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ASSESSMENT OF NUTRITIONAL QUALITY, YIELD AND ANTIOXIDANT ACTIVITY OF *TRITICUM AESTIVUM* TREATED WITH ZINC OXIDE NANOPARTICLES

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The extensive use of nanoparticles in all sectors including agriculture has led to concern about their long-term exposure effects to our ecosystem. In this research work we investigated the effect of synthesized ZnO nanoparticles (ZnO NPs) on *TRITICUM AESTIVUM*. ZnO NPs were synthesized by chemical method and further characterized by X-ray Diffraction (XRD), Fourier Transform Infrared Spectroscopy (FTIR) and Scanning Electron Microscopy (SEM). Wheat was grown in soil by applying two concentrations (10 and 20 ppm) of ZnO NPs and Bulk ZnO through seed priming method. Growth, Yield, Total Phenolic Contents (TPC) and DPPH radical scavenging activity was monitored. Atomic absorption Spectroscopy (AAS) was used to determine concentration and uptake of elements in roots, shoots and grains. XRD and SEM results confirmed the formation of pure hexagonal wurtzite structure ZnO NPs. There was increase in growth and yield parameters by ZnO NPs treatments. ZnO NPs 10 ppm treatment significantly increased plant height, biomass, number of spikelets per spike, spike length, number of grains per spike compared to control and ZnO bulk treatments. ZnO NPs treatment at 10 ppm increased TPC by 60.37% compared to control group. The AAS results indicated increased Zinc content in roots by 88.89% and 112.59% in ZnO NPs 10 ppm and 20 ppm group respectively compared to Control. The Iron concentration in ZnO NPs at 10 ppm treated group grains increased by 113 % indicating improved nutritional quality of plant. It can be concluded that ZnO NPs application in minor concentration exhibits stimulatory effect on overall plant growth and grain nutrition.

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Keywords: Wheat, DPPH radical scavenging, Total phenolic content, Atomic absorption Spectroscopy

1. Introduction

Nanoparticles have ability to change agronomic parameters such as plant height, biomass and can affect the productivity, quality and yield of edible plants grown upto full maturity. But a very limited knowledge is present in literature regarding their long-term exposure on plant growth. Nanotechnology in the agriculture field seems very promising but the risk of health safety of NPs use in agriculture is also prevalent. The NPs effects on various plant species may vary depending upon stage of plant growth, exposure method, duration, NPs size, shape, concentration, and chemical composition. As there is a very limited literature on the NPs effects on plant covering all aspects of how they interact with plants and what changes in plants morphological, biochemical parameters, and especially nutrient content and quality of product occur So, there is need to focus in this area and study in detail NPs interaction with plants.

Zinc Oxide nanoparticles (ZnO NPs) have specific physical, chemical and antibacterial applications which makes them suitable for various applications. ZnO have potential to improve growth and yield of food crops. Zinc is a very important nutrient for plant growth as it is part of

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different proteins and enzymes. Zinc plays major role in different enzymes catalytic activity in plants by either being a part or initiates action of enzymes involved in protein synthesis, metabolism of carbohydrate or cellular membranes maintenance [1]. Wheat cultivation in 2017-18 resulted in 25.492 million tonnes production which added value to 1.7% to GDP of Pakistan. The production shortfall is accounted due to different factors such as declined sowing area, acute water shortage and prolonged season of sugarcane crush. Being a staple diet, it is needed that not only wheat yield but also quality of grains should be improved. The literature shows the positive impact of ZnO NPs on various plants. ZnO NPs increased the pod yield, early flowering and higher chlorophyll content of peanut plant [2]. Increase in 1000 grain weight, seed weight per umbel and seeded fruit per umbel was observed by application of ZnO nanoparticles [3]. ZnO NPs application on wheat has been carried out to study the effect on germination and early growth. ZnO NPs enhanced the seed germination and growth in hydroponic medium [4]. ZnO NPs showed no toxic effect on wheat seed germination and root/shoot growth and increased protein and chlorophyll content [5]. Different varieties of Durum wheat treated with ZnO NPs showed no toxic effect on germination and development parameters and improved seed germination in one variety [6]. ZnO NPs were applied in different media to study their effect on wheat growth and uptake of elements. Wheat was grown in sand (for 14 days) to determine metal bioaccumulation and oxidative stress induction [7] and was also grown in alkaline and acidic soil (for 7 days) to determine effect on root/shoot length and elements uptake [8]. Some research work has been reported on ZnO NPs effect on wheat germination and early growth but only few report affect of ZnO NPs on wheat yield, especially detailed study of wheat grain and its composition is required before its consumption by end users in order to check crop biosafety. In this work we present the interaction of ZnO NPs with wheat in soil medium and its effect on growth, yield, antioxidant activity, uptake bioaccumulation of zinc, with quantitative analysis of different elements present roots, shoots and grains of wheat.

