Characterization of synthesized zinc oxide nanoparticles and their effect on growth, productivity and zinc use efficiency of wheat and field pea in the Indian Himalayan foothills

Renu Verma[1], G. K. Dwivedi[1], Jai Paul Singh[1], Ajeet Pratap Singh[1], Neha Tamta[1] and Amit Kumar[1,2,]*

[1]G.B. Pant University of Agriculture and Technology, Pantnagar 263 145, India
[2]Forest Research Institute, Dehradun 248 006, India

Nanofertilizers have emerged as an effective alternative to traditional fertilizers. They contribute to increased agricultural production by increasing input efficiency and reducing relevant losses. The present study was carried out at the G.B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand, India, during 2016–17 to study the effect of synthesized zinc oxide nanoparticles (ZnO NPs) on the growth and productivity of wheat and field pea crops. The results of the study revealed that significantly greater wheat and field pea plant height was recorded with 10, 20 and 50 ppm concentration of ZnO NPs, which decreased at 100 ppm concentration. A significantly higher yield of wheat was recorded at 50 ppm ZnO NPs concentration (3.28 ± 0.51 g plant^{-1}), followed by 20 ppm (3.05 ± 0.43 g plant^{-1}), which was at par with 100 ppm (3.02 ± 0.45 g plant^{-1}), and the minimum at 10 ppm concentration (2.70 ± 0.34 g plant^{-1}) over control. A similar trend in yield was observed for field pea. With respect to the mode of application, a higher yield of wheat was observed in the seed-soaking method (3.05 ± 0.43 g plant^{-1}); however, in the case of field pea, a higher yield was observed using foliar spray (6.21 ± 0.52 g plant^{-1}) method of ZnO application. Higher Zn content was observed in 50 ppm concentration for wheat (42.39 μg g^{-1}) and field pea (26.00 μg g^{-1}). The higher Zn use efficiency in terms of physiological efficiency was recorded at 20 ppm concentration (1.46) for wheat and 10 ppm (5.51) for field pea. Hence, it can be concluded that the applied ZnO NPs have stimulating effects on wheat and field pea crop growth and yield through increased zinc content in plants, zinc uptake and zinc use efficiency.

Keywords: Field pea nanofertilizers, growth and productivity, wheat, zinc oxide nanoparticles.

NANOTECHNOLOGY is a new, rising and appealing field of science that is presently being applied in various areas of science and has enormous potential in agriculture and associated fields[1]. In agriculture, the use of nanotechnology has the potential to increase nutrient use efficiency (NUE) through the manufacture of nanofertilizers, development of new-generation pesticides and their carriers, reclamation of salt-affected soils and many other aspects[2]. Nanotechnology can increase agricultural production and supplement the processed food industry by utilizing the unique properties of nanoparticles (NPs) in the field of agriculture[3]. The nanofertilizers inhibit the nutrients from being converted prematurely into chemical or gaseous forms that cannot be taken up by the plants and release the nutrients on demand[4]. It has the capability to bring about the second green revolution in agriculture[5].

NPs offer an extremely attractive platform for a wide range of applications in biology[6]. Novel research also targets efficient utilization of fertilizers, insecticides and water to decrease pollution and promote a more eco-friendly agriculture[7]. Alteration in agricultural technology has been a key factor determining modern agriculture[8]. The development of nano-sized materials and nano-sized devices could pave the way for new applications in agriculture and allied fields[9].

The application of huge quantities of fertilizers such as urea, phosphate and nitrate compounds is dangerous. Moreover, most of the applied fertilizers are not available to the plants due to leaching loss, which causes pollution[10]. Besides, nanocoatings give protection to the surface for larger particles[11].

The method of application and dose of NPs increase the uptake of nutrients and decrease environmental pollution. The present practices include broadcasting, top-dressing, banding and dusting which deal with problems of surface run-off because of dissolution in soil moisture and leaching[12]. Seed treatment of NPs in many crops has been studied[13]. An alternative method of application of NPs on crops is a foliar spray. NPs may be toxic to plants at higher concentrations due to their great reactivity[14].

Placement of fertilizers in huge amounts close to the seed and less soil moisture resulted in salt damage. Wheat

*For correspondence. (e-mail: amitudu@gmail.com)

(*Triticum aestivum*) is a widely cultivated cereal crop that needs a large amount of fertilizers, which may cause a reduction in soil fertility. Field pea (*Pisum sativum*) is a legume crop with rhizobium bacteria in the root nodules which fix atmospheric nitrogen[15]. Keeping the above facts in mind, wheat and field pea were chosen for the pot experiment which was conducted in a glasshouse with the following objectives: (i) Synthesis and chemical characterization of zinc oxide (ZnO) NPs using scanning electron microscope (SEM) and UV–Vis spectroscopy and (ii) Effect of varying concentration and modes of application of synthesized ZnO NPs on growth, yield and zinc use efficiency (ZnUE).

Materials and methods

Experimental details

The study was conducted at the G.B. Pant University of Agriculture and Technology (GBPUAT), Pantnagar, Uttarakhand, India located at 29°N lat., 79.3°E long. and at an altitude of 243.84 m amsl in the tarai belt of the Central Himalayan foothills. The climate of the study site is humid subtropical with cold winters and hot, dry summers. In this study, the crop was chosen based on its germination percentage. A pot experiment was laid out during the *rabi* season. Pots were filled with soil up to the mark specified in them. Ten seeds each of wheat (HD 158 variety) and field pea (Pant Pea 42 variety) were sown in each pot with three replications during the first week of November 2016–17. The treatments were control, and nano-zinc at 10, 20, 50 and 100 ppm. The experiment comprised two modes of ZnO application (seed-soaking and foliar spray). Seeds were soaked in different concentrations of ZnO for seed-soaking treatment before sowing in the pot. Seeds were sown without ZnO application for foliar spray treatment. For this treatment, solutions of ZnO NPs were made according to the desired concentration. Three foliar sprays, the first during CRI stage (9 January 2017), the second during tillering stage (28 January 2017) and the third during the flowering stage (20 February 2017) were given to the crops.

