributes was found with the highest dose of NPs applied whereas the lowest values of these parameters were found in control plants. At 100 mg $L^{-1}$ ZnO NPs, the increase in plant height, number of tillers per plant, spike length, shoot dry weight, root dry weight, and grain yield was about 16, 69, 87, 58, 36, and 185% than control plants.

*Fig. 2: Treated wheat plants with different concentration of ZnO nanoparticles*

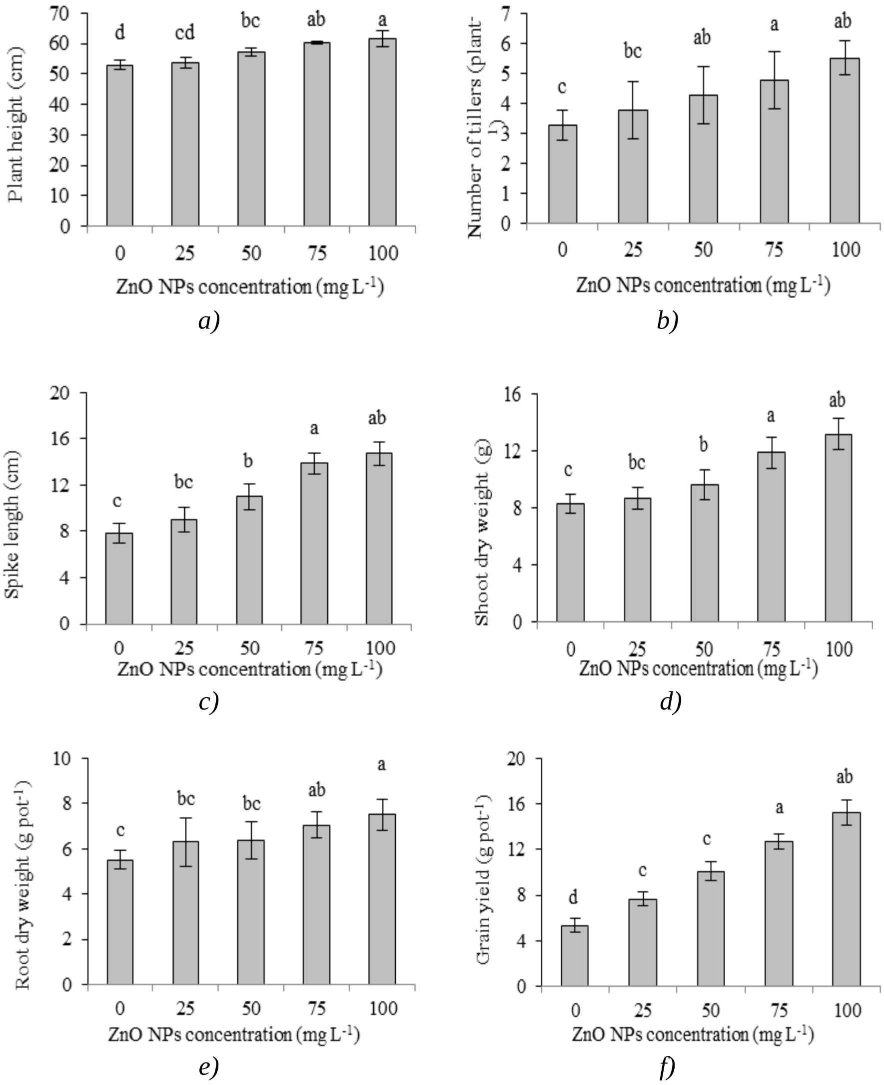



*Fig. 3. Effect of different concentrations of ZnO NPs on plant height (a), number of tillers per plant (b), spike length (c), and dry weights of shoot (d), root (e) and grains (f). Error bars indicate the standard deviation (SD) of four replicates. Different letters on the histograms indicate the significant differences among the treatments at a P < 0.05.*

### 3.3. Photosynthetic attributes and electrolyte leakage

Total chlorophyll contents, photosynthetic rate, stomatal conductance and transpiration rate increased with increasing ZnO NPs concentrations (Figure 4). The highest increase in these attributes was observed in the highest dose of NPs applied. As compared to control, photosynthetic rate increased by 25, 40, 55, and 58% and stomatal conductance increased by 25, 64, 87, and 102% in 25, 50, 75, and 100 mg $L^{-1}$ ZnO NPs respectively. Transpiration rate increased by 10, 36, 54, and 62% in 25, 50, 75, and 100 mg $L^{-1}$ ZnO NPs over control. The EL reduced in both shoots and roots with the application of NPs than control plants.

