Impact Assessment of Zinc Oxide Nanoparticles (ZnO-NPs) on Seed Germination and Seedling Development of Maize

Abstract

The rising interest in using zinc oxide nanoparticles (ZnO NPs) in agriculture raises concerns about soil contamination, which could result in phytotoxic effects on germinating seeds and seedlings.The present study was carried out to analyze the responses induced by lower, as well as higher doses of ZnO NPs (0–5120 ppm), in maize, for a period of 7 days incubation study.The results revealedthat percentseed germination, shoot, root and seedling length, fresh and dry weightand seed vigour was increased with nano seed priming at lower concentration (80 ppm) as compared to control but decline variably at higher levels (higher than 640-5120 ppm) ofzinc in maize.Thus, seed-priming with zinc oxide nanoparticles at low dosages was sufficient to elicit a good response to seedling germination and vigour, while higher doses were associated with a delay in early development metrics and caused toxic effect on the seedling.

Keywords: Zinc oxide nanoparticles, seed germination and seed vigour

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Introduction

“Nanotechnology proved as a boon to this era and is widely applied in many areas of science and technology. Agriculture is the backbone of industrial raw material and it is very important to have technologies supporting high yield, better crop production and protection in a cost effective, ecofriendly and sustainable way. In recent time nano-based materials like nanoparticles and nano formulations, nano-based fertilizers, nano-pesticides and insecticides, nano fungicides and other plant disease control formulations are available in the market. Though this technology offers many advantages, yet there are some vices which is causing the concern among the researchers and practitioners. The unregulated exposure of the nanoparticles to the soil is expected to cause adverse effects on the soil microbiota and in some cases negatively impact the important adaphic factors like soil infertility and toxicity. In the past few decades, nanoparticles (NPs) have received great attention due to their unique properties and beneficial applications in agriculture and allied sectors.The field of nanotechnology is also being explored as a new source of key improvements in the agricultural sector. Broadly, it describes the synthesis, manipulation, and characterization of materials and structures that have one or more dimensions at nanoscale i.e. <100 nm” (Santos *et al*., 2019). “Nanoparticles are favored for larger applications because of their unique characteristics. These include a very high surface area to volume ratio, increased reactivity, surface potential, and tunable physical and chemical properties. Additionally, they allow for molecular manipulation, making them more advantageous compared to their salt and bulk material counterparts” (Khan *et al*., 2019).

“Zinc plays a vital role in plant metabolism, and it is an essential element for higher plants. Its significance in agriculture is gaining more recognition” (Rattan *et al*., 2009).“Initially, the incidence of Zn deficiency was observed more in cereals, particularly in rice and wheat crops, but with the passage of time, distribution of Zn deficiency covered the whole country across the crops and cropping systems” (Shukla *et al*., 2016). “Zinc functions as a cofactor, playing a crucial role in regulating the activity of numerous enzymes in crop plants". (Barak & Helmke 1993; Lopez-Millan *et al.* 2005). "Moreover, zinc is essential for the formation of chlorophyll and plays a significant role in photosynthesis and respiration".(Aravind and Prasad 2003). "Therefore, zinc deficiency leads to a reduction in the photosynthetic rate, chlorophyll content, carbonic anhydrase activity, and protein biosynthesis". (Anwar *et al*., 2021).

Maize (*Zea mays* L.) is a staple crop of global significance, extensively cultivated for its high yield potential and versatility in food, feed, and industrial applications. Its adaptability to diverse environmental conditions and substantial biomass production makes it a critical component of agricultural systems worldwide. However, the increasing application of nanotechnology in agriculture necessitates a thorough understanding of the potential impacts of nanomaterials on crop growth and development.

