Pak. J. Agri. Sci., Vol. 56(4), 879-884; 2019
ISSN (Print) 0552-9034, ISSN (Online) 2076-0906
DOI: 10.21162/PAKJAS/19.8544
http://www.pakjas.com.pk

IMPACT OF NANOSIZED AND BULK ZnO ON GERMINATION AND EARLY GROWTH RESPONSE OF *Triticum aestivum*

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Enormous use of manufactured nanoparticles in various fields has motivated researchers to assess their potential applications in agriculture along with serious concerns of toxicity on various plant species. When we are going to study the effects caused by nanoparticles on plants, their bulk counterparts should also be investigated in order to have comprehensive comparative knowledge. In present study the effects of Zinc oxide nanoparticles (ZnO NPs) and ZnO Bulk powder on seed germination and early growth parameters of wheat plant (*Triticum aestivum*) were investigated. Co-precipitation technique was used to synthesize ZnO NPs and X-ray diffraction (XRD) and Scanning electron microscopy (SEM) were used to characterize these NPs, which indicated formation of pure wurzite hexagonal ZnO nanoparticles with average size of 35nm. These synthesized ZnO NPs and ZnO bulk powder were applied to wheat seeds of variety Ujala approved in 2016. Wheat seeds were divided into three groups i) control seeds with no treatment ii) Seeds treated with ZnO bulk and iii) Seeds treated with ZnO NPs. Experiment was performed in complete randomized design (CRD) with three replicates of each group, at two different concentrations (300ppm and 600ppm) of each treatment of ZnO NPs and ZnO bulk. Seed germination and early growth parameters were monitored and obtained results were analysed statistically using one-way ANOVA and the mean comparisons were made by Tukey HSD (Honest significant difference) test at 5% significance level. At 300ppm and 600ppm concentration, wheat mean germination time and time span of germination increased in ZnO NPs treated group. Shoot length, shoot weight and vigor index II also enhanced significantly at ZnO NPs 300 ppm treatment compared to ZnO bulk and control group. These results indicate that low concentration of ZnO NPs can have stimulatory effect on wheat germination and growth parameters.
Keywords: Nanotechnology, nanomaterial, ZnO NPs, wheat germination, vigor index, ZnO bulk

INTRODUCTION

Nanotechnology is described as the process of formation, manipulation and use of nanomaterials. Nanomaterials have widespread applications in electronics, materials science, energy, manufacturing, agriculture, medical diagnosis and health care. Nanoparticles (NPs) are in the range of 1-100 nm in at least one dimension and due to very small size and high surface to volume ratio, NPs have unique properties different from their bulk counterparts. Nanoparticles synthesis and their applications are increasing rapidly and it is predicted that by 2020 their production will increase to 58000 tons (Maynard *et al.*, 2006). So there are chances that NPs can enter into terrestrial and aquatic life accidently, which will lead to enhanced exposure to living organisms. Plants form a major portion of ecosystem, which are consumed by both humans and animals. Plants have direct exposure to nanoparticles through waste water, soil, underground water, air, land corrosion, fertilizer etc. They can play a vital role in nanoparticles movement by uptake, accumulation and final transfer to the food chain. Therefore it is important to understand the nanoparticles interaction with plants and their effects at different development stages of plant (Aslani *et al.*, 2014). Studying the effects of different nanoparticles on different plant species and crops is important to have an idea about phytotoxicity of nanoparticles which can be present in our environment and their possible dangerous long term effect on ecosystem, food and fruit quality and finally human health. On contrary, some researchers are working on the possible applications of nanomaterials in agriculture including nanosensors, nanofertilizers, nanopesticides and smart drug delivery system etc. (Duhan *et al*., 2017). These are supposed to be better than conventional methods in improving growth and production of crops, increasing the fertilizer use efficiency, minimizing nutrient loss and reducing hazardous impacts of fertilizers on environment (Liu and Lal, 2015).

Before widespread use of these nanomaterials in agriculture, a thorough study on their possible positive or toxic impact on different plant and crops species should be carried out (Prasad *et al.*, 2014).

