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Research Article

EFFECT OF GREEN-SYNTHESIZED ZINC OXIDE NANOPARTICLES ON SEED GERMINATION AND EARLY SEEDLING GROWTH OF CORN (*ZEA MAYS* L.)

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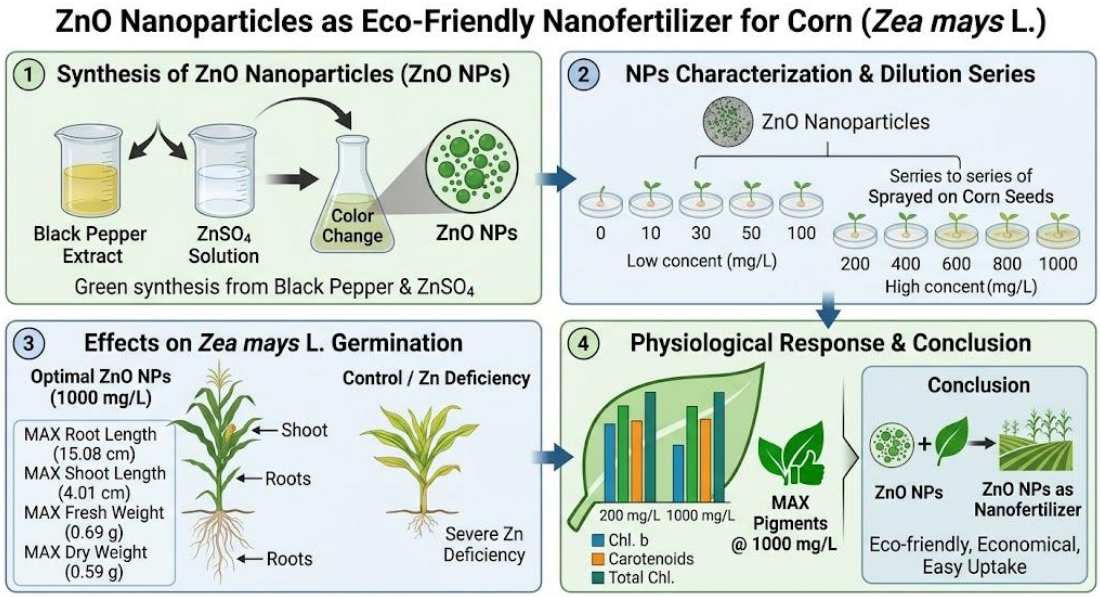
ABSTRACT

Nanoparticles are minute particles which have different properties. The effects of zinc oxide NPs were checked on *Zea mays* L. commonly known as corn or maize present in many parts of Punjab in different varieties. Corn is one of the most sensitive plants to zinc deficiency. In soils with severe zinc deficiency, a chlorotic stripe appears on one or both sides of the central veins in the first two weeks of growth. In the present study, ZnO nanoparticles were formed by the mixture of plant extract (black pepper) and $ZnSO_4$ solution in different concentrations. Change in color of the extracts indicated the formation of ZnO nanoparticles. Zinc ions were reduced to form nanoparticles. There were reverse sequences dilutions of nanoparticle solutions (10, 30, 50, 100 and 0 mg/L at lower concentrations and 200, 400, 600, 800 and 1000 mg/L at higher concentrations) were prepared and sprayed on the corn seeds of different replica petri plates. The germination of seedlings was observed after 9 days. Maximum root length (15.08^{a}±1.585 cm), shoot length (4.01^{a}±0.28 cm), fresh weight (0.69^{a}±0.07 g) and dry weight (0.59^{a}±0.05 g) were observed at the 1000mg/L concentration. Chl. a content was best seen in 200 mg/L solution (74.69^{ab}±4.48 mg/g). Other maximum chl. b (58.16^{b}±4.09 mg/g), carotenoids (10.27^{b}±1.27 mg/g) and total chl. (66.35^{a}±1.32 mg/g) were also best observed in the 1000 mg/L concentration. It can be concluded that ZnO nanoparticles can be used as nanofertilizers in agriculture as compared to other $ZnSO_4$ fertilizers because these are environment friendly, economical and can be easily uptake by the plants.

KEY WORDS: Corn (*Zea mays* L.), Nanoparticles, Nanopriming, Morphological and physiological parameters.

GRAPHICAL ABSTRACT:



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1: INTRODUCTION:

Nanotechnology opens a large scope of novel applications in the fields of biotechnology and agricultural industries, because nanoparticles (NPs) have unique physiochemical properties, i.e., high surface area, high reactivity, tunable pore size, and particle morphology. Nanoparticles can serve as "magic bullets", containing herbicides, nano-pesticide fertilizers, or genes, which target specific cellular organelles in plant to release their content. Despite the plenty of information available on the toxicity of nanoparticles to plant system, few studies have been conducted on mechanisms, by which nanoparticles exert their effect on plant growth and development. Moreover, nanoscience contributes new ideas leading us to understand the suitable mode of action of nanoparticles in plants.

The appropriate elucidation of physiological, biochemical, and molecular mechanisms of nanoparticles in plant leads to better plant growth and development (Siddiqui *et al.,* 2015).