"In farming, seeds are a crucial input that determines crop yield and productivity. Therefore, applying seed priming agent technologies to enhance seed quality and physiological parameters presents a significant opportunity.Compared to bulk materials, nanoparticles possess unique properties that significantly impact nearly every branch of science and technology". (Mirza *et al.,* 2019). "In recent decades, the application of nanotechnology has spurred researchers to explore and develop various applications within agricultural systems.Seed priming is a pre-sowing treatment that induces physiological changes in seeds, allowing them to germinate more rapidly" (Bruce *et al*., 2007). "This technique enhances seed quality, boosts crop yields, increases tolerance to environmental stresses, and can improve weak and damaged seeds" (Butler *et al*., 2009)."Priming using nanoparticles (nano-priming) has been proven to be more promising than traditional priming approaches for achieving feasible agricultural yields" (AbbasiKhalaki*et al.,* 2021). "Recently, nano-priming is an innovative method for improving seed germination and reduces seed aging"(Mahakham*et al.,* 2017).Zinc oxide nanoparticles (ZnO NPs) have garnered attention for their unique physicochemical properties, including antimicrobial activity and potential as nano-fertilizers. Despite these promising benefits, the application of ZnO NPs in agriculture also raises concerns regarding their potential toxicity and environmental impact. Understanding the balance between their beneficial effects and possible adverse outcomes is crucial for developing safe and effective agricultural practices. This study aims to investigate the impact of ZnO NPs on the seed germination and seedling growth of maize (*Zea mays* L.), a staple crop of global importance. By examining both the positive and negative effects of ZnO NPs, this research seeks to contribute to the development of sustainable nanotechnology-based solutions in agriculture.

Material and Methods

Chemical

Analytical reagent-grade Zinc sulfateheptahydrate, Sodium hydroxide (NaOH) and Carboxymethyl Cellulose powder were purchased from Merck Chemicals Ltd., Mumbai, India.

Characterization of ZnO nano-particles

ZnO nanoparticles were synthesized at the Nanotechnology Laboratory of Anand Agricultural University, Anand campus, India with particle sizes of less than 100 nm. The morphology of these ZnO nanoparticles was examined using scanning electron microscopy (ZEISS EVO 18) (Figure 1).The particle size distribution of the nano-particles in aqueous system was measured by Photon Collision Spectroscopy (dynamic light scattering techniques), using ZETA sizer Analyzer (Medel: Malvern Zeta-sizer, nano ZS 90), Zeta

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potential measurements were determined using a ZETA sizer (Beckman coulter) from the electro-phoretic mobilities of particles using the Smoluchowski equation (Figure 2). All measurements were performed at 25^{o}c.

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Seeds

The maize seeds of variety GAYMH 1 (Gujarat Anand Yellow Maize Hybrid 1) were procured from the Main Maize Research Station, Godhra, Anand Agricultural University, Anand, Gujarat, India. The average germination rate of the seeds was 85 % as shown by a preliminary study. The seeds selected were of uniform size to minimize errors in seed germination and seedling vigor. The seeds were surface sterilized for 15 min using sodium hypochlorite (10% v/v) and washed three times with sterilized deionized water. Each treatment consisted of 50 randomly selected seeds with three replications. For seed priming, 150 seeds were soaked in various concentrations of zinc nanoparticles (as per treatment concentrations mention in table 1) for 2 hours, then shade-dried for an hour. A set of seeds was used without providing any treatment as a control and soaked in distilled water.

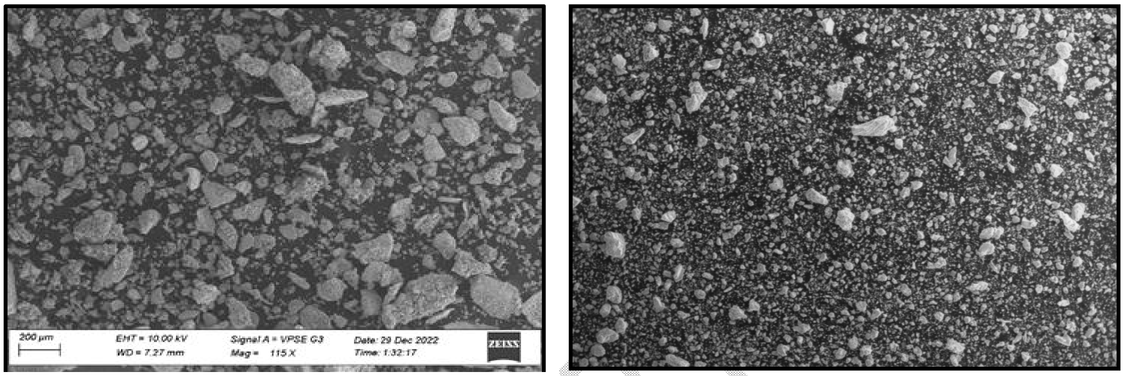

Figure. 1 Scanning electron microscopy (SEM) micrographs of zinc oxide nanoparticles