Zinc Oxide (ZnO) NPs are one of the most abundantly synthesized and commercially used NPs due to wide applications. Therefore their ecotoxicity should be investigated carefully. ZnO NPs have potential to increase agriculture yield as they have great physical and antimicrobial properties and also zinc is the important micronutrient for plants growth (Noulas *et al.*, 2018). It is a vital micronutrient for plant as it is part of different oxidation- reduction reactions, metabolic processes, and enzymatic reactions. Zinc deficiency in plants can result in stunted growth, chlorosis, decreased tiller numbers, small leaves, spikelet sterility, delay in crop maturity and poor quality crop products (Hafeez *et al.*, 2013).

Many researchers have investigated the interaction of nanoparticles with different plants. Previous studies show that ZnO NPs can increase growth and yield of crops (Sabir *et al.*, 2014). Application of carbon nanotubes enhanced the seed germination and growth rates of tomato. The ability of these nanoparticles to penetrate the seeds was observed which enhanced germination by increasing water uptake (Khodakovskaya *et al.*, 2009). TiO_2 NPs enhanced the germination percentage, germination rate index, vigor index, shoot and root fresh weight, plant length, and also chlorophyll content of parsley seedlings. (Dehkourdi and Mosavi, 2013). ZnO NPs enhanced the protein and chlorophyll content significantly in wheat seeds (Ramesh *et al.*, 2014). The effect of different ZnO nanoparticles concentrations on tomato, cucumber and alfalfa showed that seed germination of cucumber seeds was enhanced (de la Rosa *et al.*, 2013). The translocation, uptake and effect of nanoparticles varies with plant species, mode of application (seed priming, foliar), growth medium (hydroponic, soil, sand, MS medium), nanoparticles properties (stability, size, shape, coating, surface) stage of plant and overall environmental conditions (Yang *et al.*, 2017).

In this research work, we have selected wheat for investigating effect of ZnO NPs because it is the important staple food of many people in Pakistan and the by-products are used in various industries all across the world. Low concentration of nanoparticles was used in this experiment and tested on latest wheat variety i.e., Ujala 2016, being commonly used by Pakistani farmers. To best of our knowledge, there is no published report about effect of ZnO nanoparticles on wheat variety Ujala-2016 so this work can provide useful information for their future use in the growth of wheat. The bulk counterpart (ZnO) in same concentration was also investigated for comparison under similar conditions, in order to know the efficiency of synthesized nanoparticles.

MATERIALS AND METHOD

***Synthesis of nanoparticles*:** Zinc Oxide nanoparticles (ZnO NPs) were prepared in the laboratory by chemical synthesis method (Gnanasangeetha and Sarala, 2013). 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 70°C for 2 hours and then kept in muffle furnace at 400°C for 2 hours, to obtain crystalline ZnO NPs. The obtained NPs were grinded using pestle and mortar and further characterized by using powder sample. X-ray Diffractometer (Pro PANalytical) with Cu Kα- radiation (λ= 1.5496 Å) at 40 KV was used. The step counting method was used to record intensity data (with a 0.05°/s scanning speed) in the 2θ range (from 20 to 80°). Also surface morphology was characterized by scanning electron microscopy (JSM-6490 JEOL). The obtained peaks were matched with standard JCPDS card and particle size and lattice parameters were calculated (Raoufi, 2013).