The main characteristic of engineered nanoparticles is their size (<100 nm), which falls in the transitional zone between individual atoms or molecules and the corresponding bulk materials. This can modify the physico-chemical properties of the material as well as create the opportunity for increased uptake and interaction with biological systems. This combination effect can generate adverse biological effects in living cells that would not otherwise be possible with the same material in large form. Therefore, organisms and especially those that interact strongly with their immediate environments are expected to be affected because of their exposure to engineered nanoparticles. However, to date, plants as important ecological receptors, have not received enough toxicity research. At present, there is only limited phytotoxicity studies reported both positive and negative effects of nanoparticles on higher plants (Kumar and Udayasoorian, 2014).

The use of engineered nanoparticles (NPs) in medicine, cosmetics, energy, agriculture and machinery, *etc.* has increased rapidly. Considering that engineered NPs can be released to the environment, accidentally or incidentally, nanotoxicity is receiving increasing attention currently. Despite the fact that more and more researchers have reported nanotoxicity in plants, the studies are still at the emerging stage and knowledge on the effects of NPs in plant systems needs further investigation , especially in crop plants, since the engineered NPs may be a threat to human health through the food chain. Maize (*Zea mays* L.) is one of the three most important food crops worldwide. Rice (*Oryza sativa* L.), as another important staple food, feeding more than half of the World's population. Due to their important roles in the food security of humankind, the assessment of the phytotoxicity of metal oxide NPs on these two agriculturally important crop plants is crucial to human health (Yang *et al.,* 2015).

2: MATERIALS AND METHODS

The nanoparticles synthesis and their effects on corn seedlings were determined by using the protocol by Razzaq *et al.* (2016).

Following materials and methods were used for the experimentation.

2.1: Source of Plant material

Black pepper was obtained from a local market, and corn seeds were brought from NARC.

2.2: Formation of plant extract

Five grams of black pepper were weighed on weighing balance and then put that in a beaker. Distilled water was taken 100ml and added in that beaker and then mixed well with the help of a stirrer.

The solution was heated for 30 seconds in oven and again mixed well with the help of a stirrer. The solution was filtered through disc filter paper with the help of a glass funnel in another beaker. The plant extract was thus prepared.

2.3: Formation of 0.4M $ZnSO_4$ solution

On weighing balance, 0.65 grams of $ZnSO_4$ salt was weighed and put in a beaker. Then 100ml of distilled water was added in the salt and mixed well with the help of a stirrer. The solution was then heated for 30 seconds in the oven and again mixed well. Thus 0.4M solution was prepared.

2.4: Formation of ZnO-NPs nanoparticles

The plant extract with amounts of 10, 20 and 30 mL was taken in 3 separate beakers. Then 10 mLl of $ZnSO_4$ solution was added in each beaker and mixed well with the help of a stirrer. These solutions then put aside for some time. The solutions were observed first after 15 minutes. Then the solutions were observed after half an hour and then again observed after one and a half hour for the last time. The colour change in three solutions was observed. Then the solutions were left for 24 hours. After 24 hours, 9 eppendorfs were taken and 1 ml of these solutions were taken in each eppendorf.

2.5: Centrifugation

Eppendorfs were taken to the centrifuge and rotated at 10000rpm for 10 minutes. The zinc oxide (ZnO) nanoparticles were formed under the base of the eppendorfs and upper layer was supernatant. That supernatant was removed and nanoparticles were taken.

2.6: Formation of nanoparticle solutions

The nanoparticles were dried and then took the pellete with the help of a sterilized needle. Weighed the nanoparticles on weighing balance. Nanoparticles were dissolved in the distilled water, and stock solution was prepared which was 200 mg/L. For this, 200 mg nanoparticles were dissolved in one litre of distilled water. Then further dilutions of 100 mg/L, 50 mg/L, 30 mg/L and 10 mg/L were prepared with the same procedure in flasks. These five solutions were used experimentally. In another flask, distilled water was taken which was used as control (0mg/L).

2.7: Treatment details

There were 6 treatments; each having 3 replicas. First treatment was control which has distilled water treated seeds. All the other treatments were experimental. Treatment 2 had the seeds treated with 200mg NPs in 1 liter of distilled water. Treatment 3 had the seeds treated with 100mg NPs in 1 liter of distilled water. Treatment 4 had the seeds treated with 50mg NPs in 1 liter of distilled water. Treatment 5 had the seeds treated with 30mg NPs in 1 liter of distilled water and treatment 6 had the corn seeds treated with 10mg NPs in 1 liter of distilled water.

2.8: Other treatments

In next experiment, the new concentrations of nanoparticles solutions were used which were the treatments of 1000mg/L stock solution and then the dilutions of 800mg/L, 600mg/L and 400mg/L and the seeds were treated in the same manner along with three replicas of each treatment.

2.9: Sterilization

Eighteen petri plates were taken; 3 for each solution and sterilized giving 170 degrees temperature in the oven for almost 3-4 hours so that no germs were left. It was the sterilization of petri plates which must be used for experimentation.

Almost 300 seeds of *Zea mays* L. were taken and sterilized in 70% ethanol for almost 20 minutes. This is called surface sterilization. Then the seeds were washed 2 to 3 times with distilled water.