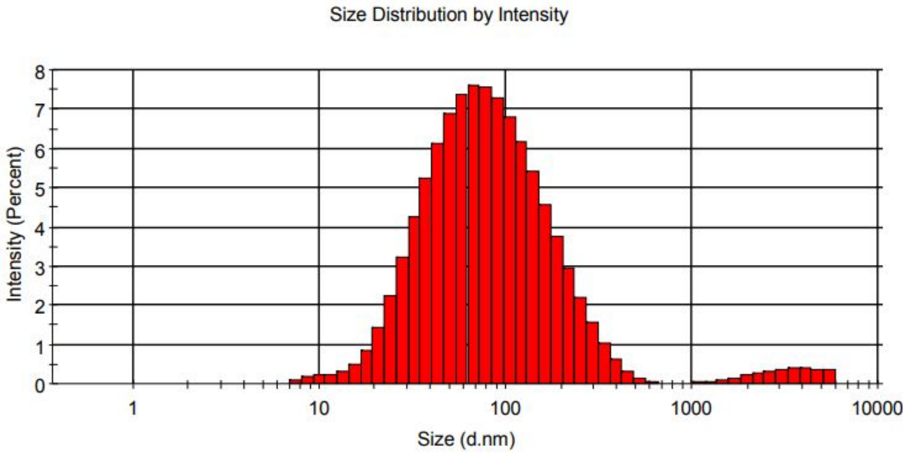

Figure 2. Intensity distribution of ZnO nano-particles through photon collision spectroscopy (dynamic light scattering techniques).

Study area description

A laboratory study conducted at the Department of Seed Science and Technology, BansilalAmrutlal College of Agriculture, Anand Agricultural University, Anand campus (Latitude 22.56°N and Longitude 72.95°E) during the *kharif* season, 2023to assessed the effects of zinc oxide nanoparticles on seed germination and growth of maize seedling. In contrast, zinc oxide nanoparticles were synthesized at the Nanotechnology Laboratory of

Anand Agricultural University, Anand campus, India. The ZnO nanoparticles had an average size of 64.87 ± 0.2386 nm and a zeta potential of -40.9 mV.

Details of Laboratory Experiments

The laboratory experiments involved various concentrations of zinc, specifically nano zinc (ZnO NPs), with thirteen different concentrations (0, 2.5, 5.0, 10, 20, 40, 80, 160, 320, 640, 1280, 2560, and 5120 ppm). The experiment was designed using a completely randomized design (CRD) with three repetitions.

Table 1. Treatment details of the experiment

Treatment Details	
Treatment No.	Concentration of Zn (ppm)
T_1	0.0
T_2	2.5
T_3	5.0
T_4	10
T_5	20
T_6	40
T_7	80
T_8	160
T_9	320
T_{10}	640
T_{11}	1280
T_{12}	2560
T_{13}	5120

Preparation of Seed Priming Solutions

Different concentrations (0, 2.5, 5.0 10, 20, 40, 80, 160, 320, 640, 1280, 2560, and 5120 ppm) of zinc nanoparticles (ZnONPs) prepared in ultrapure water and the NPs dispersed by ultrasonic vibrations for 20 min. A stock solution of nano zinc with a concentration of 6000 ppm, utilize this stock solution to create working solutions as specified in Table 1.All dilutions were freshly prepared before use.