***Experimental design*:** Wheat (*Triticum aestivum* var. Ujala 2016) seeds were taken from Ayub Agriculture research institute Faisalabad, Pakistan. The healthy seeds with uniform size were selected randomly to minimize errors. The seeds were surface sterilized in 5% sodium hypochloride solution for 15 min and then rinsed with distilled water for several times to remove excess of chemical. Selected seeds were divided in three major groups: Control, treated with ZnO NPs and treated with ZnO bulk. A complete randomized design (CRD) with three replicates of each treatment was used to understand the effects of different size and concentration of ZnO NPs on wheat germination parameters. The experiment included two concentrations (300 ppm) and (600 ppm) of both ZnO NPs and ZnO bulk along with control where only distilled water was used. Sterilized Petri dishes of 9 cm diameter were taken and double layer of filter paper was fitted in them. Wheat seeds of each group were soaked in corresponding treatment solutions for 4 hours. For control group only distilled water was used. Six seeds in each petri dish were placed at equal distances. 2 ml of corresponding treatment solution was given to each petri dish and only distilled water was used for control. These petri dishes were sealed and placed in the dark for next 24 hours of germination. The experiment was performed in growth room of Virology laboratory, Centre of Agricultural Biochemistry and Biotechnology (CABB), University of Agriculture, Faisalabad under controlled laboratory conditions. The germinated seeds were checked daily and growth parameters were recorded for 7 days. After 7 days the experiment was terminated and fresh roots and shoots weights were noted. Further these were dried in solar oven for 2 days and final dry root and shoot weight was recorded. Final germination percentage (FGP) was determined using Equation 1.

$$FGP = \frac{\sum N_G}{\sum N_T} \times 100 \quad (1)$$

Where N_G is the number of seeds germinated and N_T is the number of total tested seeds. Mean germination time (MGT) indicates the rate at which the seed germinated

$$MGT = \frac{\sum D.N}{\sum N} \quad (2)$$

Where N = seeds germinated on day D. Germination Rate Index shows germination percentage on each germination day. Higher GRI value shows faster germination (Pereira *et al.*):

$$GRI = \frac{\text{No.of germinated seeds on day 1}}{\text{Day of first count}} + \ldots + \frac{\text{No. of germinated seeds on final day}}{\text{Day of final count}} \quad (3)$$

First day of germination (FDG), Last day of germination (LDG) and Time spread of Germination (TSG) was also noted. Vigor index I and Vigor index II were also calculated using given formulas.

Vigor index I = germination% ×seedling length (root+shoot) (4)

Vigor index II = germination % ×seedling weight (root+shoot) (5)

The experiment was performed using completely randomized design and the statistical analysis was carried out using R language software. One-way ANOVA was used to measure significant difference levels and the means comparison was made by Tukey HSD (Honest significant difference) test at 5% significance level.

RESULTS AND DISCUSSION

The crystallanity and average particle size of the synthesized Zinc Oxide nanoparticles (ZnO NPs) was determined using XRD and SEM analysis. The XRD pattern of the ZnO NPs is given in Figure 1 showing different peaks at (100), (002), (101), (102), (110), (103), (112) and (201).

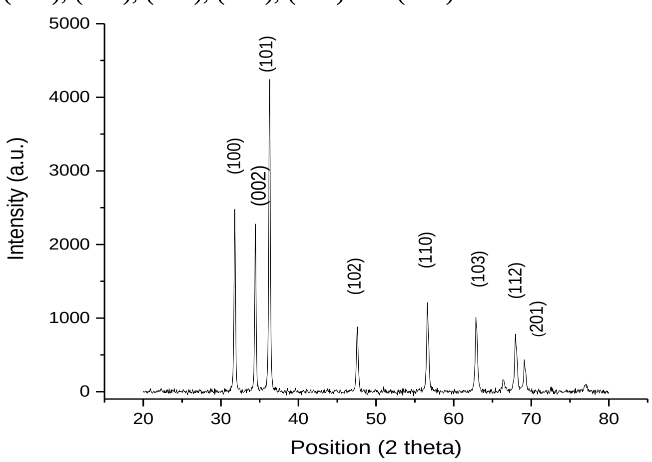

Figure 1. XRD data of synthesized ZnO NPs showing peaks corresponding to 2θ values at 31.7909, 34.445, 36.2783, 47.5789, 56.6264, 62.9141, 67.9717 and 69.1358.