2. Materials and methods

2.1. Synthesis of nanoparticles

Zinc Oxide nanoparticles (ZnO NPs) were prepared in the laboratory by chemical synthesis method [9]. This procedure was adopted with modifications as it is comparatively cheap and easy to handle. Zinc acetate dihydrate was used as precursor and sodium hydroxide as a basic solution. The basic solution (4g/100ml) was added drop-wise to the precursor solution (4.23/100ml), as a result white precipitates were formed which were filtered and later washed using distilled water 3 times. The obtained powder was oven dried at 60 ºC for 2 hours. To study the effect of post-synthesis calcination temperature on NPs size three samples were prepared adopting similar procedure and calcination was performed in muffle furnace at 400 ºC, 500 ºC and 600 ºC for 2 hours, to obtain crystalline ZnO NPs. The obtained NPs were grinded using pestle and mortar and further characterized by X-ray Diffractometer (Pro PANalytical) with Cu Kα- radiation (λ= 1.5496 Å) at 40 KV. The step counting method was used to record intensity data (with a 0.05º/s scanning speed) in the 2θ range (from 20º to 80º). The obtained peaks were matched with standard JCPDS card and particle size and lattice parameters were calculated [10]. Surface morphology was studied by scanning electron microscopy (JSM-6490 JEOL). For FTIR analysis the sample was used in solid pellet form and was analyzed by spectrum 2, PerkinElmer.

2.2. Experimental design

To determine the effect of ZnO NPs on wheat (var. Ujala approved in 2016) growth and yield soil was used as growth medium. The fertile field soil was prepared for experiment by heating, grinding and sieving and making a homogeneous soil. Equal amount of soil was added in each plastic pot. Recommended dose of NPK fertilizer was added in the soil and water was added according to field capacity. Seed priming method was used for treatment of wheat seeds. ZnO NPs and ZnO bulk solutions were prepared by adding measured amount of powder in distilled water and sonication was done for 10 minutes. Two treatments of ZnO NPs (10 ppm and 20 ppm) and

two treatments of ZnO Bulk (10 ppm and 20 ppm) were used along with one control treatment (without any Zinc, only distilled water). Seeds were washed and disinfected before use. Seeds were soaked in the corresponding solution for 4 hours and were then transferred to the pots. Seven seeds were sown in each pot at equal distances. Three replicates of each treatment were used. The experiment was monitored regularly, and distilled water was given to each pot according to field capacity when required. After harvesting of wheat, morphological and yield parameters such as plant height, plant weight, Spike length, number of spikelets/ spike, Grain yield per spike, number of grains per spike, 1000 Grain weight, number of grains per pot, Grain yield per pot were recorded. Flowchart of the conducted experiment is shown in Fig. 1.