2.10: Seed germination

Seeds were put in petri plates in the following way for germination:

2.11: Control (0 mg/L)

Enough distilled water was taken in a beaker, and 45 corn seeds were dipped in that for almost 2 hours. The 3 sterilized petri plates were taken and put double disc filter paper in them. Marked those 3 replicas as control (R_1, R_2, R_3). Distilled water was taken in a dropper and poured onto the filter papers so that they became soaked. The dipped seeds from distilled water were removed and put 15 seeds in each of the petri plate with 1-2cm distance between two seeds. Again, the distilled water was poured onto the seeds, so the the seeds became wet and not flooded heavily. The petri plates were covered and sealed tightly with the help of paper tape so that there would not be the fungal attack on the corn seeds.

2.12: Experimental (nanoparticle solutions)

The upper five dilutions of nanoparticles were taken in a small amount in separate beakers, and 45 seeds were dipped in each of the solutions (15 for each petri plate) for almost 2 hours. Then sterilized petri plates were taken and put the double disc filter paper in each petri plate. Three replicas (R_1, R_2, R_3) of each solution were made and marked according to their concentrations. Then the 200 mg/L solution was taken in a dropper and poured almost 5ml in its 3 replica petri plates. Same procedure is done by other 100 mg/L, 50 mg/L, 30 mg/L and 10 mg/L solutions in other 12 petri plates (3 for each solution). Then the dipped seeds of *Zea mays* L. were removed from their solutions and put them into their petri plates of respective concentrations and replica. The distance between two seeds was about 1-2cm. Again, pour the respective solutions onto the seeds in normal amounts. The seeds were not flooded by the solutions heavily. Only that number of solutions was poured which was sufficient for the soaking of filter papers and seeds. Then the petri plates were covered and sealed with the paper tape tightly so that there would not be the attack of fungal spores on the seeds. The experimental petri plates were left for some days so that the seeds could be grown.

2.12: BIOCHEMICAL ANALYSIS

a) Chlorophyll and Carotenoids:

The topmost leaves from each treatment were taken and 0.05g of those leaves were measured from each replica with the help of a weighing balance separately. These leaves were crushed separately in pestle and mortar one by one and put in glass vials. Then 80% acetone solution was prepared by adding 20ml distilled water in 80ml of acetone. In the vials containing leaves, 5ml of 80% acetone solution was added one by one and mixed very well and then filtered it with the help of disc filter paper. Absorbance was recorded at 470nm, 645nm and 663nm through the spectrophotometer. The values of chlorophyll a, chlorophyll b, total chlorophyll and carotenoids were measured by the following formulas (Arnon, 1949).

Chlorophyll a = 12.21 (A663) - 2.81 (A645)

Chlorophyll b= 20.13 (A645) – 5.03 (A663)

Total chlorophyll= 20.4 (A645) + 8.02 (A663)

Carotenoids (µg/ml) =

$$\frac{1000\ (A_{470}) - 3.27\ (\text{chl. a}) - 10(\text{chl.b})}{227}$$

To determine the concentration per gram of plant tissue, following formula was used:

$$C*V/1*G$$

Where C is the amount of pigment (mg/L), V is the volume of acetone used and G is the weight of leaves taken for test, Chl. a/b ratio was also calculated.

2.13: Morphological parameters of *Zea mays* L.

The growth of seeds was analyzed on daily bases and noted the number of seeds grown per day according to their respective concentrations and replica. After some days, several roots and shoots of corn seeds were grown in the petri plates. The plant morphological parameters, i.e. root length, shoot length, fresh seed weight and dry seed weight treated with ZnO nanoparticles were carried out by standard procedures. The root and shoot lengths were measured by measuring scale and fresh and dry seed weights were measured by weighing balance. For the dry weight, the seeds were first dried in the dry heat oven at 170 degrees for about 3-4 hours. Then the dry seeds were weighed by weighing balance. The final data was analyzed and tabulated.

2.14: Statistical analysis:

Data was collected from three replicates (R_1, R_2, R_3). The data collected was analyzed through one way analysis of variance by using COSTAT and SPSS software. Duncans multiple range test was applied to check the significance level.

3: RESULTS

3.1: Effect of ZnO NPs on the germination and growth of corn seedlings

Table 1 shows the effect of Zinc oxide (ZnO) nanoparticles of concentrations with different ranges of 10, 30, 50, 100, 200 mg/L on seed germination of *Zea mays* L. Seed germination was studied with the parameters i.e. shoot length, root length, fresh seedling weight and dry seedling weight.

Data was collected from three replicates (R_1, R_2, R_3). The collected data was analyzed through one way analysis of variance by using COSTAT software. Duncans multiple range test was applied to check the significance level. The results were presented as mean ± standard deviation.

It is clear from Table 1 that Zinc oxide nanoparticles have negative effects on fresh seedling weight and shoot length and these nanoparticles have no significant effects on root length and dry seedling weight of *Zea mays* L at lower concentrations. The best growth of seedlings was obtained by control (distilled water having no NPs).