The presence of these peaks confirmed the hexagonal wurtzite structure of ZnO powder confirmed by JCPDS 36-1451 data. Purity of the samples was also evident as no additional peaks related to impurities were observed. The average size of ZnO NPs was calculated by using Debye Scherrer's formula (equation 6), which was found to be 35nm.

$$D = \frac{k\,\lambda}{\beta \cos\theta} \quad (6)$$

The lattice parameter a and c were also calculated (Kahouli *et al.*, 2015) which were found to be 5.2 Å and 3.2 Å respectively comparable with JCPDS 36-1451. 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. SEM image of aggregated particles in 10 µm scale is shown in Figure 2.

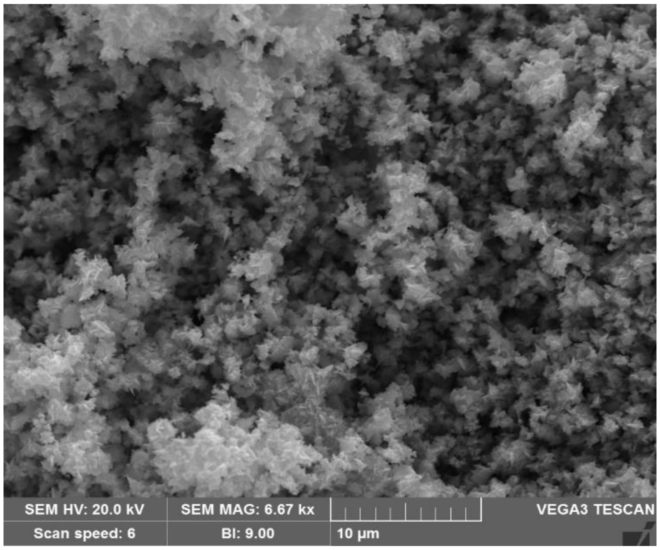

Figure 2. SEM image of synthesized ZnO NPs.

Figure 3. Wheat seedling of control, ZnO Bulk and ZnO NPs groups at each concentration.

Germination is an important parameter for determining the final plant yield along with time required for seed maturation. Earlier plant germination can improve plant productivity and growth. A comparison picture of both treatment groups (ZnO NPs and ZnO Bulk) with control group is shown in Figure 3. Our results indicated that final germination percentage was not affected by treating wheat plant with ZnO. However, mean germination time and germination rate index improved for both ZnO NPs and ZnO bulk treatments as compared with control (Water). ZnO NPs treatment at 300 ppm and 600 ppm showed more stimulatory effect as compared to ZnO bulk treatment at 300 ppm and 600 ppm. Germination parameters of all the treatment groups are given in Table 1.

Table 1. Wheat germination data of all the treatment groups along with control group.

Treatment	FGP	MGT	GRI	FDG	LDG	TSG
Control	100	1.22	5.44	1.00	2.00	2.00
ZnO NPs (300 ppm)	100	1.00	6.00	1.00	1.00	1.00
ZnO NPs (600 ppm)	100	1.00	6.00	1.00	1.00	1.00
ZnO Bulk (300 ppm)	100	1.06	5.83	1.00	1.33	1.33
ZnO Bulk (600 ppm)	100	1.11	5.67	1.00	1.67	1.67

Each value corresponds to average of three replications in each treatment group.

ZnO NPs were reported to increase mean germination time and percentage, root length, shoot length, seedling length, dry weight and seedling vigor index of green gram (Lakshmi *et al.*, 2017). Low concentrations of ZnO enhanced the seed germination and seedling growth of onion seeds. Root /shoot length enhanced at low concentration but reduced at high concentration compared to untreated seeds (Raskar and Laware, 2014). Wheat seedlings of all treatment groups along with control group are shown in Figure 3. Root length and shoot length enhanced for both ZnO bulk and ZnO NPs groups as compared with control. Data of Tukey test is shown in Table 2 indicating significant difference (less than 0.05) between control group and ZnO NPs 300 ppm group for shoot length. ZnO NPs 600 ppm, ZnO Bulk 300 ppm and ZnO bulk 600 ppm groups also indicated stimulatory effect on wheat shoot length compared to the control group but was not statistically significant.