In Vitro Germination of Seeds

Treated maize seeds were shade-dried for 1 hour. Then the 50 seeds were placed in one piece of sterilized germination paper and water was added (as per the recommendations of theInternational Seed Testing Association (1976). Germination paper was covered and placed in an incubator at 26 ± 1^0C for sevendays. Watering was given to all germination papers. After sevendays, maximum seeds were germinated and developed into normal seedlings. Germination was calculated based on the number of seeds germinated in a germination paper having fifty seeds and expressed as germination percentage. The seedling vigorindex (SVI) was calculated bythe formula described by Abdul-Baki and Anderson (1973).

Measurement of Physiological Indexes

Germination percentages, shoot length, root length, seedling length, seedling vigor index-I and II (SVI), were calculated on the sevendays. A seed was considered as germinated after the emergence of radicles or plumules from the seed coat (United States Environmental Protection Agency, 1996 and Ahmed *et al.*, 2019).

a) Shoot and root length: On the sevenday of the experiment, 10 seedlings from the germination paper were randomly selected to measure the shoot and root lengths using a ruler with a centimeter and millimeter scale (Rawat *et al.*, 2018);

b) Seedling length = the sum of shoot length and root length of a seed (Rawat *et al.,* 2018);
c) Germination percentage (%) = (average number of germinated seeds/total number of seeds) × 100 (Lawre, 1985).
d) Seedling vigor index (SVI) = [average root length (cm) + average shoot length (cm)] × average germination percentage (Abdul-Baki and Anderson1973 and Ushahra and Malik, 2013).;
e) Seedling dry weight = Seedlings were dried in an oven at 80^0C for 24h. Samples were cooled in an air-tight closed glass desiccator containing silica gel for 30 min. and dry weight of seedlings was recorded and expressed as g/10 seedlings.

Statistical Analysis

The raw data observed during the laboratory experiment were put for statistical analysis by following the CRD to draw the valid differences among the treatments using SPSS software. Duncan's multiple range test (Duncan, 1955) was performed to test the significance of the difference between the treatments. All the data presented are an average with standard error (S.E.).

Results

Effect of Zinc Oxide Nanoparticle on Seed Germination, Shoot Length, Root Length and Seedling Length

The results showed that the application ZnO NPs had a significant effect on seed germination, shoot, root and seedling length of maize.Table 2 designates the effect of seed priming of ZnO NPs on maize seed germination, shoot, root and seedling length.The data regarding germination showed that maize seed responded variably towards the treatment at various concentrations of nanoscale ZnO particles.The seed priming with 80 ppm nanoscale ZnO recorded significant germination (91.33%), the maximum shoot length (15.72 cm) was recorded at 160 ppm Zn concentration and also root length (15.40 cm) and seedling length (31.12 cm)was significantly higher at 160 ppm Znthrough nanoscale ZnOfollowed by T_6, T_5, $T_4$and T_3 for germination; T_7 and T_9 for root length and also for seedling length.However, with the addition and increase in ZnO NPs concentration, germination, shoot and root length as well as seedling length decreased which ultimately adversely affect the seedling growth. Seed germination, shoot, root and seedling length varied noticeably among different ZnO NPs concentration (Figure 3 and 4). However, the lower seed germination (63.33 %), root length (9.47 cm) and seedling length (10.88 cm) was observed at higher concentration of Zn through zinc oxide nanoparticles (5120 ppm Zn)and the lowest shoot length (9.41 cm) was also observed at higher concentration of Zn (5120 ppm) followed by T_{12} for germination, root length and seedling length. which may indicate that higher concentration of zinc oxide nanoparticles may cause adversely affected on seedling growth. It might be due to nanoparticle toxicity.