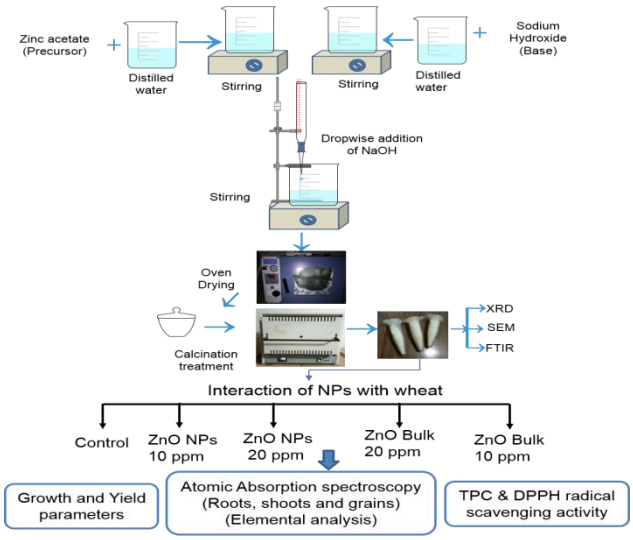

Fig. 1. Experimental layout of ZnO nanoparticles synthesis, characterization and investigation of their effect on wheat growth, yield, Elemental composition and biochemical parameters.

2.3. Statistical analysis

Completely randomized design was used as experimental layout with three replicates of each treatment. One way Anova was used to find the significance of each treatment at p value less than 0.05. For pairwise comparison means of each treatments LSD (least significance difference) test at $p < 0.05$ was employed.

2.4. DPPH (1,1 diphenyl 2 -picrylhydrazyl) scavenging activity

The antioxidant activity of wheat leaves was performed by using crude leaf extract along with its polar fraction and measuring their ability of scavenging of free radicals of DPPH. The procedure was followed with slight changes as adopted by [11]. Equal volume of crude leaf extract along with polar fraction was added in 500 µl ethanolic DPPH solution (0.1mM). The incubation of sample was done at room temperature for 30 minutes and afterwards absorption was measured at 517 nm. For standard control BHT was used. The DPPH assay was carried out as percentage inhibition of DPPH free radical using the following relation.

$$\% \text{ inhibition} = \frac{[(\text{Abs}_{\text{control}} - \text{Abs}_{\text{Sample}})]}{\text{Abs}_{\text{control}}} \times 100 \quad (1)$$

where $\text{Abs}_{\text{sample}}$ is the test sample absorbance and $\text{Abs}_{\text{control}}$ is control reaction mixture absorbance (excluding test compounds).

2.5. Total Phenolic content (TPC) determination

The Folin Ciocalteu reagent technique was used to determine TPC as adopted by [12]. For this purpose, FC reagent (200µl) was added to in 100 µl of sample and thoroughly vertex. 800 µl of Na_2CO_3 (700mM) was added to sample and incubated for 2 hours at room temperature. In a clear 96 well-plate prepared solution was transferred and at 765 nm absorbance was recorded. TPC amount was calculated with the use of Gallic acid calibration curve. The results are expressed in the form of Gallic acid equivalent (GAE) per dry matter.

2.6. Elemental analysis

To monitor the uptake, the concentration of different macronutrients and micronutrients (Zinc, Iron, Calcium and copper) in different parts of wheat was determined by atomic absorption spectroscopy (AAS). The samples were prepared through wet acid digestion method. For this purpose powder sample of different parts of wheat plant (root, shoot and grain) were prepared separately for each treatment by drying and grinding to make fine powder. A measured amount of sample powder (250 mg) was taken and 2.5 ml of nitric acid (HNO_3) and perchloric acid ($HClO_4$) were added in 2:1 ratio. The sample was covered and kept overnight. Then the sample was heated on a hot plate. The fumes arouse when sample was fully boiled and later on sample became colorless. At this point the sample was removed from hot plate and was filtered by adding pure distilled water. Total volume of 25 ml of each sample was made by adding distilled water. The sample was stored in the bottle and labeled. The elements present in the samples prepared by above mentioned method were determined using Atomic Absorption Spectrophotometer (Hitachi Polarized Zeeman, Z-8200, Japan). The elements were detected at the following wavelengths: Zinc (213.9 nm), Calcium (422.7 nm), Iron (248.3 nm) and Copper (324.8 nm).