Figure 1(a) shows the root and shoot lengths of the corn seeds under the lower concentrations of zinc oxide nanoparticles i.e. 200, 100, 50, 30, 10 mg/L and control (0mg/L). The maximum root length (5.37^{a}±0.31cm) and shoot length (0.71^{a}±0.05 cm) were developed in the control series and the minimum root length (2.95^{a}±0.20 cm) and shoot length (0.09^{b}±0.05 cm) was seen in the 100mg/L series. Overall lower concentrations of ZnO NPs have negative effect on the root and shoot lengths of *Zea mays* L. seedlings. There is no significant effect of these nanoparticles concentrations (100, 50, 30 and 10 mg/L) on shoots of seeds and roots showed some growth in these concentrations. But the roots and shoot lengths somehow increased in 200 mg/L concentration.

Figure 1(b) shows the fresh and dry weights of the corn seedlings after growth and germination under the lower concentrations of zinc oxide nanoparticles i.e. 200, 100, 50, 30, 10 mg/L and control (0 mg/L). The maximum (0.46^{a}±0.02g) fresh weights of corn seedlings were observed in the control series and the minimum (0.38^{b}±0.27g) values were seen in the 100 mg/L series. The maximum (0.26^{a}±0.002g) dry weights were observed in the 30 mg/L series. The values of maximum fresh and dry weights of the seedlings of 30 mg/L and 10 mg/L almost resembled each other. The minimum dry weights (0.24^{a}±0.006g) were seen in the control and 200 mg/L series which was same. Overall lower concentrations of ZnO NPs have negative effect on the fresh and dry weights of *Zea mays* L. seedlings. There is no significant effect of these nanoparticles' concentrations on the corn seeds. Overall, it is obvious that the lower concentrations of the zinc

oxide nanoparticles affect the fresh and dry seedlings weights of *Zea mays* L. negatively.

Table 2 shows the effect of different concentrations of Zinc oxide (ZnO) nanoparticles (200, 400, 600, 800 and 1000 mg/L) on seed germination of *Zea mays* L. Seed germination was studied with the parameters i.e. shoot length, root length, fresh seedling weight and dry seedling weight. Data was collected from three replicates (R_1, R_2, R_3). The data collected was analyzed through one way analysis of variance by using SPSS software. Duncans multiple range test was applied to check the significance level. The results were presented as mean ± standard deviation. It is clear from Table 2 that Zinc oxide nanoparticles have positive effects on fresh seedling weight and dry seedling weights and these nanoparticles have no significant effects on root length and shoot length of *Zea mays* L. at higher concentrations. The best growth of seedlings was obtained by 1000 mg/L.

Table 1: Effect of lower concentrations of zinc oxide nanoparticles on the growth and germination of Corn (*Zea mays* L.) seedlings.

Sr. no	Dose of ZnO NPs (mg/L)	S.L (cm)	R.L (cm)	F. Wt (g)	D. Wt (g)
1	200	0.45^{ab}±0.09	4.46^{a}±0.52	0.43^{ab}±0.06	0.24^{a}±0.006
2	100	0.09^{b}±0.05	2.95^{a}±0.20	0.38^{b}±0.27	0.25^{a}±0.02
3	50	0.24^{ab}±0.09	4.14^{a}±0.60	0.43^{ab}±0.01	0.26^{a}±0.004
4	30	0.16^{b}±0.08	3.37^{a}±0.55	0.42^{ab}±0.02	0.26^{a}±0.002
5	10	0.17^{b}±0.06	3.25^{a}±0.12	0.42^{ab}±0.03	0.26^{a}±0.02
6	0	0.71^{a}±0.05	5.37^{a}±0.31	0.46^{a}±0.02	0.24^{a}±0.006

Table 2: Effect of higher concentrations of zinc oxide nanoparticles on the growth and germination of Corn (*Zea mays* L.) seedlings

Sr. no	Dose of ZnO NPs (mg/L)	S.L (cm)	R.L (cm)	F.Wt (g)	D.Wt (g)
1	1000	4.01^{a}±0.28	15.08^{a}±1.585	0.69^{a} ±0.07	0.59^{a}±0.05
2	800	3.01^{a}±1.37	11.49^{a} ±1.83	0.56^{ab}±0.07	0.45^{a}±0.06
3	600	2.14^{a}±0.22	8.34^{a} ±1.57	0.54^{ab}±0.02	0.51^{a}±0.04
4	400	2.73^{a}±1.10	5.83^{a} ±1.102	0.58^{ab}±0.08	0.43^{ab}±0.03
5	200	0.68^{a}±0.22	2.26^{a} ±1.06	0.47^{b}±0.23	0.37^{ab}±0.15
6	0	1.36^{a}±0.90	1.76^{a} ±1.87	0.46^{ab}±0.05	0.42^{b}±0.06

S.L: shoot length; **R.L**: root length; **F.Wt:** fresh seedling weight, **D.Wt:** dry seedling weight