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Table 2: Effect of different concentration of seed priming of ZnO NPs on germination, shoot, root and seedling length of maize

Sr. No.	Concentration(ppm)	Germination (%)	Shoot length(cm)	Root length(cm)	Seedling length(cm)
1	0	84.67^{cd} ± 0.66	10.79^{f} ± 0.47	11.36^{g} ± 0.30	22.15^{def} ± 1.97
2	2.5	85.33^{bcd} ± 1.76	11.17^{f} ± 0.27	11.50^{fg} ± 0.32	22.67^{de} ± 0.51
3	5.0	87.33^{abc} ± 0.66	11.83^{e} ± 0.27	12.16^{efg} ± 0.18	23.99^{cd} ± 0.84
4	10	90.00^{ab} ± 1.15	12.46^{d} ± 0.23	12.66^{def} ± 0.12	25.12^{cd} ± 1.49
5	20	90.00^{ab} ± 1.15	13.09^{cd} ± 0.02	13.76^{bcd} ± 0.68	26.85^{bc} ± 1.23
6	40	90.67^{a} ± 1.76	13.10^{cd} ± 0.06	14.22^{bc} ± 0.99	27.32^{bc} ± 0.66
7	80	91.33^{a} ± 0.66	14.56^{b} ± 0.09	14.60^{ab} ± 0.07	29.16^{ab} ± 2.28
8	160	84.00^{cd} ± 2.30	15.72^{a} ± 0.05	15.40^{a} ± 0.03	31.12^{a} ± 1.09
9	320	80.67^{de} ± 0.66	14.96^{b} ± 0.14	14.54^{ab} ± 0.10	29.50^{ab} ± 0.23
10	640	78.67^{e} ± 2.40	13.60^{c} ± 0.12	13.31^{cde} ± 0.07	26.91^{bc} ± 0.18
11	1280	68.67^{f} ± 1.33	13.20^{c} ± 0.06	12.35^{efg} ± 0.10	25.55^{cd} ± 0.16
12	2560	67.33^{fg} ± 1.76	10.13^{g} ± 0.05	9.95^{h} ± 0.01	20.08^{ef} ± 0.07
13	5120	63.33^{g} ± 1.76	9.41^{h} ± 0.28	9.47^{h} ± 0.22	18.88^{f} ± 0.20

Note: Treatment means in a column with the letters in common are not significant by Duncan’s New Multiple Range Test at 5% level of significance.

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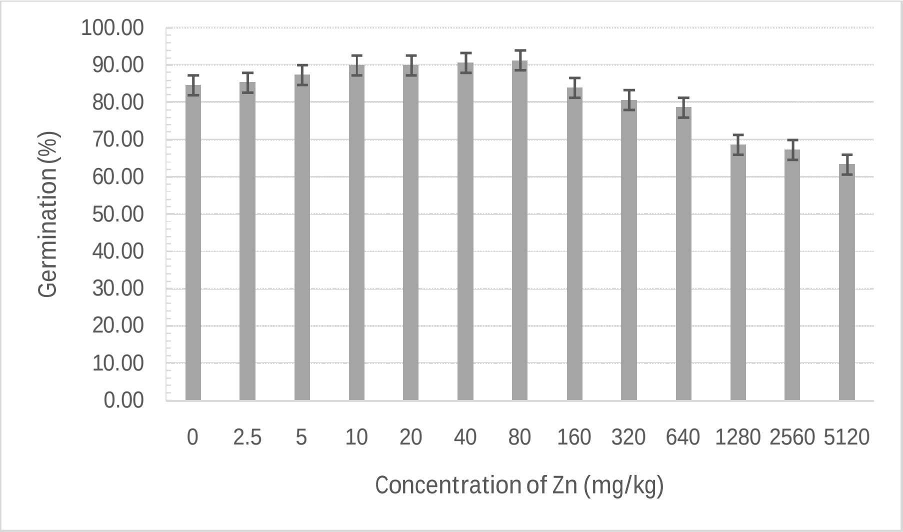

Figure 3: Effect of different concentration of ZnO NPs on germination maize seeds after priming