3. Results and discussions

Fig. 2 shows the pattern of the synthesized Zinc Oxide nanoparticles (ZnO NPs) using XRD. Different peaks at (100), (002), (101), (102), (110), (103), (112) and (201) confirmed the hexagonal wurtzite structure of ZnO powder confirmed by JCPDS 36-1451 data. Purity of the samples was evident as there were no additional peaks related to impurities. The average size was calculated by using Debye Scherrer's formula (equation 2), which was found to be 33 nm.

$$D=\frac{k\lambda}{\beta\cos\theta} \tag{2}$$

The lattice parameter a and c were also calculated [13]. Different crystalline parameters calculated for the ZnO NPs synthesized at different calcination temperatures are given in Table 1. The results show that crystalline size increased from 33.67 nm at 400 ºC to 57.53 nm at 600 ºC. It can be observed that crystalline size increased with increase in calcination temperature due to fusion of the atoms present at the surface.

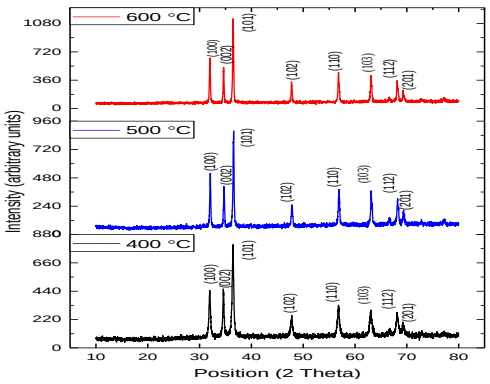

Fig. 2. XRD pattern of synthesized ZnO NPs at postsynthesis calcination temperature of 400 ºC, 500 ºC and 600 ºC.

Table 1. Crystalline size, lattice parameters (a and c), volume and density of sample calculated from XRD data of ZnO NPs samples synthesized at different calcination temperature.

Furnace temperature	**Crystalline size**	**Lattice parameter a (Å)**	**Lattice parameter c (Å)**	**c/a ratio**
400 ºC	33.67	3.23	5.17	1.60
500 ºC	51.37	3.23	5.17	1.60
600 ºC	57.53	3.24	5.17	1.595

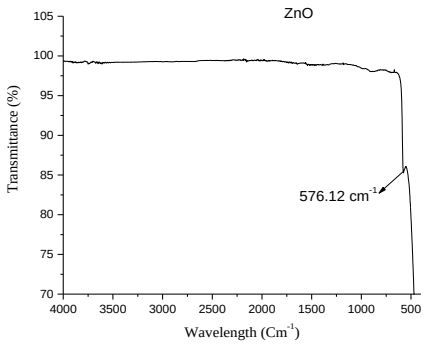

Fig. 3. FTIR spectrum of synthesised ZnO nanoparticles.

The FTIR spectrum of synthesized ZnO NPs in Fig. 3 shows a peak at 576.12 cm^{-1} which represents metal-O bond showing the formation of ZnO NPs. SEM image of ZnO NPs indicated individual as well aggregated particles. The analysis showed relatively spherical shape ZnO NPs having diameter range of 30-40 nm. Aggregated particles in 10 μm scale are shown in Fig. 4.

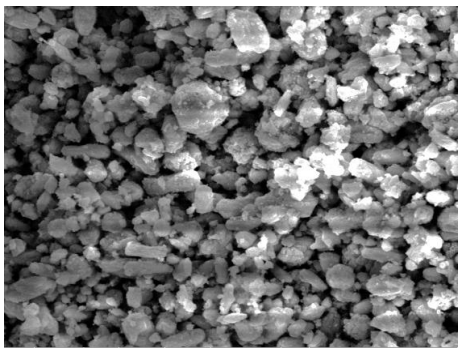

Fig. 4. SEM image of synthesized ZnO NPs.