Shoot and root weight of all the treatment groups increased as compared with control group. Shoot weight of ZnO NPs at 300ppm and ZnO NPs at 600 ppm indicated a significant increase with control group as shown in Figure 4. ZnO NPs treatment group showed better results as compared to ZnO bulk group at both concentrations. Seedling vigor index I was enhanced by ZnO NPs and ZnO bulk as compared to control group, showing the highest value at 300ppm ZnO NPs treatment. It was reported that ZnO NPs increased the seed germination and also seedling vigor index at concentration of 1000 ppm of peanut plant (Prasad *et al.*, 2012).

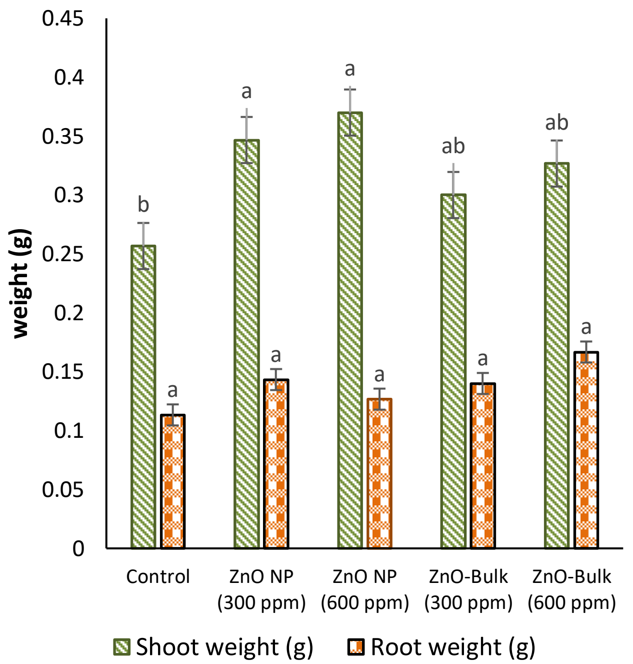

Figure 4. Effect of ZnO nanoparticles and ZnO Bulk on wheat root and shoot weight (The data in Columns is presented as mean ±S.E.).

Our results were in accordance with other studies reporting that TiO_2 nanoparticles application affected only the mean germination time of wheat seeds among germination parameters and enhanced shoot and seedling length as compared to the control and bulk TiO_2 treated seeds (Feizi *et al.*, 2012). Vigor index II for both ZnO NPs 300ppm and ZnO

Table 2. Tukey HSD test at 95% confidence level of wheat shoot length values along with standard deviation of all the treatment groups and control group.

Tukey multiple comparisons Test	Diff	lwr	upr	p adj
ZnO NPs (300 ppm)-Control	2.239	0.060	4.418	0.040
ZnO NPs (600 ppm)-Control	1.317	-0.863	3.496	0.543
ZnO Bulk (300 ppm)-Control	1.961	-0.218	4.140	0.107
ZnO Bulk (600 ppm)-Control	1.722	-0.457	3.901	0.220
ZnO NPs (600 ppm)-ZnO NPs (300 ppm)	-0.922	-3.101	1.257	0.865
ZnO Bulk (300 ppm)-ZnO NPs (300 ppm)	-0.278	-2.457	1.901	1.000
ZnO Bulk (600 ppm)-ZnO NPs (300 ppm)	-0.517	-2.696	1.663	0.992
ZnO Bulk 300 ppm)-ZnO NPs (600 ppm)	0.644	-1.535	2.824	0.974
ZnO Bulk (600 ppm)-ZnO NPs (600 ppm)	0.406	-1.774	2.585	0.998

Table 3. Shoot length, root length, seedling length along with vigor index I and II corresponding to all treatment groups and control group.