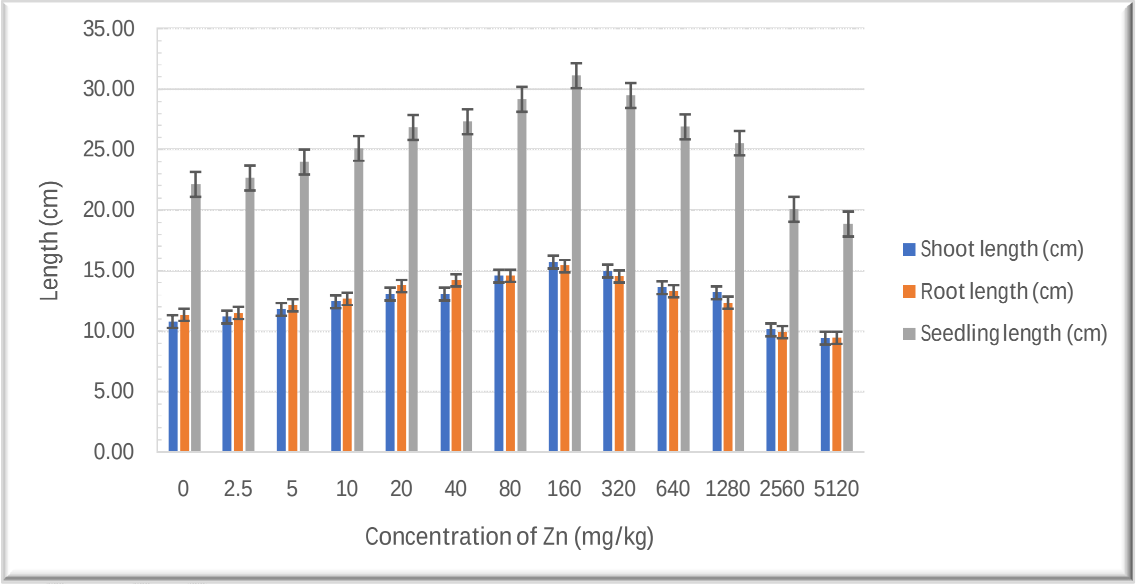

Figure 4: Effect of different concentration of ZnO NPs on Shoot, root and seedling length of maize after priming

Table 3: Effect of different concentration of ZnO NPs on freshweight, dry weight and seed vigour of maize seedlings

Sr. No.	Concentration(ppm)	Fresh weight (g)	Dry weight (g)	Seed vigour index-I	Seed vigour index-II
1	0	$7.92^{de} \pm 0.45$	$1.257^{d} \pm 0.0033$	$1897^{e} \pm 66$	$106.39^{de} \pm 3.57$
2	2.5	$8.77^{bcde} \pm 0.22$	$1.293^{cd} \pm 0.006$	$1967^{de} \pm 63$	$110.35^{cde} \pm 1.88$
3	5.0	$8.95^{bcd} \pm 0.16$	$1.297^{cd} \pm 0.018$	$2130^{c} \pm 47$	$113.23^{bcde} \pm 1.55$
4	10	$9.15^{abc} \pm 0.07$	$1.347^{cd} \pm 0.056$	$2275^{b} \pm 31$	$121.31^{abcd} \pm 11.70$
5	20	$9.25^{abc} \pm 0.07$	$1.373^{c} \pm 0.038$	$2334^{b} \pm 22$	$123.54^{abc} \pm 3.58$
6	40	$9.42^{abc} \pm 0.10$	$1.393^{bc} \pm 0.034$	$2398^{b} \pm 42$	$126.38^{ab} \pm 4.68$
7	80	$9.65^{ab} \pm 0.03$	$1.487^{ab} \pm 0.018$	$2648^{a} \pm 8$	$135.77^{a} \pm 2.77$
8	160	$9.73^{ab} \pm 0.16$	$1.527^{a} \pm 0.017$	$2540^{a} \pm 63$	$128.21^{ab} \pm 3.31$
9	320	$10.20^{a} \pm 0.50$	$1.570^{a} \pm 0.043$	$2292^{b} \pm 16$	$126.70^{ab} \pm 7.36$
10	640	$9.15^{abc} \pm 0.77$	$1.260^{d} \pm 0.03$	$2050^{cd} \pm 50$	$99.03^{e} \pm 2.57$
11	1280	$8.46^{cde} \pm 0.34$	$1.053^{e} \pm 0.049$	$1622^{f} \pm 36$	$72.41^{f} \pm 4.40$
12	2560	$7.75^{e} \pm 0.11$	$0.980^{e} \pm 0.04$	$1312^{g} \pm 38$	$66.12^{fg} \pm 4.30$
13	5120	$6.02^{f} \pm 0.36$	$0.880^{f} \pm 0.02$	$1187^{g} \pm 24$	$55.68^{g} \pm 1.04$

Note: Treatment means in a column with the letters in common are not significant by Duncan's New Multiple Range Test at 5% level of significance.