The growth and yield parameters are presented in Fig. 5. Among wheat growth parameters the maximum height (56.50 cm) was achieved by ZnO NPs 10 ppm treatment while lowest value (50.80 cm) was obtained by control (untreated) samples. ZnO NPs at 10 ppm increased plant height compared to control by 11.24% while ZnO bulk at 20 ppm increased plant height by 8.96% compared to control. ZnO NPs at 10 ppm increased total biomass by 12.85% compared to control samples while ZnO bulk at 10 ppm increased biomass by 7.14% compared to control. Different methods of ZnO NPs application (seed priming, foliar spray, seed priming with foliar spray) increased tomato plant root length, shoot length and their dry weight and yield compared to control and Bulk $ZnSO_4$ [14].

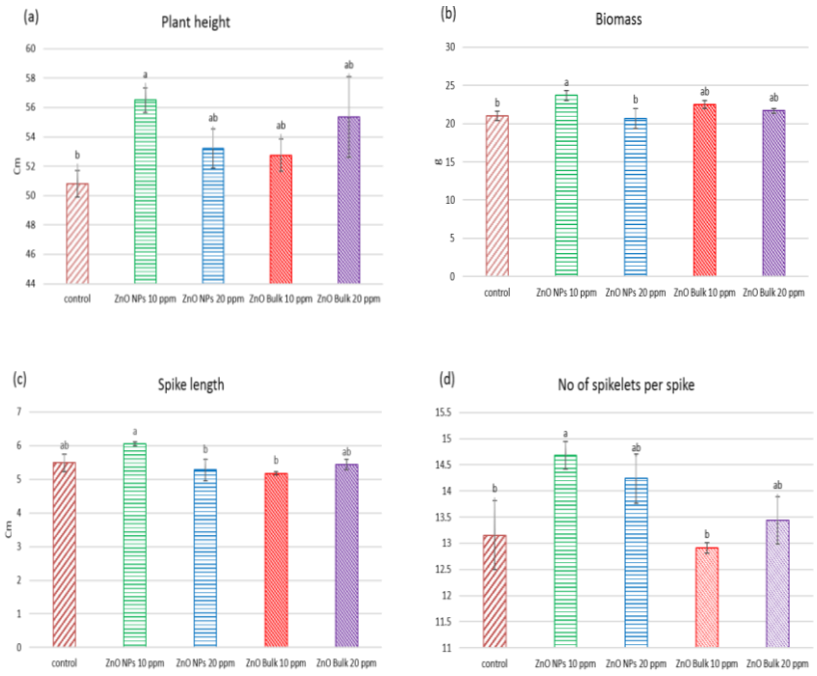

Fig. 5. Growth and Yield parameters of Control and treated wheat plants.

Table 2. Effect of ZnO nanoparticles and ZnO bulk on various yield parameters of wheat.

Treatment	Grains per spike	No. of Grains per pot	Grain weight per pot	1000 Grain weight
Control	36.15^{b}	142^{ab}	4.31^{a}	33.7^{a}
ZnO NPs 10 ppm	43.98^{a}	167.33^{a}	4.59^{a}	37.2^{a}
ZnO NPs 20 ppm	35.92^{b}	134.66^{b}	4.11^{a}	34.4^{a}
ZnO Bulk 10 ppm	36.62^{b}	162^{ab}	4.56^{a}	34.1^{a}
ZnO Bulk 20 ppm	38.68^{ab}	152.33^{ab}	4.42^{a}	34.2^{a}