Chemical synthesis of ZnO NPs

In order to synthesize ZnO NPs, 0.02 M (0.219 g) zinc acetate solution was made by dissolving it in 50 ml distilled water under continuous stirring. Then, 2.0 M (4 g) NaOH aqueous solution was added drop by drop to reach pH 12 at room temperature. The whole solution was placed in a hot plate magnetic stirrer for 2 h at 70°C. The white precipitate obtained was centrifuged at 8000 rpm for 10 min (Centrifuge (REMI)). The supernatant was discarded and the remaining product was thoroughly washed three times with distilled water followed by ethanol to remove impurities. The precipitate was then dried in a hot-air oven overnight at 60°C to complete the conversion of $Zn(OH)_2$ to ZnO NPs. The formation of ZnO NPs with an average size of about 30 nm was confirmed by the SEM images[16].

Characterization of ZnO NPs

Chemically synthesized ZnO NPs were characterized by two methods.

Scanning electron microscopy: To ensure the size of NPs, the final sample was studied using SEM (JEOL, JSM-6610LV) at the College of Veterinary Sciences, GBPUAT, Pantnagar, for structure and particle size analysis. SEM analysis was done by placing NP samples on the slides after ultra-sonication for 30 min followed by gold-coating of the samples (JEC gold-coater). Finally, the size and other characteristics of NPs were observed under SEM.

UV-Vis spectroscopy: To determine the formation of ZnO NPs under a UV-Vis spectrophotometer 108 (Systronics), 10 ppm concentration solutions of synthesized ZnO NPs, reagent-grade zinc acetate and zinc nitrate were read in the wavelength range 200–800 nm separately using distilled water as blank. The absorption spectra were obtained at 5 min intervals.

Growth and developmental measurement

The growth and development parameters such as germination percentage, plant height, leaf area index (LAI) and yield were estimated using appropriate methods[17]. Plant height was measured at 20, 40 and 60 days after sowing (DAS). However, LAI was recorded at 40 DAS.

Zinc content and total zinc uptake by the plant at harvest stage

The dried plant samples were digested using diacid digestion mixture of nitric acid and perchloric acid (9: 4) on a hot plate. The contents were filtered and put into a volumetric flask and made up with distilled water. Then zinc content was obtained directly using an atomic absorption spectrophotometer (AAS)[18]. Total zinc uptake by the plant was estimated by multiplying zinc content with the dry weight of the plant[19].

Zinc use efficiency

Zinc use efficiency is expressed as agronomic efficiency (AE), which can be calculated as follows[20]

$$AE = (Y - Y_0)/F,$$

where Y is the yield of crop with zinc applied, Y_0 the yield of crop with no zinc applied and F is the amount of zinc applied.

Zinc use efficiency is also expressed as physiological efficiency (PE), which can be calculated as follows

$$\mathrm{PE} = \frac{(Y - Y_0)}{(U - U_0)},$$

where Y is the yield of crop with zinc applied, Y_0 the yield of crop with no zinc applied, U the total nutrient uptake with zinc applied and U_0 is the total nutrient uptake with no zinc applied.

Statistical analysis

The experiment consisting of two methods of application, four levels of concentration and one untreated control was laid out in two factorial randomized block designs with three replications. The effects of different ZnO NP concentrations and modes of application on plant growth and productivity were compared using two-way ANOVA. The comparison of means was carried out using Tukey's HSD ($P < 0.05$). All data were analysed using the *R* (4.1.2) statistical software and the standard error of treatment means was used for separation of means.

Results

Characterization of synthesized ZnO NPs

SEM and UV-Vis spectroscopy: The SEM images confirmed that the synthesized ZnO NPs, ranged in size from 0.20 to 0.30 μm. They were spherical in shape with mono-dispersion NPs. They were also found in aggregates or clumps, depending on the period of sonication (Figure 1). The absorbance peak was observed at 365 nm for ZnO NPs, which is a typical feature of these NPs. The absorbance peaks were found at 371 nm for zinc nitrate and 376 nm for zinc acetate.

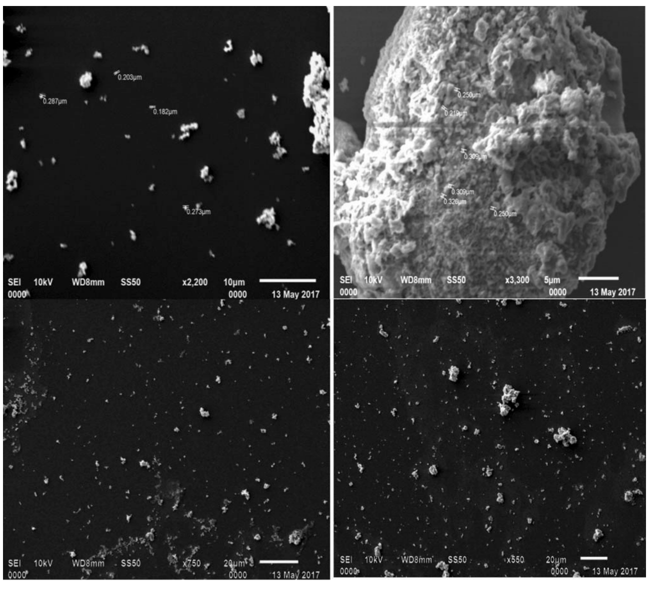

Figure 1. Scanning electromicroscope (SEM) images of zinc oxide (ZnO) nanoparticles (NPs) at different magnitudes.