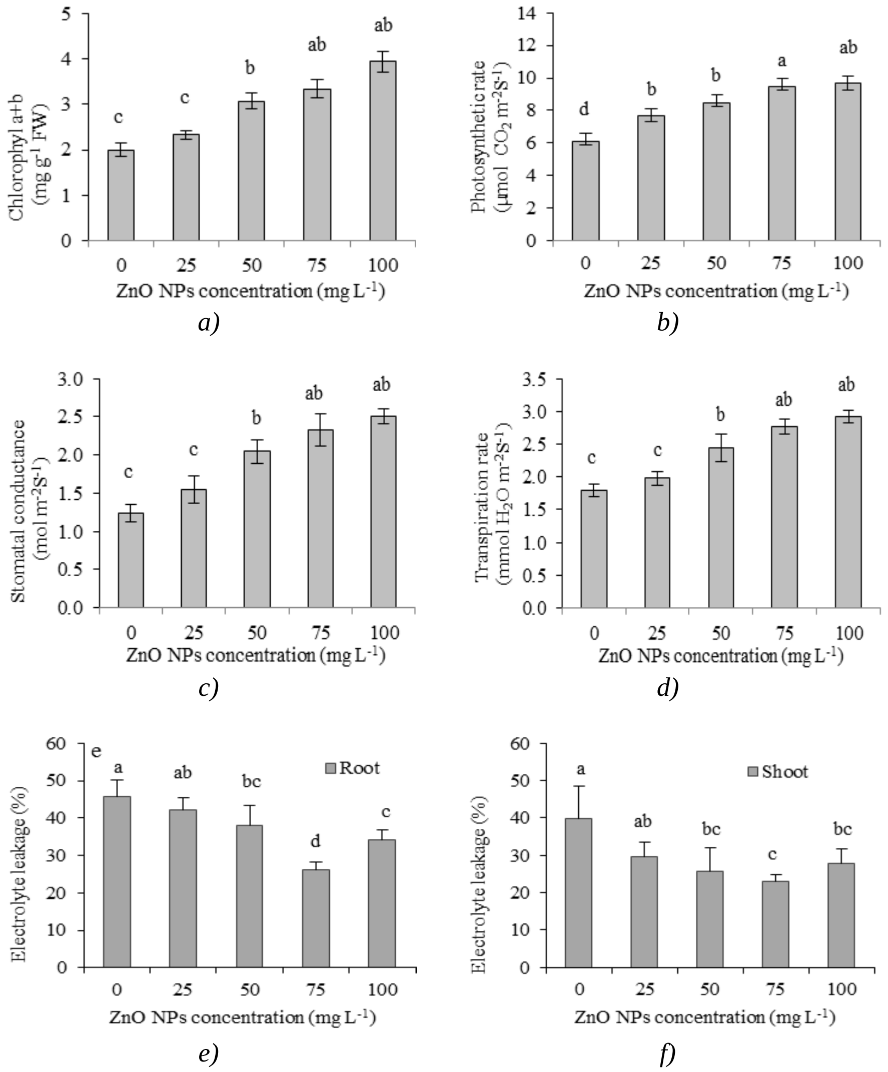



*Fig. 4. Effect of different concentrations of ZnO NPs on chlorophyll contents (a), photosynthetic rate (b), stomatal conductance (c), transpiration rate (d), and root and shoot electrolyte leakage (e, f) of wheat. Error bars indicate the standard deviation (SD) of four replicates. Different letters on the histograms indicate the significant differences among the treatments at a P< 0.05.*

### 3.4. Zinc concentration in plants

Zinc concentration linearly increased in shoot, root and grains than control plants (Figure 5). As compared to control, Zn concentration in shoots increased by 25, 43, 51, 65%, in roots increased by 20, 21, 29, 43%, and in grains increased by 8, 35, 50, and 64% respectively.