Treatment	Shoot Length (cm)	Root Length (cm)	Seedling length (cm)	Vigor index I	Vigor index II
Control	4.128[b]	5.211[a]	9.47[a]	947.333[a]	37.00[b]
ZnO NPs (300 ppm)	6.367[a]	6.404[a]	12.78[a]	1278.667[a]	49.00[a]
ZnO NPs (600 ppm)	5.444[b]	5.349[a]	10.82[a]	1081.667[a]	49.67[a]
ZnO Bulk (300 ppm)	6.089[b]	5.909[a]	11.98[a]	1197.667[a]	44.00[ab]
ZnO Bulk (600 ppm)	5.850[b]	5.603[a]	11.44[a]	1143.667[a]	49.33[a]

Note. Different letters indicate statistically significant values.

NPs 600ppm groups was enhanced (statistically significant) as compared with control group. Even ZnO Bulk 300ppm and ZnO bulk 600ppm treatment showed better results as compared with control group but the obtained results were not significant statistically.

It was reported that ZnO NPs enhanced significantly seed germination of chilli seeds at high concentrations. Root length, shoot length, and seedling length was maximum at higher concentrations but decreased at low values (Afrayeem and Chaurasia, 2017). An increase in shoot/root length and fresh biomass was reported in *Triticum aestivum* seedlings treated by ZnO NPs as compared to control grown in hydroponic solution (Awasthi *et al.*, 2017). Similar results were stated for TiO_2 NPs, which enhanced the seedling length, fresh weight and vigor index of wheat seedlings and weighted germination index and germination rate was also enhanced (Mahmoodzadeh and Aghili, 2014). In our case vigor index II of ZnO NPs group at 300ppm concentration was 49 and ZnO Bulk group at similar concentration was 44, which was much better than control group having value of 37. It should be noted that all the values (root/shoot length, root/shoot weight, vigor index I and II) were calculated statistically considering three replicates of each group. The average values were presented in Table 3.

The reported work shows that ZnO NPs at higher concentration (1500-2000mg/L) showed toxic effects on plants (Prasad *et al.*, 2012). How ZnO NPs will affect the seeds/plants and their relative dissolution and uptake strongly depends on the medium properties (Hydroponic, MS medium, Soil etc.), NPs characteristics and their application mode. It is reported that soil properties also play a vital role in determining the possible toxicology of the NPs. Acidic and calcareous soil with upto 500 ppm dose of ZnO NPs were investigated. ZnO NPs showed more toxicity to wheat in acidic soil than alkaline soil at same concentration of 500 mg/Kg (Watson *et al.*, 2015). Also the wheat grown in sand was affected by ZnO NPs treatment by reducing its root/shoot growth and weight (Dimkpa *et al.*, 2012).

Interaction of nanoparticles also depends on plant variety. Three different varieties of wheat (Boussallem, Gtadur and Ouarsenis) were exposed to ZnO nanoparticles upto 500 ppm concentration. No toxic effect was observed except decrease in root elongation at higher concentration (Chiahi *et al.*, 2016). Our studies suggest that no toxic effect of ZnO nanoparticles were observed on wheat (Var. Ujala) at lower concentration. In fact presence of ZnO NPs at 300ppm and 600ppm concentration provoked a stimulating effect on seeds. However besides possible studies of ZnO NPs effect on different plants, we should also thoroughly investigate a detailed comparison with their bulk counterparts in farm fields to know exactly if nanoparticles can prove better in future applications.

***Conclusion*:** Determining the dose response of wheat plants to nanoparticles (NPs) is expected to improve our ability to use NPs to increase crop yields and to boost plant deficiencies against pathogens. In accordance with recent literature review and our studies, we have come to conclusion that the use of ZnO NPs in low concentration of 300 ppm and 600 ppm can have stimulatory effect on wheat seedlings. No toxic effects were observed at these concentrations on wheat seedlings. Main factors, which influence the effects of NPs on plants, are characteristics of the NPs (size/concentration/stability/category/shape/coating material), the plant variety (species), growth medium (soil/hydrophobic/MS/sand) and the plant growth stage at the time of application of nanoparticles.

***Acknowledgment*:** This work was done under the financial help of research grant to Dr Ayesha Younus, funded by Higher Education Commission of Pakistan NRPU Project No. 4602.

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