Plant growth parameters of wheat

The plant height of wheat significantly increased with an increase in the concentration up to 50 ppm and decreased thereafter at 100 ppm (Table 1). Maximum shoot length (29.42 cm) was recorded at 50 ppm concentration at 20 DAS which was 63.4% more than control. Height of the wheat plants at 20, 40 and 60 DAS increased significantly in all ZnO NP-treated plants over control. There was a significant effect of the mode of application on plant height at 20 and 60 DAS. At 60 DAS, the effect of foliar spray was more pronounced than seed-soaking. The interaction effect of concentration and mode of application had a significant effect on plant height during all stages, except 40 DAS (Appendix 1). At 20, 40 and 60 DAS, plant height had significantly increased with an increase in concentration up to 50 ppm, which thereafter decreased at 100 ppm.

Leaf area index (LAI) of wheat plants increased significantly in all ZnO NP-treated plants over control (Table 1). It had significantly increased with an increase in concentration up to 50 ppm and thereafter decreased at 100 ppm. Significantly higher LAI was recorded at 50 ppm (284.7%) over control. However, no significant impact between the modes of application on LAI was observed. The interaction effect of concentration and mode of application had a significant effect on LAI (Appendix 1).

Height of the field pea plants at 20, 40 and 60 DAS increased significantly for all ZnO NP concentrations over control (Table 2). At 20, 40 and 60 DAS, plant height had significantly increased with an increase in concentration up to 50 ppm, which thereafter decreased at 100 ppm concentration. The effect of zinc concentration was 42.1%, 32.4% and 21.9% at 50 ppm during 20, 40 and 60 DAS respectively, which reduced drastically at 100 ppm. There was a significant effect of the mode of application on plant height at 20, 40 and 60 DAS. The interaction effect of concentration and mode of application had a significant impact on plant height in all stages (Appendix 2). Seed-soaking was more suitable at lower concentrations, while foliar spray was at higher concentrations.

LAI of field pea had significantly an increased with increase in concentration up to 50 ppm (Table 2). Significantly higher LAI was observed at 50 ppm concentration (116.90%) followed by 20 ppm (111.32%), 100 ppm (64.15%) and 10 ppm (11.32%) over control. There was a significant effect of mode of application on LAI; foliar spray showed significantly higher LAI (0.97) than seed-soaking (0.93). The interaction effect of concentration and mode of application had a significant effect on LAI (Appendix 2).

Table 1. Effect of zinc oxide (ZnO) nanoparticles (NPs) on plant height and leaf area index (LAI) of wheat crop

Treatment	20 DAS (cm)	40 DAS (cm)	60 DAS (cm)	LAI
Concentration (ppm)				
Control	$18.00^{d} \pm 0.92$	$28.00^{e} \pm 0.95$	$46.58^{e} \pm 1.08$	$0.59^{d} \pm 0.12$
10	$22.67^{c} \pm 1.05$	$35.00^{c} \pm 1.09$	$54.42^{c} \pm 1.42$	$1.35^{c} \pm 0.34$
20	$25.08^{b} \pm 0.98$	$38.58^{b} \pm 1.17$	$61.50^{b} \pm 1.87$	$1.45^{b} \pm 0.28$
50	$29.42^{a} \pm 1.24$	$48.17^{a} \pm 1.25$	$63.50^{a} \pm 2.05$	$2.27^{a} \pm 0.42$
100	$22.00^{c} \pm 0.84$	$33.00^{d} \pm 1.02$	$48.67^{d} \pm 1.32$	$1.37^{c} \pm 0.35$
Mode of application				
Foliar spray	$23.33^{b} \pm 0.92$	$36.17^{b} \pm 0.97$	$55.37^{a} \pm 1.45$	$1.41^{a} \pm 0.28$
Seed soaking	$23.53^{a} \pm 1.01$	$36.93^{a} \pm 1.14$	$54.50^{b} \pm 1.62$	$1.39^{a} \pm 0.23$

DAS, Days after sowing. Different letters denote significant differences among treatments. Data with the same letters in the superscript do not differ significantly.

Table 2. Effect of ZnO NPs on plant height at 20, 40 and 60 DAS of field pea at different growth stages

Treatment	20 DAS (cm)	40 DAS (cm)	60 DAS (cm)	LAI
Concentration (ppm)				
Control	$27.50^{e} \pm 1.24$	$53.17^{e} \pm 1.23$	$106.17^{e} \pm 2.41$	$0.53^{e} \pm 0.08$
10	$31.50^{c} \pm 1.32$	$57.33^{d} \pm 1.47$	$114.00^{d} \pm 2.17$	$0.59^{d} \pm 0.08$
20	$34.25^{b} \pm 1.12$	$63.75^{b} \pm 1.95$	$122.25^{b} \pm 3.42$	$1.12^{b} \pm 0.19$
50	$39.08^{a} \pm 1.47$	$70.42^{a} \pm 2.04$	$129.38^{a} \pm 3.57$	$1.15^{a} \pm 0.18$
100	$30.83^{d} \pm 0.98$	$59.00^{c} \pm 1.47$	$115.33^{c} \pm 2.68$	$0.87^{c} \pm 0.09$
Mode of application				
Foliar spray	$32.80^{a} \pm 1.02$	$60.70^{a} \pm 1.67$	$117.89^{a} \pm 2.41$	$0.97^{a} \pm 0.15$
Seed soaking	$32.47^{b} \pm 1.14$	$60.77^{a} \pm 1.85$	$116.97^{b} \pm 2.37$	$0.93^{b} \pm 0.12$

Different letters denote significant differences among treatments. Data with the same letters in the superscript do not differ significantly.