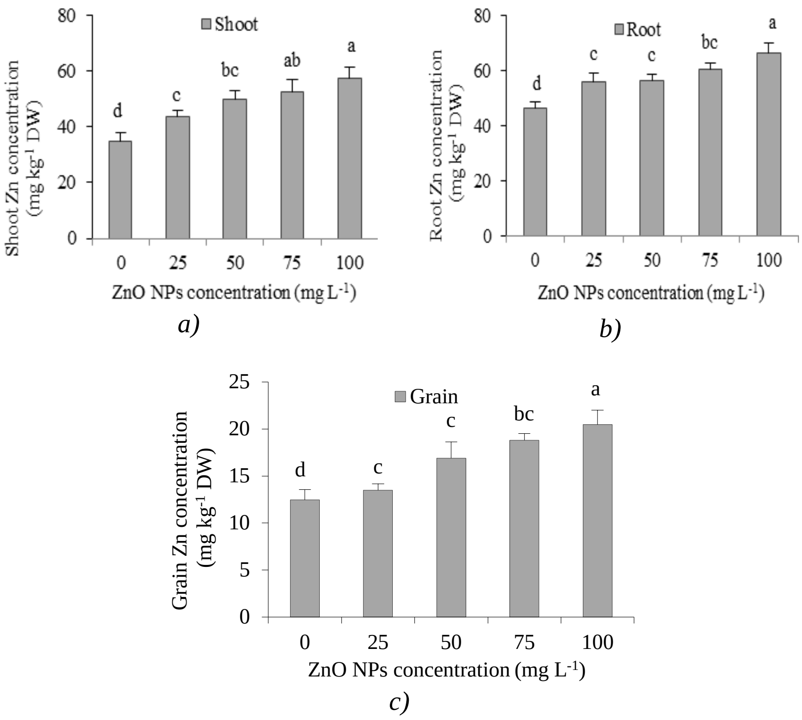



*Fig. 5. Effect of different concentrations of ZnO NPs on Zn concentrations in shoots (a), roots (b), and grains (c) of wheat. Error bars indicate the standard deviation (SD) of four replicates. Different letters on the histograms indicate the significant differences among the treatments at a $P< 0.05$.*

## 4. Discussion

The solution evaporation technique has been selected for the synthesis of ZnO NPs due to its unique features of controllable synthesis at ambient temperature, economical and cost effective methods compared to physical vapor deposition (PVD) and chemical vapor deposition (CVD). The XRD analysis indicates (Fig.1a) the formation of ZnO NPs (34.4nm) of highly crystalline hexagonal wurtzite structure which is most stable among zinc blende, and rocksalt structure. Whereas the SEM analysis (Fig.1b) demonstrate the formation of nanoparticles of different shape and size. These nanoparticles of different shape and size are linked to each other resulted in the formation of complex nanostructure.

Our results showed that ZnO NPs increased the plant growth and photosynthesis than control (Figure 2-4). The current results clearly indicate that ZnO NPs are effective in enhancing plant growth and yield. It was reported that foliar application of Zn nano fertilizer increased the growth, leaf protein, and chlorophyll contents of peal milt than control and ordinary Zn application [23]. [24] showed that 50 ppm Zn NPs reduced the seed germination and root length of lettuce than control. This reduction in growth might be due to experimental conditions and the increase in growth in current study. (Figure 2-4) indicates that seed priming might be a suitable method to enhance plant growth with Zn supply. The higher plant growth with NPs might be due to the mobilization of nutrients in the soil such as phosphorus as well as increase in microbial population especially in the rhizosphere [25].

Seed priming with ZnO NPs increased the Zn concentrations in roots, shoots and grains than control (Figure 5). The effect of Zn uptake from NPs depends upon the doses and experimental conditions [8]. It was reported that Zn concentrations in shoots of cucumber plants were lower with Zn NPs (125 mg $kg^{-1}$) than Zn concentration of 25 mg $kg^{-1}$ which might be due to root deformation under higher Zn levels [26]. On the other hand, it was reported that the increase in Zn concentrations in plants with NPs application might be due to higher penetration of NPs into plant cells [9] but the penetration and transport mechanisms of NPs different size (1~100 nm) and shape of nanostructure are not quite clear and more studies are needed in this regard [8].

Thus, our results showed that seed priming might be an effective method for enhancing grain Zn concentrations in plants. Overall, our results can help the fertilizer industries to decide the production of nanofertilizers especially ZnO NPs for plant nutrition which will help to reduce the Zn deficiency in plants and finally humans.

## 5. Conclusions

ZnO nanoparticles were synthesized using solution evaporation method. The XRD analysis showed the hexagonal wurtzite structure of NPs with an average crystalline size of 34.4nm.The SEM analysis revealed that NPs contained small, bunch of irregular and round shape particles.

Plant growth, photosynthesis, biomass and Zn concentrations in roots, shoot and grains increased linearly with the seed priming (0, 25, 50, 75, 100 ppm) of ZnO NPs than control. Thus, ZnO NPs could be used as a source of Zn to reduce Zn deficiency in cereals. However, field studies under different nanostructure sizes, shapes, conditions and plants may further enhance the mechanistic understanding of the applicability of NPs in this field.

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