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Fresh and dry weight

The results revealed that seed priming through ZnO NPs significantly influenced on fresh and dry weight. Figure 5 designates the effect of different concentration of ZnO NPs on fresh and dry weight of maize seedling after priming. Results showed that at 320 ppm ZnO NPsrecorded the maximum fresh weight (10.20 g) and dry weight (1.570 g) as compared to the control (0 ppm) followed by T_8, T_7, T_6, T_5, T_4 and $T_{10}$for fresh weight and $T_8$and $T_7$for dry weight (Fig. 5). However, increasing the concentration of ZnO NPs decreases the fresh and dry weight. The lowest fresh weight (6.02 g) and dry weight (0.880 g) irrespective of ZnO NPswere noticeable at higher concentrations of 5120 ppm.

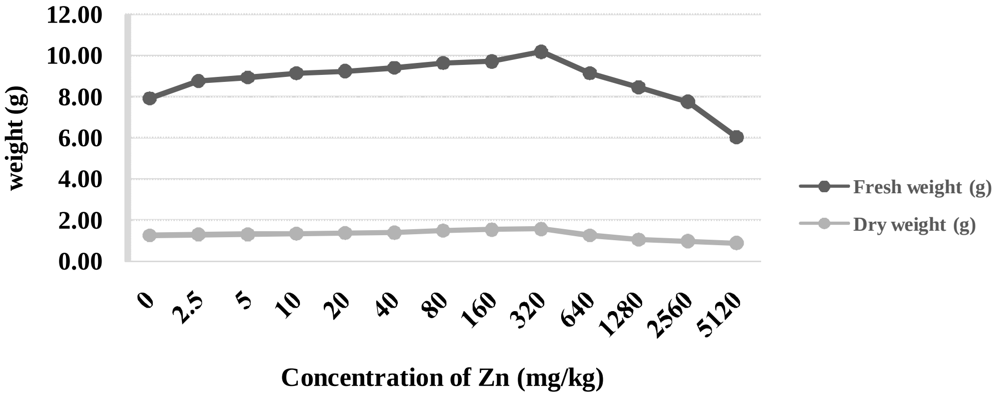

Figure 5: Effect of different concentration of ZnO NPs on fresh and dry weight of maize seedlings after priming

Seed vigour

The seed priming with different concentration ofZnO NPswas significantly influenced on seed vigour index. Figure 6 designates the effect of different concentration of seed priming of ZnO NPs on seed vigour index. Figure 6 shows that seed vigour was highly significantly influenced by sources of zinc and its concentrations. The results showed that the significantly higher seed vigour index – I and II was noticed at seed priming with 80 ppm irrespective of nano Zn(2648 and 135.77, respectively) followed by T_8 for vigour index-I and T_8, T_9, T_6, T_5 and T_4 for vigour index-II. The lower seed vigour index-I (1187) and II (55.68) was recorded at higher concentrations of Zn irrespective of nano Zn (5120 ppm) followed by T_{12} for seed vigour index-I and II.