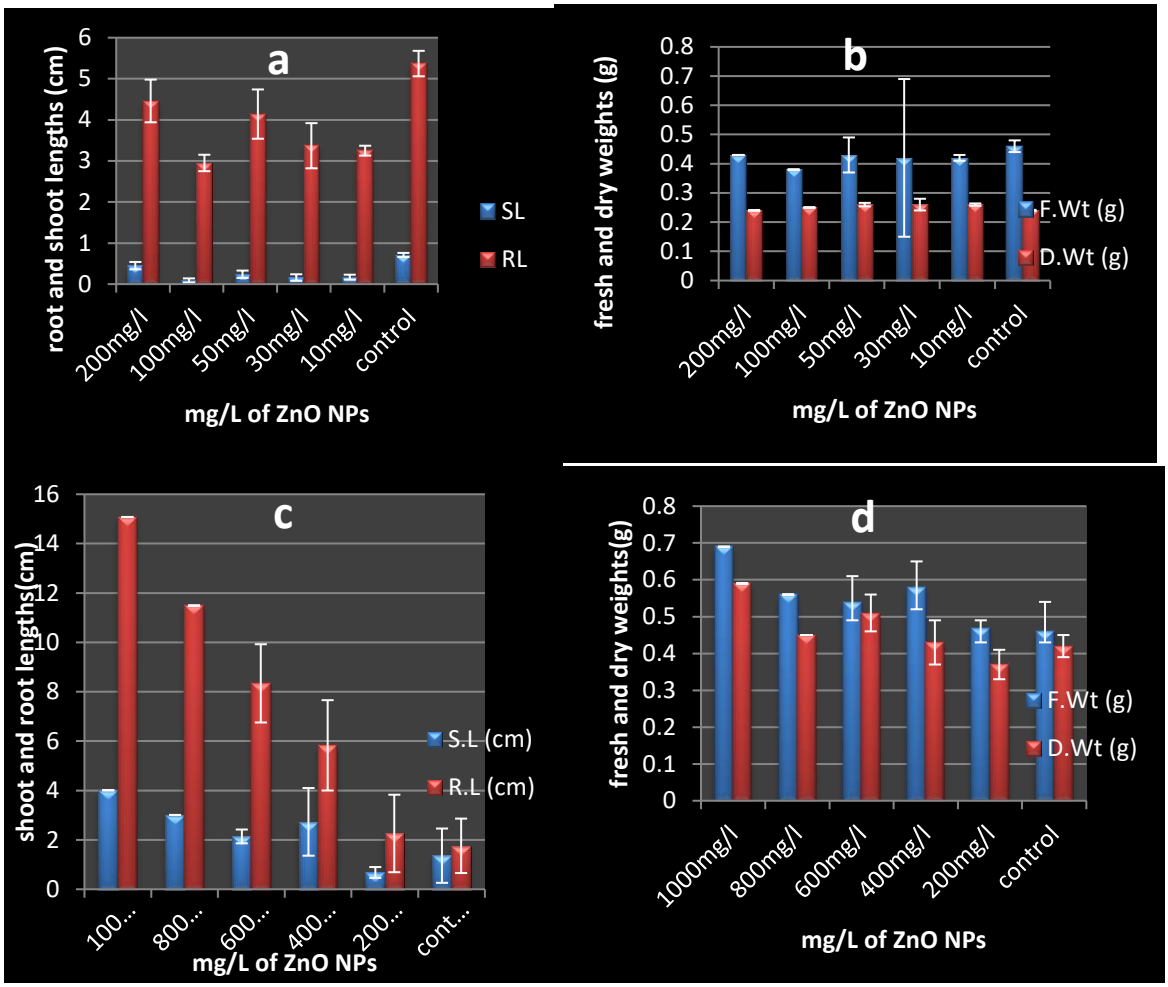

Figure 1: Effect of Lower Concentrations of ZnO nanoparticles on a) root and shoot lengths b) fresh and dry weights; effect of higher concentrations of ZnO nanoparticles on c) root and shoot lengths d) fresh and dry weights of *Zea mays* L. seeds

Figure 1 (c) shows the root and shoot lengths of the corn seeds under the higher concentrations of zinc oxide nanoparticles i.e. 1000, 800, 600, 400, 200 mg/L and control (0 mg/L). The maximum root length ($15.08^{a}\pm1.585$cm) and shoot length ($4.01^{a}\pm0.28$ cm) developed in the 1000 mg/L concentration and the minimum root length ($1.76^{a}\pm$ 1.87 cm) and shoot length ($1.36^{a}\pm0.90$ cm) was seen in the control series. Overall higher concentrations of ZnO NPs have positive effect on the root and shoot lengths of *Zea mays* L. seeds. There is no significant effect of distilled water on the corn seeds as compared to the different concentrations of the zinc oxide nanoparticles. Overall, it is obvious that the higher concentrations of the zinc oxide nanoparticles affect the seeds of *Zea mays* L. positively and these particles made the growth of root and shoot lengths more in higher amounts. The figure 1 (d) shows the fresh and dry weights of the corn seedlings after growth and germination under the higher concentrations of zinc oxide nanoparticles i.e. 1000, 800, 600, 400, 200 mg/L and control (0 mg/L). The maximum ($0.69^{a}\pm0.07$g) fresh weights of corn seedlings were observed in the 1000 mg/L series and the minimum ($0.46^{ab}\pm0.05$g) values were seen in the control series. The maximum ($0.59^{a}\pm0.05$g) dry weights were observed in the 1000 mg/L and the minimum values ($0.37^{ab}\pm0.15$ g)

of dry weights were observed in the 200 mg/L series. Overall higher concentrations of ZnO NPs have positive effect on the fresh and dry weights of *Zea mays* L. seedlings. There is normal effect of control or distilled water on the corn seedlings. Overall, it is obvious that the lower concentrations of the zinc oxide nanoparticles affect the fresh and dry seedlings weights of *Zea mays* L. positively and these NPs enhanced the seedling weights in higher concentrations.