ZnO NPs treatment improved various yield parameters of wheat. The spike length increased only for ZnO NPs 10 ppm treated wheat group by 9.26% compared to control samples. The no. of Spikelets per spike increased in ZnO NPs 10 ppm and 20 ppm treated groups by 11.59% and 7.89% respectively compared to control wheat plants. ZnO bulk 10 ppm showed a decrease in number of spikelets per spike but ZnO bulk 20 ppm did not affect the number of spikelets/spike. The number of grains per spike increased by ZnO NPs 10 ppm treatment while slightly decreased for ZnO NPs 20 ppm treatment. ZnO NPs 10 ppm treatment increased no. of grains per spike by 21.65% while for ZnO bulk 20 ppm increased by 6.99%. ZnO NPs 10 ppm increased grains/spike by 13.7% compared to ZnO bulk 20ppm treatment (Table 2).

ZnO NPs 10 ppm treatment increased no of grain/pot by 17.38% compared to control while increase by 14.08% and 7.27% was observed by ZnO bulk at 10 ppm and 20 ppm respectively compared to control. Application of copper NPs in soil significantly enhanced growth of wheat at 30 ppm concentration [15]. Grain weight per pot increased by ZnO NPs 10 ppm treatment compared to control. ZnO bulk 10 ppm also enhanced grain weight/pot. ZnO NPs and

ZnO bulk 10 ppm increased grain weight/pot by 6.49% and 5.8% respectively. ZnO NPs 10 ppm treatment increased 1000 grain weight among all treatments. No significant increase was observed by other treatments compared to control. An increase of 10.39% was observed by ZnO NPs 10 ppm compared to control treatment. An increase in wheat plant height and shoot biomass has been observed in soil amended with nano-CeO_2. Among yield traits increase in spikelet's, number per spike, grain number per spike, grain yield per spike and yield per pot was observed [16]. Different concentrations of silver NPs (25-150 ppm) in soil increased yield parameters of wheat. No. of grains per spike was significantly high (29) at 25 ppm and weight of 100 grain was maximum (4.73g) at 50 ppm followed by 4.66g at 25 ppm [17].

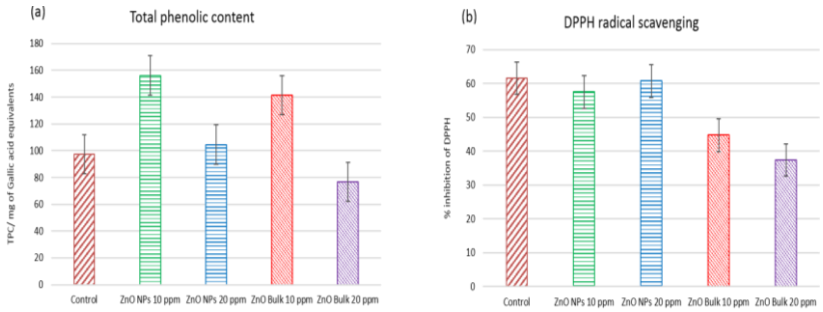

Fig. 6. Effect of different treatments on Total phenolic content and % inhibition of DPPH.

All treatments of ZnO NPs and ZnO bulk increased total phenolic content in wheat leaves except ZnO bulk 20 ppm treatment. The highest TPC (156.2) value was of ZnO NPs 10 ppm treated wheat plant while lowest value (76.8) was obtained for ZnO bulk 20 ppm samples. ZnO NPs treatment at 10 ppm and 20 ppm increased TPC by 60.37% and 7.39% compared to control samples. Also ZnO bulk increased TPC by 45.17% at 10 ppm concentration while decreased by 21.15% at 20 ppm concentration. The literature shows that foliar spray of ZnO NPs at 300 and 500 ppm concentration significantly increased phenolic content by 20 and 22% in potato [18]. ZnO NPs treatment of Glycyrrhia glabraa seedling at 10µM concentration showed higher phenolic content compared to bulk ZnO and control treatment [19].