Table 3. Effect of ZnO NPs on grain, straw and total yield of wheat crop

Treatment	Grain yield (g plant^{-1})	Straw yield (g plant^{-1})	Total yield (g plant^{-1})
Concentration (ppm)			
Control	$2.15^{e} \pm 0.35$	$0.22^{d} \pm 0.04$	$2.37^{d} \pm 0.34$
10	$2.31^{d} \pm 0.42$	$0.39^{c} \pm 0.06$	$2.70^{c} \pm 0.34$
20	$2.59^{b} \pm 0.22$	$0.46^{b} \pm 0.06$	$3.05^{b} \pm 0.43$
50	$2.81^{a} \pm 0.41$	$0.47^{b} \pm 0.07$	$3.28^{a} \pm 0.51$
100	$2.37^{c} \pm 0.37$	$0.65^{a} \pm 0.11$	$3.02^{b} \pm 0.45$
Mode of application			
Foliar spray	$2.32^{a} \pm 0.28$	$0.39^{b} \pm 0.08$	$2.71^{a} \pm 0.36$
Seed soaking	$2.56^{a} \pm 0.31$	$0.49^{a} \pm 0.09$	$3.05^{a} \pm 0.43$

Different letters denote significant differences among treatments. Data with the same letters in the superscript do not differ significantly.

Yield parameters

The grain yield of wheat was also significantly higher in all ZnO NP concentrations, except at 100 ppm (Table 3). Significantly higher grain yield was recorded at 50 ppm (30.7%) over control. For the mode of application, there was no significant difference between foliar spray and seed-soaking. The interaction effect between concentration and mode of application had a significant impact on grain yield. Straw yield had significantly increased at all ZnO NP concentrations up to 100 ppm, except at 10 ppm. Significantly higher straw yield was recorded at 100 ppm (0.65 g). For the mode of application, seed-soaking had a significantly higher straw yield (25.6%) than foliar spray. The interaction effect between concentration and mode of application had a significant impact on grain yield. Total yield had significantly increased with increase in concentration up to 50 ppm, which thereafter decreased at 100 ppm. The maximum total yield was recorded at 50 ppm, which was 3.28 g (38% over the control). The effect of mode of

Table 4. Effect of ZnO NPs on grain, straw and total yield of field pea

Treatment	Grain yield (g $plant^{-1}$)	Straw yield (g $plant^{-1}$)	Total yield (g $plant^{-1}$)
Concentrations (ppm)			
Control	$2.82^{e} \pm 0.49$	$0.33^{d} \pm 0.04$	$3.15^{e} \pm 0.35$
10	$4.82^{c} \pm 0.61$	$0.79^{c} \pm 0.08$	$5.61^{c} \pm 0.47$
20	$6.53^{b} \pm 0.47$	$0.82^{c} \pm 0.11$	$7.35^{b} \pm 0.68$
50	$7.66^{a} \pm 1.02$	$1.11^{a} \pm 0.17$	$8.77^{a} \pm 0.85$
100	$3.90^{d} \pm 0.68$	$1.09^{b} \pm 0.15$	$4.99^{d} \pm 0.52$
Mode of application			
Foliar spray	$5.38^{a} \pm 0.85$	$0.83^{a} \pm 0.08$	$6.21^{a} \pm 0.52$
Seed soaking	$4.91^{b} \pm 0.62$	$0.75^{b} \pm 0.09$	$5.66^{b} \pm 0.38$

Different letters denote significant differences among treatments. Data with the same letters in the superscript do not differ significantly.

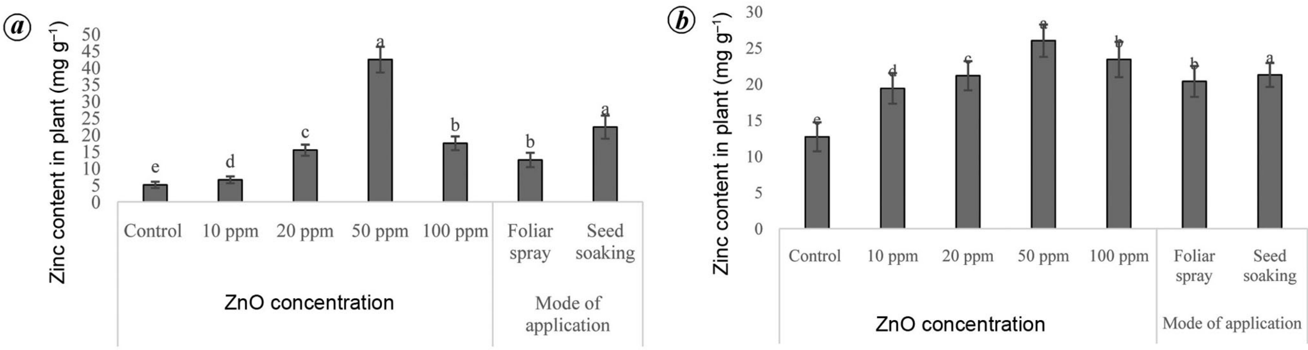

Figure 2. Effect of ZnO on zinc content of (***a***) wheat and (***b***) field pea.

application on total yield was non-significant. Interaction effect between concentration and mode of application had a significant impact on grain yield (Appendix 3).

The grain yield of field pea was significantly higher (7.66 g/plant) at 50 ppm concentration; it was 172% higher than control (Table 4). However, it greatly reduced (96%) at 100 ppm concentration than 50 ppm. Interaction effect of concentration and mode of application had significant impact on grain yield. A significantly higher straw yield was also recorded at 50 ppm concentration (1.11 g $plant^{-1}$) followed by 100 ppm (1.09 g $plant^{-1}$) and 20 ppm (0.82 g $plant^{-1}$), which was statistically at par with 10 ppm (0.79 g $plant^{-1}$). The impact of mode of application on straw yield was significant, and foliar spray had 11% higher impact than seed-soaking. The interaction effect of concentration and mode of application had a significant impact on straw yield. A significantly higher total yield was recorded at 50 ppm (8.77 g) than control and other ZnO NP concentrations. The impact of mode of application on total yield was significant and foliar spray was 10% superior compared to seed soaking. The interaction effect of concentration and mode of application had a significant impact on total yield (Appendix 4).