3.2: Effect of ZnONPs on biochemical parameters of *Zea mays* L.

The figure 2 (a) shows the chlorophyll "a" content in the leaves of the corn seedlings after growth and germination under the higher concentrations of zinc oxide nanoparticles i.e. 1000 mg/L, 800 mg/L, 600 mg/L, 400 mg/L, 200 mg/L and control (0 mg/L). The maximum ($74.69^{ab} \pm 4.48$mg/g) chl. 'a' content of corn seedlings was observed in the 200 mg/L series and the minimum ($3.006^{b} \pm 0.63$mg/g) value was seen in the 1000 mg/L series. Overall higher concentrations of ZnO NPs have no significant effect on the chl. 'a' content of *Zea mays* L. seedlings. There was no significant effect of distilled water on the chl. 'a' content of corn seedlings.

The figure 2 (b) shows the chlorophyll "b" content in the leaves of the corn seedlings after growth and germination under the higher concentrations of zinc oxide nanoparticles i.e. 1000 mg/L, 800 mg/L, 600 mg/L, 400 mg/L, 200 mg/L and control (0 mg/L). The maximum ($58.16^{b} \pm 4.09$ mg/g) chl. 'b' content of corn seedlings was observed in the 1000 mg/L series and the minimum ($7.31^{ab} \pm 0.79$mg/g) value was seen in the 200mg/L series. Overall higher concentrations of ZnO NPs have no significant effect on the chl. 'b' content of *Zea mays* L. seedlings. There are also not more significant effects of distilled water on the chl. 'b' content of corn seedlings but the chl. 'b' content in it is less than 1000 mg/L and more than other series.

The figure 2 (c) shows the number of carotenoids in the leaves of the corn seedlings after growth and germination under the higher concentrations of zinc oxide nanoparticles i.e. 1000 mg/L, 800 mg/L, 600 mg/L, 400 mg/L, 200 mg/L and control (0 mg/L). The maximum ($10.27^{b} \pm 1.27$mg/g) carotenoid content of corn seedlings was observed in the 1000mg/L series and the minimum ($4.3^{ab} \pm 0.42$mg/g) value was seen in the 400mg/L series. Overall higher concentrations of ZnO NPs have positive effect on the carotenoid content of leaves of *Zea mays* L. seedlings. There are not more significant effects of distilled water on the carotenoid content of corn seedlings.

The figure 2(d)' shows the amount of total chlorophyll in the leaves of the corn seedlings after growth and germination under the higher concentrations of zinc oxide nanoparticles i.e. 1000 mg/L, 800 mg/L, 600 mg/L, 400 mg/L, 200 mg/L and control (0mg/L). The maximum ($66.35^{a} \pm 1.32$mg/g) content of total chl. of corn seedlings was observed in the 1000mg/L series and the minimum ($21^{ab} \pm 3.52$mg/g) value was seen in the 400mg/L series. Overall higher concentrations of ZnO NPs have positive effect on the total chlorophyll content of leaves of *Zea mays* L. seedlings. There are not more significant effects of distilled water on the total chlorophyll content of corn seedlings.