The Fig. 6 shows that DPPH radical scavenging activity did not change significantly by ZnO NPs treatments but a significant reduction in ZnO bulk treated plant leaves is observed. The highest DPPH inhibition percentage value was of control samples while lowest value was of ZnO bulk 20 ppm treated samples. DPPH inhibition value decreased for ZnO NPs by 6.55% while no significant difference was observed for ZnO NPs 20 ppm treated samples. Percentage inhibition of DPPH decreased in ZnO bulk 10 ppm and ZnO bulk 20 ppm by 27.33% and 39.2% respectively compared to control samples. The variation in radical scavenging activity was observed in seedling of Brassica nigra treated by ZnO NPs as DPPH inhibition % increased and decreased at different NPs concentration while phenolic content increased with ZnO NPs treatment [20]. It is found that ZnO NPs treatment affected antioxidant activity by increasing phenolic content compared to control which shows activated defense system of treated wheat plants against external factors that may affect wheat metabolism.

The AAS results (Fig. 7) show that ZnO NPs at 10 ppm and 20 ppm increased Zinc content in roots by 88.89% and 112.59% respectively compared to control while ZnO Bulk 10 ppm treatment increased Zn content by 18.52% compared to control. This can be due to the small size of ZnO NPs compared to bulk ZnO which helps in better penetration and uptake of particles. Zinc content in shoots was only increased in ZnO bulk 20 ppm while decreased in all other treatments. ZnO NPs 20 ppm treatment increased Zinc content by 10.81% compared to control. The elemental analysis results of wheat seeds showed that no significant change was observed in Zinc content except for ZnO NP 10 ppm treatment. Among all treatments only ZnO NPs 10 ppm treatment increased Zinc content by 7.55% compared to control. ZnO NPs 20 ppm and ZnO bulk

20 ppm treatments did not show any significant impact on Zinc content while a decrease was observed at ZnO bulk 10 ppm treatment. This shows that the ZnO NPs taken by plant roots are not translocated in plants shoots and grains. It shows the safety of the crop yield and product. Further these NPs can be used to enhance certain mineral content in grains, can be used for biofortification of food products.

In roots Iron concentration increased by ZnO NPs at 10 ppm and 20 ppm. ZnO bulk 10 ppm increased Iron concentration while ZnO bulk 20 ppm reduced Iron concentration in roots. ZnO NPs and ZnO Bulk treatments at 10 ppm concentration increased Fe content by 61.85% and 35.26% respectively compared to control. In shoots Fe concentration increased only for ZnO NPs 20 ppm treatment and it reduced for all other treatments in shoots. ZnO NPs 20 ppm treatment increased Fe content by 139.41% compared to control. In seeds Iron content increased in all treatments compared to control. ZnO NPs at 10 ppm and 20 ppm increased Fe content by 113% and 10.76% while ZnO bulk at 10 ppm and 20 ppm increased Fe content by 84.30% and 48.88% respectively compared to control. Zinc and iron uptake are related to each other. In our experiment we find a significant increase in iron and zinc content in roots of ZnO NP 10 ppm treatment group compared with all other treatments.