Zinc content and total zinc uptake

Significantly higher zinc content was recorded in 50 ppm ZnO NPs concentration (42.39 µg g^{-1}) followed by 100 (17.45 µg g^{-1}), 20 (15.40 µg g^{-1}) and 10 ppm (6.55 µg g^{-1}) in wheat (Figure 2 *a*). The effect of mode of application on zinc content was also significant and seed-soaking had 78.4% higher zinc content than foliar spray. The interaction effect of concentration and mode of application had a significant effect on zinc content (Appendix 5).

Significantly higher zinc uptake was recorded at 100 ppm (129.97 µg g^{-1}) followed by 50 (63.59 µg g^{-1}), 20 (41.44 µg g^{-1}) and 10 ppm (18.28 µg g^{-1}) in wheat (Figure 3 *a*). The effect of mode of application on zinc uptake was also significant; seed-soaking showed higher zinc uptake (71.68 µg g^{-1}) than foliar spray. The interaction effect of concentration and mode of application had a significant effect on total zinc uptake (Appendix 5).

The zinc content of field pea was the highest at 50 ppm, followed by 100, 20 and 10 ppm (Figure 2 *b*). Significantly higher zinc content was recorded at 50 ppm concentration (26.00 µg mg^{-1}), followed by 100 (23.40 µg mg^{-1}), 20 (21.15 µg mg^{-1}) and 10 (19.40 µg mg^{-1}). There was significant impact of mode of application on zinc content. The seed-soaking-treatment (21.26 µg mg^{-1}) had a significant effect on zinc content than foliar spray (20.36 µg mg^{-1}). The interaction effect of concentration and mode of application had a significant impact on zinc content (Appendix 6).

Significantly higher zinc uptake was recorded in 50 ppm concentration (169.57 µg mg^{-1}) and lower in 10 ppm (103.67 µg mg^{-1}) by field pea (Figure 3 *b*). The effect of mode of application on zinc uptake was not significant.

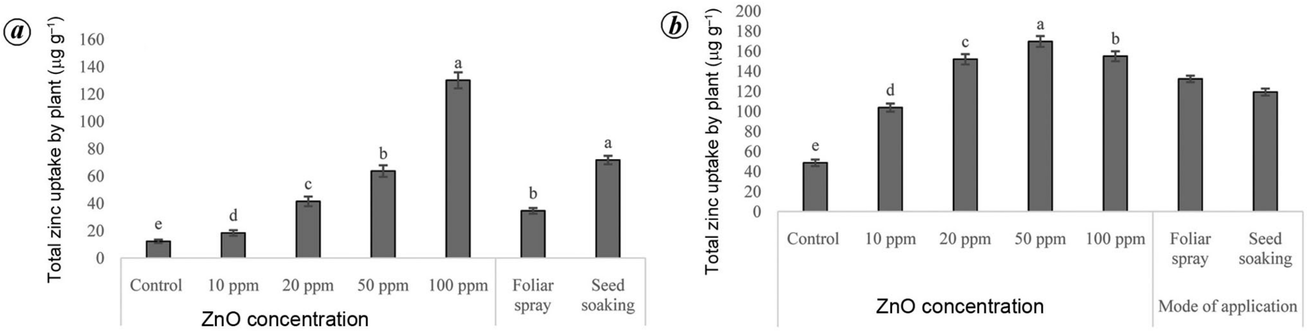

Figure 3. Effect of ZnO NPs on total zinc uptake by (***a***) wheat and (***b***) field pea.

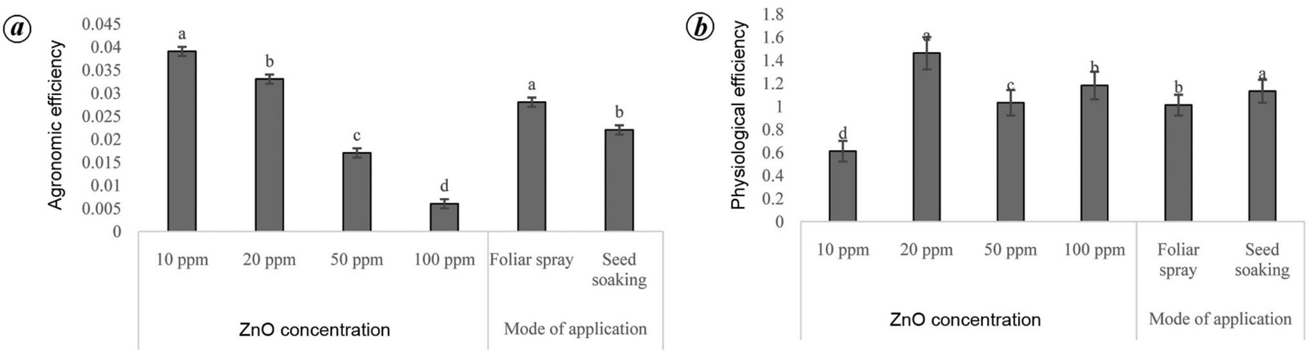

Figure 4. Effect of ZnO on zinc use efficiency in terms of agronomic and physiological efficiency in wheat.

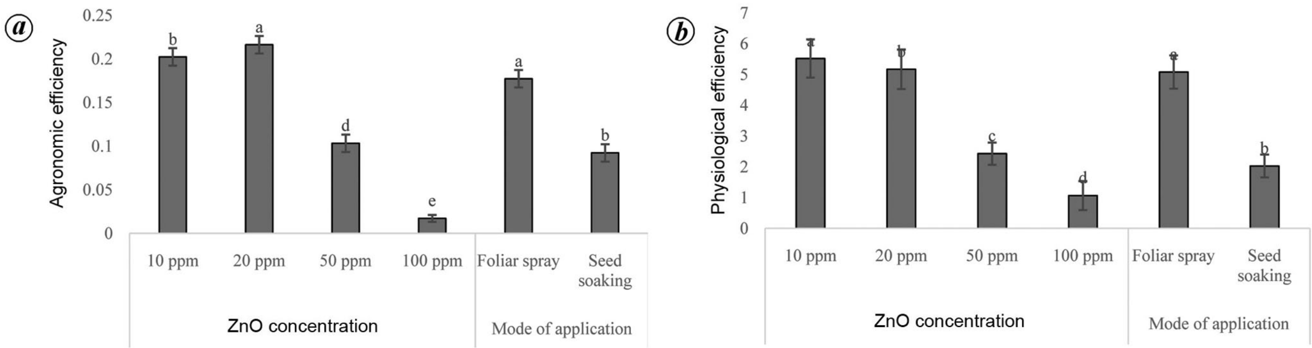

Figure 5. Effect of ZnO NPs on zinc use efficiency in terms of agronomic and physiological efficiency in field pea.