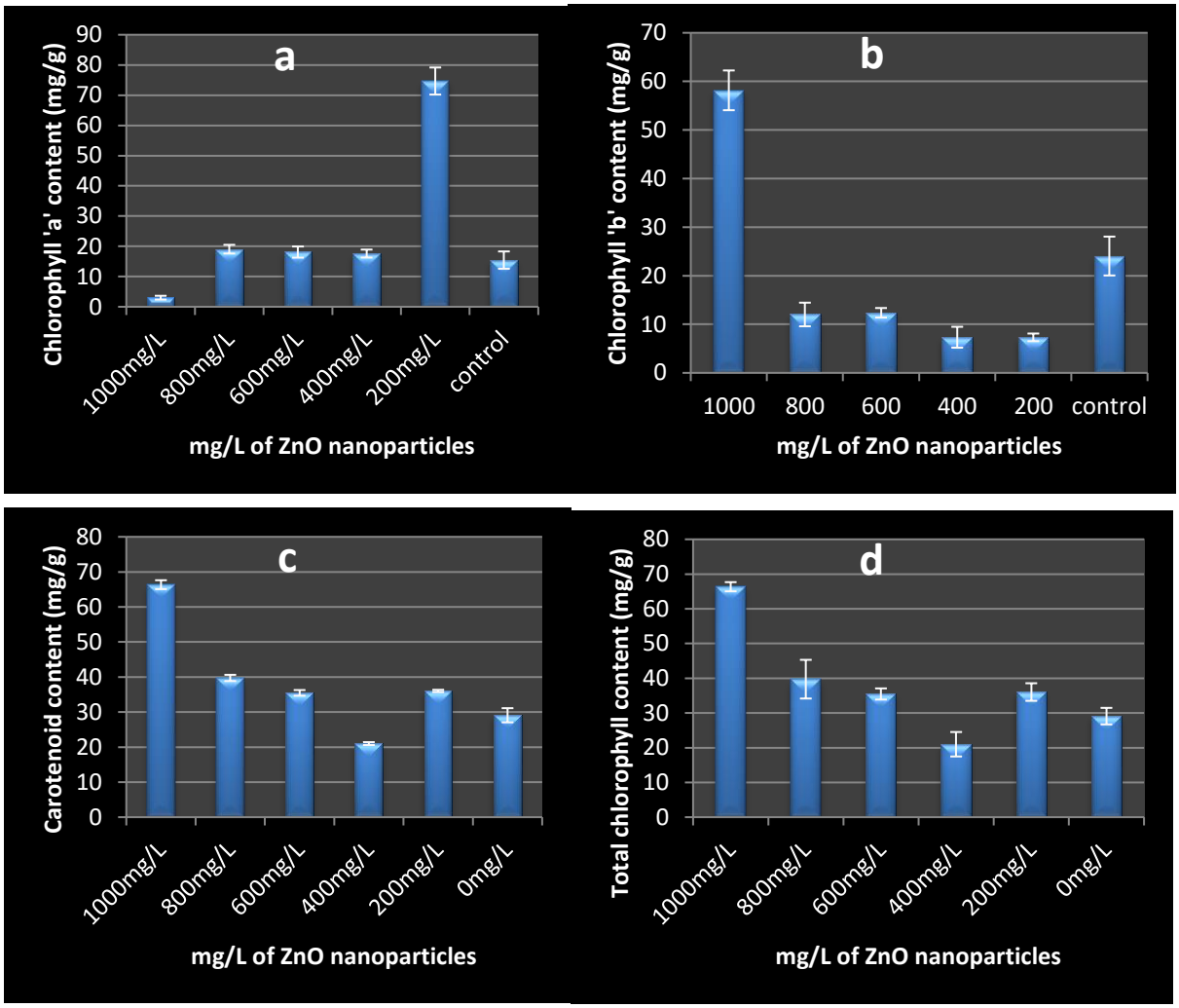

Figure 2: The effect of higher concentrations of ZnO nanoparticles on a) Chlorophyll "a" content b) Chlorophyll "b" content c) carotenoids d) amount of total chlorophyll in leaves of *Zea mays* L. seedlings

4: DISSCUSION

Nanotechnology opens a large scope of novel application in the fields of biotechnology and agricultural industries, because nanoparticles (NPs) have unique physicochemical properties, i.e., high surface area, high reactivity, tunable pore size, and particle morphology. Nanoparticles can serve as "magic bullets", containing herbicides, nano-pesticide fertilizers, or genes, which target specific cellular organelles in plant to release their content. (Siddiqui *et al.,* 2015). Nanotoxicity, an emerging concept, is receiving increasing attention with the fast development of nanotechnology. Increasing application of nanotechnology highlights the need to clarify nanotoxicity. (Kumar and Udayasoorian, 2014).

Maize (*Zea mays*), also called corn, is believed to have originated in central Mexico 7000 years ago from a wild grass, and Native Americans transformed maize into a better source of food. Maize contains approximately 72% starch, 10% protein, and 4% fat, supplying an energy density of 365Kcal/100g and is grown throughout the world, with the United States, China, and Brazil being the top three maize-producing countries in the world, producing approximately 563 of the 717 million metric tons/year. The way in which maize is processed and consumed varies greatly from country

to country, with maize flour and meals being two of the most popular products. The actual human consumption of these cereals is somewhat lower than the estimated figures because of waste, use in nonfood products, and because milling removes some of the outer layers, or bran, which is generally used as animal feed. As with all cereals, most micronutrients are concentrated in the outer layers of the maize grain; thus, removing these layers in the milling process results in the loss of most vitamins and minerals (Ranum *et al.,* 2014).

In the present studies, the effects of Zinc oxide (ZnO) nanoparticles on the growth and germination of *Zea mays* L. seeds were observed. The nanoparticle solutions were prepared and observed just after 15 minutes, then half an hour and then after 1 and a half hour. There was change in colour of the solutions as time passed. Then nanoparticles were prepared by the process of centrifugation. Five parameters, i.e. root length, shoot length, fresh seedling weight, dry seedling weight and chlorophyll and carotenoid contents were measured.

The shoot lengths of maize in lower concentrations (200, 100, 50, 30, 10 and 0mg/L) have no significant effects on the shoot lengths of corn seedlings as nanoparticles inhibit the growth. The growth was good in control as compared to NPs treatments. Better root length was observed at nanoparticle treatments. The snificant effects were seen in fresh and dry seedling weights by nanoparticles too.

At higher concentrations of ZnO nanoparticles, i.e. 1000, 800, 600, 400, 200 and 0 mg/L, the excellent root lengths were seen. Better shoot lengths were also observed. These lengths increased as the concentration of ZnO NPs increased. Maximum root and shoot lengths were observed in 1000mg/L dilution. There were also good values of fresh and dry seedling weights that were demonstrated as the nanoparticles enhanced the growth and germination at higher concentrations. The values at control (0mg/L) were not significant as compared to NPs solutions.

At higher concentrations of ZnO NPs, chlorophyll and carotenoid content of corn seedlings were also observed by measuring the absorbance through spectrophotometer. Then the values of chl.a, chl.b, carotenoids and total chlorophyll were measured.