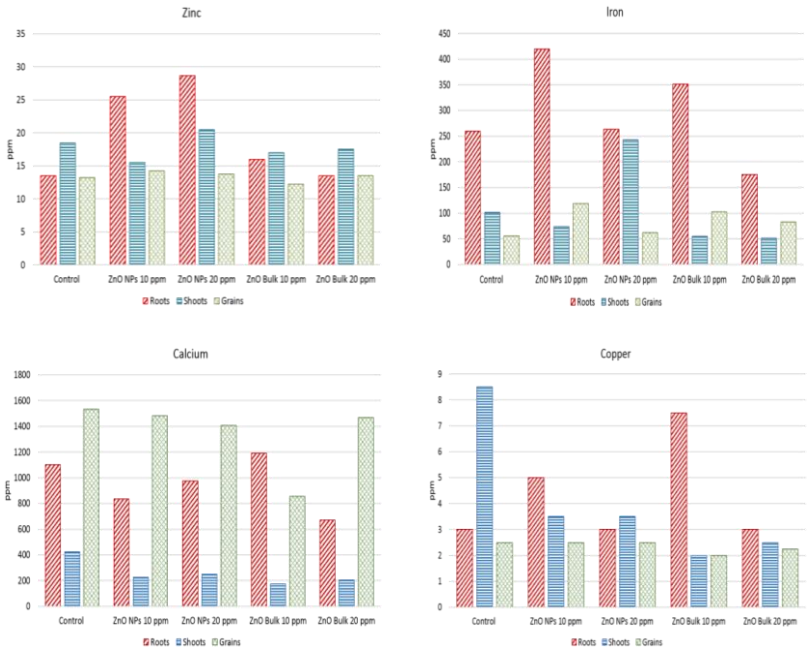

Fig. 7. Concentration of different elements (Iron, Zinc, calcium and copper) in roots, shoots and grains of control and treated Wheat groups.

In roots ZnO NPs 20 ppm treatment increased Ca content by 45.5 % compared to ZnO bulk 20 ppm treatment. In shoots ZnO NPs 10 ppm treatment increased Ca content by 28.57 % compared to ZnO bulk 10 ppm treatment while ZnO NPs 10 ppm increased Ca content by 21.95 % compared to ZnO bulk 20 ppm treatment. In grains ZnO NPs 10 ppm treatment showed increased Ca content compared to ZnO bulk 10 ppm treatment by 73.1%. The maximum calcium content in grains was of control (1531.25 ppm) while minimum value was of ZnO bulk 20 ppm treatment (856.25 ppm). It can be observed that ZnO NPs 10 ppm treatment increased Ca content in shoots and grains compared to ZnO bulk 10 ppm treatment while ZnO NPs 20 ppm increased Ca content in roots and shoots compared to ZnO bulk 20 ppm treatment. The copper concentration was not affected significantly in grains by any treatment and all ZnO NPs and ZnO bulk treatments showed same Cu concentration compared to control.

Various studies have shown NPs accumulation in the roots of plants and also translocation to leaves, seeds and fruits is observed only sometimes [21, 22]. The extent of NPs accumulation depends on physicochemical parameters (shape, size, agglomeration state, zeta potential, chemical composition) and plant species. It can be concluded that ZnO NPs treatment to wheat plant in soil

increased Zn content in roots due to direct interaction. The increase in Zn concentration in ZnO NPs treated wheat roots was higher compared to ZnO bulk treatment which can be due to small size and high reactivity of ZnO NPs. The concentration of Zn in shoots did not increase compared to roots which shows less translocation of ZnO NPs from root to shoot. Furthermore, in grains Zn concentration did not vary among treated and control wheat samples which may be associated with no translocation of Zn from shoots to grains. Our results correlate to results reported by Lin and Xing, 2008 which described the uptake of ZnO NPs with 20nm diameter in hydroponic medium by ryegrass roots and very limited NPs translocation to shoots was seen . It can be seen that ZnO NPs increased Fe content in grains and Calcium content was also in suitable amount compared to control. So it can be concluded that ZnO NPs did not induced any toxicity and showed only positive stimulatory effects by improving growth, yield and food quality.

4. Conclusion

It is concluded that the synthesized ZnO NPs were of appropriate size and shape for agricultural application. ZnO NPs showed positive stimulatory effect on wheat plant growth and yield parameters. The antioxidant activity increased which showed activated defense system of wheat. The ZnO NPs treatment improved the uptake of Zinc in roots as compared to ZnO Bulk group. Nutritional quality of wheat grains was improved without elevating Zinc content as zinc translocation from roots to shoots and further grains was not observed. Use of ZnO NPs in optimum concentration can have stimulatory effect on plant and further they can be used for biofortification of various crops.

Disclosure statement

No potential conflict of interest was reported by the authors.

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