However, the foliar spray had a 9% higher zinc uptake than seed-soaking. The interaction effect of concentration and mode of application had a significant impact on zinc uptake by field pea (Appendix 6).

Zinc use efficiency

Zinc use efficiency is expressed as PE, i.e. the efficient acquisition of Zn and utilization or re-translocation within a plant. AE in terms of biomass produced per microgram of zinc applied was 0.039 at 10 ppm, indicating 0.039 g biomass is produced by application of 1 μg zinc. Similarly, AE was 0.033 at 20 ppm, 0.017 at 50 ppm and 0.006 at 100 ppm under wheat crop (Figure 4 *a*). For mode of application, significantly higher AE was recorded for foliar spray than seed-soaking. Also, significantly higher PE was recorded at 20 ppm (1.46) followed by 100 (1.18), 50 (1.03) and 10 ppm (0.61) in wheat (Figure 4 *b*). For mode of application, seed-soaking had significantly higher PE (1.13) than foliar spray (1.01).

The AE of field pea was 0.216, 0.103 and 0.017 at 20, 50 and 100 ppm respectively (Figure 5 *a*). For mode of application, AE of foliar spray (0.18) was significantly higher than seed-soaking (0.09). PE in field pea ranged from

Appendix 1. Interaction between modes of zinc oxide (ZnO) application and different concentrations on plant height and LAI of wheat

Concentration (ppm)	20 DAS (cm)		40 DAS (cm)		60 DAS (cm)		LAI	
	Foliar spray	Seed soaking	Foliar spray	Seed soaking	Foliar spray	Seed soaking	Foliar spray	Seed soaking
Control	$15.67^{j} \pm 0.35$	$20.33^{h} \pm 0.92$	$24.50^{j} \pm 1.03$	$31.50^{i} \pm 0.86$	$42.33^{j} \pm 1.28$	$50.83^{h} \pm 1.22$	$0.70^{h} \pm 0.11$	$0.47^{i} \pm 0.09$
10	$22.00^{g} \pm 0.95$	$23.33^{ef} \pm 0.87$	$34.00^{g} \pm 1.34$	$36.00^{de} \pm 0.92$	$54.33^{ef} \pm 1.65$	$54.50^{e} \pm 1.43$	$1.63^{de} \pm 0.32$	$1.07^{g} \pm 0.16$
20	$23.83^{e} \pm 1.02$	$26.33^{c} \pm 1.08$	$35.83^{def} \pm 1.42$	$41.33^{c} \pm 1.04$	$62.33^{b} \pm 2.14$	$60.67^{d} \pm 2.06$	$1.67^{d} \pm 0.28$	$1.23^{f} \pm 0.24$
50	$30.83^{a} \pm 1.32$	$28.00^{b} \pm 1.22$	$50.33^{a} \pm 2.18$	$46.00^{b} \pm 1.05$	$65.50^{a} \pm 2.45$	$61.50^{c} \pm 2.01$	$2.27^{a} \pm 0.44$	$2.27^{ab} \pm 0.39$
100	$24.33^{d} \pm 0.68$	$19.67^{i} \pm 0.62$	$36.17^{d} \pm 1.72$	$29.83^{gh} \pm 0.68$	$52.33^{g} \pm 2.04$	$45.00^{i} \pm 1.47$	$0.80^{g} \pm 0.21$	$1.93^{c} \pm 0.27$

Different letters denote significant differences among treatments. Data with the same letters in the superscript do not differ significantly. DAS, Days after sowing. LAI, Leaf area index.

Appendix 2. Interaction between modes of ZnO application and different concentrations on plant height and LAI of field pea

Concentration (ppm)	20 DAS		40 DAS		60 DAS		LAI	
	Foliar spray	Seed soaking	Foliar spray	Seed soaking	Foliar spray	Seed soaking	Foliar spray	Seed soaking
Control	$26.00^{j} \pm 1.28$	$29.00^{ghi} \pm 1.15$	$51.33^{hi} \pm 2.14$	$55.00^{g} \pm 1.08$	$103.67^{i} \pm 3.18$	$108.67^{g} \pm 3.05$	$0.53^{hi} \pm 0.09$	$0.53^{h} \pm 0.08$
10	$30.50^{g} \pm 1.65$	$32.50^{def} \pm 1.24$	$56.00^{ef} \pm 2.17$	$58.67^{e} \pm 1.26$	$111.33^{g} \pm 3.47$	$116.67^{f} \pm 4.12$	$1.07^{e} \pm 0.15$	$1.10^{bcd} \pm 0.17$
20	$33.33^{d} \pm 1.58$	$35.17^{c} \pm 1.39$	$61.17^{d} \pm 2.34$	$66.33^{c} \pm 2.05$	$119.33^{e} \pm 3.87$	$125.17^{c} \pm 5.18$	$1.13^{bc} \pm 0.14$	$1.10^{bcd} \pm 0.16$
50	$41.83^{a} \pm 1.85$	$36.33^{b} \pm 1.34$	$73.33^{a} \pm 2.84$	$67.50^{b} \pm 2.14$	$132.43^{a} \pm 3.44$	$126.33^{b} \pm 5.98$	$1.17^{a} \pm 0.17$	$1.13^{b} \pm 0.24$
100	$32.33^{de} \pm 1.67$	$29.33^{fg} \pm 1.26$	$51.67^{h} \pm 1.87$	$56.33^{e} \pm 2.17$	$122.67^{d} \pm 2.38$	$108.00^{gh} \pm 5.02$	$0.93^{f} \pm 0.12$	$0.80^{g} \pm 0.09$

Different letters denote significant differences among treatments. Data with the same letters in the superscript do not differ significantly.