Highest values of chl.a were observed at 200mg/L treatment and of chl.b at 1000mg/L concentrations. There were no significant effects of other treatments of NPs and control at chl.a and chl.b. Values of carotenoids and total chl. were excellent at zinc oxide nanoparticle solutions because there was more pigmentation as compared to control.

Overall results showed that the germination and growth of the *Zea mays* L. seeds was better at the higher concentrations of the zinc oxide nanoparticles because at higher amounts, the nanoparticles enhanced the growth of the corn seeds and the growth at control was low relatively. The growth at lower concentrations of the nanoparticles was relatively lower than higher concentrations as the zinc oxide nanoparticles inhibit the growth of the seeds at lower amounts. The growth was better at control treatment as compared to lower amounts of ZnO NPs.

Kumar and Udayasoorian, (2014) observed that higher root and shoot lengths of maize was present in control as compared to metal oxide nanoparticles i.e. ZnO, Al_2O_3 and TiO_2 NPs solutions. It means that the nanoparticles of different metal oxides inhibit the growth of the *Zea mays* L. seeds. (Xiang *et al.,* 2015) showed the toxicity of four zinc oxide nanoparticles (i.e., spheric ZnO-30, spheric ZnO-50, columnar ZnO-90, and hexagon rod-like ZnO-150) to the seed germination of Chinese cabbage (*Brassica pekinensis* L.). The results showed that zinc oxide nanoparticles (nano-ZnOs) did not affect

germination rates at concentrations of 1-80 mg/L but significantly inhibited the root and shoot elongation of Chinese cabbage seedlings, with the roots being more sensitive. (Zafar *et al.,* 2016) demonstrated that ZnO NPs concentration ranging from 500 to 1500 mg/L adversely affects the *Brassica nigra* seed germination and seedling growth and lead to an increase in the antioxidative activities and non-enzymatic antioxidants. While, culturing the stem explants of *B. nigra* on Murashige and Skoog (MS) medium at lower concentration of ZnO NPs (1–20 mg/L) resulted in the production of white thin roots with thick root hairs. At 10 mg/L ZnO NPs, shoots emergence is also observed. (Adhikari *et al.,* 2015) studied the effect of zinc oxide nanoparticles (<100 nm) on growth of maize (*Zea mays* L.) plant, as one of the major agricultural crops, in a solution culture system. Various concentrations of zinc (Zn) were applied through nano-zinc oxide (ZnO) particles (<100 nm) in suspension form and in ionic form through zinc sulfate ($ZnSO_4$) salt in Hoagland solution culture. Experimental results showed that nano zinc oxide particles could enhance and maintain the growth of maize plants as well as conventional Zn fertilizer (as $ZnSO_4$). The plant parameters like plant height, root length, root volume, fresh and dry matter weight were all improved due to application of zinc oxide nanoparticle. These findings indicate that plant roots might have the unique mechanism of assimilating nano-Zn and using for its growth and development. (Feizi *et al.,* 2013) compared concentrations of nanosized TiO2 at 0, 5, 20, 40, 60 and 80 mg L (-1) with bulk TiO2 for phytotoxic and stimulatory effects on fennel seed germination and early growth stage. After 14 d of seed incubation, germination percentage highly improved following exposure to 60 ppm nanosized TiO2. Similar positive effects occurred in terms of shoot dry weight and germination rate. Application of bulk TiO2 particles in 40 ppm concentration greatly decreased shoot biomass up to 50% as compared to the control. (Mukherjee *et al.,*2016) investigated the impact of different zinc oxide (ZnO) NPs on green pea (*Pisum sativum* L.). Plant fresh and dry biomass and changes in leaf pigment concentrations of green peas were quantified upon harvest at 65 days. Except for the coated 1000 mg/kg NP treatment, fresh and dry weight were unaffected by Zn exposure. Although, all treated plants showed higher tissue Zn than controls, those exposed to Al_2O_3ZnO NPs at 1000 mg/kg had greater Zn concentration in roots and seeds, compared to bulk Zn and the other NP treatments, keeping Al and Si uptake largely unaffected. Higher Zn accumulation in green pea seeds had resulted in coated ZnO at 250 mg/kg treatments. In leaves, Al_2O_3ZnO NP at 250 mg/kg significantly increased Chl-*a* and carotenoid concentrations relative to the bulk, ionic, and the other NP treatments.

The reason is that the ZnO nanoparticles may show dose dependent cytotoxicity, but if managed properly, will enhance the crop yield as they support photosynthetic machinery and many types of enzymes involved in different processes. As a result, nanoparticles enhance the metabolic activities of plants (Watson *et al.,* 2015). Zinc deficiency in plants shows the delay in leaf responses and it causes delay in plant maturity (Taheri *et al.,* 2015). Cytotoxic effect is parallel related to concentration of nanoparticles (Ramesh *et al.,* 2014).

CONCLUSIONS:

ZnO nanoparticles may show cytotoxicity to plants, but if managed properly, will enhance the production of crops as they support photosynthetic machinery and a few enzymes. As a result, they enhance the metabolic activities of plants. Zinc deficiency in plants shows delay in leaf responses

and causes delay in plant maturity. Cytotoxic effect is parallelly related to concentration of NPs.

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