Appendix 3. Interaction between modes of ZnO application and different concentrations on yield of wheat

Concentration (ppm)	Grain yield (g $plant^{-1}$)		Straw yield (g $plant^{-1}$)		Total yield (g $plant^{-1}$)	
	Foliar spray	Seed soaking	Foliar spray	Seed soaking	Foliar spray	Seed soaking
Control	$2.12^{hij} \pm 0.24$	$2.17^{h} \pm 0.18$	$0.16^{j} \pm 0.04$	$0.28^{hi} \pm 0.04$	$2.28^{j} \pm 0.31$	$2.47^{i} \pm 0.14$
10	$2.14^{hi} \pm 0.27$	$2.47^{e} \pm 0.24$	$0.39^{def} \pm 0.07$	$0.40^{de} \pm 0.05$	$2.79^{f} \pm 0.38$	$2.74^{g} \pm 0.19$
20	$2.51^{cd} \pm 0.31$	$2.66^{b} \pm 0.29$	$0.43^{d} \pm 0.09$	$0.49^{c} \pm 0.07$	$2.94^{d} \pm 0.42$	$3.15^{c} \pm 0.36$
50	$2.53^{c} \pm 0.35$	$3.09^{a} \pm 0.34$	$0.64^{b} \pm 0.12$	$0.30^{h} \pm 0.09$	$2.92^{de} \pm 0.23$	$3.49^{a} \pm 0.28$
100	$2.30^{g} \pm 0.39$	$2.44^{ef} \pm 0.42$	$0.34^{g} \pm 0.08$	$0.96^{a} \pm 0.13$	$2.64^{h} \pm 0.28$	$3.39^{b} \pm 0.37$

Different letters denote significant differences among treatments. Data with the same letters in the superscript do not differ significantly.

1.06 to 5.51 for different ZnO NP concentrations. Significantly higher PE was recorded 10 ppm (5.51) followed by 20 (5.16), 50 (2.42) and 100 ppm (1.06) in field pea (Figure 5 *b*). For mode of application, foliar spray had significantly higher PE (5.07) than seed-soaking (2.02). Thus, field pea had higher AE and PE than wheat when 1 g zinc was applied.

Discussion

SEM and UV–Vis spectroscopy

SEM analysis showed that the average particle size of ZnO NPs was 0.20–0.30 μm. The particle size increased from 30 to 500 nm as the temperature was increased from 80°C to 100°C (ref. 21). Kolekar *et al.*[22] also reported an absorption peak in the range 250–400 nm under UV–Vis spectroscopy. In another study, the peak was recorded at 370 nm, which is a characteristic feature of ZnO NPs (ref. 23). Kulkarni *et al.*[23] also confirmed the formation of ZnO NPs as the absorption peak was found at 361.75 nm wavelength.

Plant growth parameters

Plant growth traits in terms of plant height and LAI increased significantly with an increase in ZnO NPs concentration up to 50 ppm, which thereafter decreased at 100 ppm. This indicates that the concentration of ZnO NPs up to 50 ppm is effective in stimulating plant growth and development. The increased plant growth caused by NPs could be attributed to mobilization of nutrients such as phosphorus in the soil, as well as an increase in the microbial population, particularly in the rhizosphere[24]. However, the decrease in plant height with an increase in NPs concentration could be ascribed to the toxic effect of higher concentration of NPs[25]. Maximum plant height of 63.4%, 72% and 36.3% was recorded with 50 ppm concentration over control at 20, 40

Appendix 4. Interaction between modes of ZnO application and different concentrations on yield of field pea

	Grain yield (g $plant^{-1}$)		Straw yield (g $plant^{-1}$)		Total yield (g $plant^{-1}$)	
Concentration (ppm)	Foliar spray	Seed soaking	Foliar spray	Seed soaking	Foliar spray	Seed soaking
Control	$2.02^{j} \pm 0.22$	$3.61^{h} \pm 0.27$	$0.46^{hi} \pm 0.04$	$0.20^{j} \pm 0.02$	$2.48^{j} \pm 0.38$	$4.08^{h} \pm 0.37$
10	$4.27^{g} \pm 0.36$	$5.37^{e} \pm 0.32$	$0.76^{fg} \pm 0.06$	$0.47^{h} \pm 0.05$	$5.03^{g} \pm 0.41$	$5.57^{f} \pm 0.54$
20	$6.78^{b} \pm 0.41$	$6.28^{d} \pm 0.39$	$0.78^{f} \pm 0.06$	$0.85^{de} \pm 0.09$	$8.05^{b} \pm 0.54$	$7.13^{d} \pm 0.57$
50	$8.60^{a} \pm 0.52$	$6.71^{bc} \pm 0.37$	$1.27^{ab} \pm 0.15$	$0.94^{c} \pm 0.14$	$9.38^{a} \pm 0.68$	$7.49^{c} \pm 0.46$
100	$5.21^{ef} \pm 0.35$	$2.59^{i} \pm 0.24$	$0.88^{d} \pm 0.09$	$1.30^{a} \pm 0.18$	$6.09^{e} \pm 0.62$	$3.89^{i} \pm 0.35$

Different letters denote significant differences among treatments. Data with the same letters in the superscript do not differ significantly.

Appendix 5. Interaction between modes of ZnO application and different concentrations on zinc content and total zinc uptake by wheat plant

	Zinc content in plant (μg g^{-1})		Total zinc uptake by plant (μg g^{-1})	
Concentration (ppm)	Foliar spray	Seed soaking	Foliar spray	Seed soaking
Control	$1.30^{j} \pm 0.28$	$8.80^{h} \pm 0.68$	$2.93^{j} \pm 0.31$	$21.46^{h} \pm 1.08$
10	$2.80^{i} \pm 0.34$	$10.30^{g} \pm 0.94$	$8.23^{i} \pm 0.53$	$28.33^{g} \pm 1.45$
20	$12.20^{ef} \pm 0.98$	$18.60^{d} \pm 0.84$	$35.83^{f} \pm 1.28$	$47.04^{e} \pm 2.14$
50	$24.70^{b} \pm 1.57$	$60.09^{a} \pm 2.58$	$68.89^{b} \pm 2.85$	$58.28^{c} \pm 2.57$
100	$21.40^{c} \pm 1.62$	$13.50^{e} \pm 0.95$	$56.64^{cd} \pm 2.74$	$203.29^{a} \pm 5.98$

Different letters denote significant differences among treatments. Data with the same letters in the superscript do not differ significantly.

Appendix 6. Interaction between modes of ZnO application different concentration on zinc content and total zinc uptake by field pea plant

	Zinc content in plant (μg g^{-1})		Total uptake by plant (μg g^{-1})	
Concentration (ppm)	Foliar spray	Seed soaking	Foliar spray	Seed soaking
Control	$10.90^{j} \pm 1.05$	$14.50^{i} \pm 1.95$	$26.51^{j} \pm 1.38$	$70.61^{i} \pm 4.78$
10	$19.90^{g} \pm 1.35$	$18.90^{h} \pm 2.17$	$111.96^{g} \pm 5.68$	$95.38^{h} \pm 5.47$
20	$20.90^{f} \pm 2.25$	$21.40^{e} \pm 2.54$	$168.13^{b} \pm 6.87$	$135.55^{f} \pm 6.85$
50	$27.60^{a} \pm 2.38$	$24.40^{b} \pm 2.46$	$186.56^{a} \pm 9.58$	$152.58^{d} \pm 5.24$
100	$22.50^{d} \pm 3.05$	$24.30^{bc} \pm 2.58$	$168.09^{bc} \pm 8.57$	$141.57^{e} \pm 4.25$

Different letters denote significant differences among treatments. Data with the same letters in the superscript do not differ significantly.

and 60 DAS respectively. It was observed that the application of silver NPs at a concentration of 60 ppm significantly increased maize plant height compared to control. However, after 60 ppm, plant height decreased as the concentration increased[26]. Fathi *et al.*[27] also observed a significant increase in LAI and plant height with ZnO NPs application in wheat.

Grain yield

The higher yield of wheat and field pea in different ZnO NPs might be due to increased plant growth. Significantly higher grain yields were obtained up to 50 ppm concentration of ZnO NPs because the medium concentration of these NPs increased the absorption and accumulation of macro- and micronutrients, resulting in higher grain yield in field crops[28]. Tarafdar *et al.*[29] also found that grain yield of pearl millet significantly increased at 10 mg/l concentration of ZnO NPs with increased absorption and accumulation of macro- and micro-soil nutrients. Application of ZnO NPs either through foliar spray or seed-soaking had a significant positive effect on physiological and yield-parameters. Zinc NPs had varying effects depending on their concentration[30].

Zn content in plants

The Zn content in plants and its uptake from NPs are affected by the dose and experimental conditions[31]. Our results show that zinc content in plant samples and zinc uptake by plants is higher at lower concentration of ZnO NPs. On the other hand, it has been reported that the increase in Zn content in plants and its uptake with NPs application could be attributed to increased NPs penetration into plant cells[32]. However, NPs penetration and transport nanostructures mechanisms of different sizes (1–100 nm) and shapes are unclear; further regard is warranted on this aspect[33].

The present study found that seed-soaking is an effective method for increasing Zn content in plants and its uptake. Overall, our findings can assist the fertilizer industry in the production of nanofertilizers, particularly ZnO NPs for plant nutrition, which will help reduce Zn deficiency in plants[34].

Zinc use efficiency

Zinc use efficiency has been defined in terms of AE and PE. Apparent zinc use efficiency was found to decrease significantly as ZnO concentration increased[35]. This is due to the opposite interaction between utilization and application rate of ZnO NPs. The decrease in zinc use efficiency with an increase in ZnO concentration was also due to an increase in grain yield at higher levels of ZnO (ref. 36). The efficiency of biomass accumulation and production per unit of nutrient absorbed can be measured by use efficiency. Foliar spray of ZnO fertilizers through the use of nanocarriers is a novel technology in cereals and pulses. This demonstrates increased use efficiency of zinc in the plants through active uptake, translocation and accumulation of ZnO NPs[36]. Maximum zinc use efficiency may be due to optimal Zn uptake in different ZnO NP treatments. Zinc use efficiency was high in treatments that received ZnO as a foliar spray, which was also due to higher zinc uptake[37].

Conclusion

ZnO NPs were synthesized using SEM and UV–Vis spectroscopy. The results confirmed that the synthesized ZnO NPs ranged in size from 0.20 μm to 0.30 μm. SEM results revealed that NPs were made up of tiny clusters of irregular and spherical particles. Our findings reveal that increasing ZnO NPs concentration up to 50 ppm increases plant height, LAI and yield of wheat and field pea; but these show a decrease at 100 ppm concentration. The application of ZnO NPs through various methods, such as foliar spray and seed-soaking, is also effective in the absorption of macro- and micro-nutrients, which increases plant growth and yield in both crops. Medium concentration of ZnO NPs (50 ppm) improves the growth and yield of both food crops (wheat and field pea), but very low (10 ppm) and very high (100 ppm) concentrations reduce growth and yield in the crops.

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