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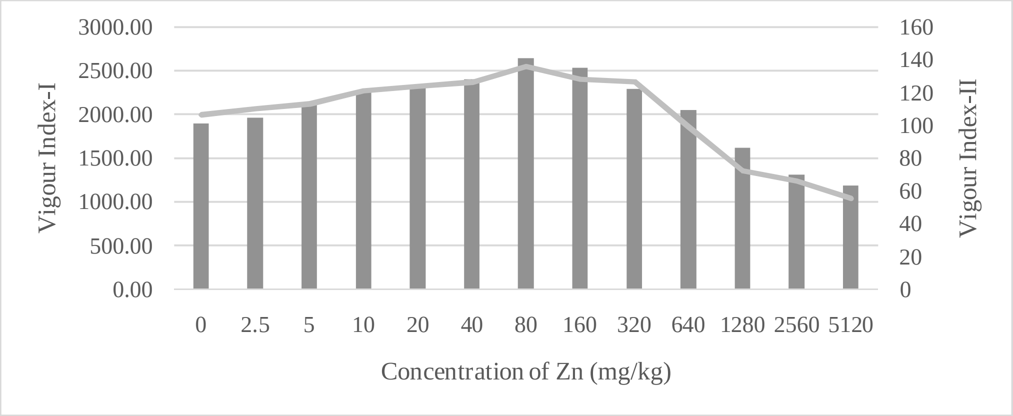

Figure6: Effect of different concentration of ZnO NPs on seed vigour after priming

Discussion

“Zinc is essential for maintaining cell membrane integrity” (Welch *et al*., 1991; Cakmak, 2000). “As a versatile micronutrient, zinc plays critical roles in various biological processes, including protein synthesis, membrane function, cell elongation, and tolerance to environmental stresses” (Cakmak, 2000). Nanoparticles can penetrate seed pores more easily compared to bulk zinc.Nanoparticles (NPs) affect the germination and vigor of plants by the stimulation and improvement of seed metabolic rate, vigor index and seedling characteristics as reported by Abbasi *et al.,* (2021), especially in the case of ZnO NPs priming.“The reason behind improvement on germination was nano-priming at a suitable concentration can stimulate seed germination of seeds by increasing α-amylase activity and starch metabolism” (El-Badri*et al.,* 2021). “The increased in germination percentage in nanoparticles treated seeds can be attributed to the role of Zn in inducing a range of biochemical changes in seed, required to start the germination process, such as breaking of dormancy, hydrolysis or metabolization of inhibitors, imbibition and enzyme activation” (Harris *et al*., 2007; Samad *et al*., 2014).The probable reason for decreased in germination at higher Zn concentration could be the increased absorption and accumulation of these nano Zn both in extracellular space and within the cells resulted in reduction in cell division, cell elongation and inhibition of the hydrolytic enzymes involved in food mobilization during the process of seed germination. Similar results were noticed by Raskar and laware (2014), they observed that seed germination increased in lower concentration, however showed decreased in values at higher concentration. Germination indices showed increased values in lower concentration; however, these decreased significantly at higher concentrations.

“Results from the study suggested that seed priming with ZnO NPs at optimum concentration had a greater impact on seedling growth and development, probably due to greater capacity of nanoparticles to be absorbed and assimilated by the seeds more efficiently owing to their nanometric size and lower solubility. Similar findings were” also found by Pavani*et al.* (2014).“They reported an increased growth in seedlings treated with ZnO NPs, while retardation in the growth of seedlings treated with $ZnSO_4$.However, the enhancement by nanoscale ZnO at cellular level has to be understood by further in depth investigations” (Prasad *et al*., 2012).“It might be due to increased cell division and cell elongation under the impact of increased IAA activity may account for the notable increases in plant length as seen at varying nanoparticle concentration. higher quantities of Zn nanoparticles were associated with a decrease in groundnut plant height, as reported” by Prasad *et al*. (2012). Similar results were recorded by Mahajan *et al*. (2011) and Burman *et al*. (2013) “and they reported that increasing the concentration of Zn nanoparticles had a negative effect on plant height, possibly due to nanoparticle toxicity. Low dosages of Zn nanoparticles were sufficient to elicit a good response in a separate investigation on chickpea seedlings, while higher doses were associated with a delay in early development metrics”.

The mechanism of improvement of seed germination and growth under these conditions could be attributed to the fact that zinc nanoparticles enhance water uptake by seeds which trigger enzymatic reactions to stimulate the germination process and improved the fresh weight and seed vigour index.This might be due to increase in germination percentage, germination rate and increase in seedling shoot and root lengths in ZnNP seeds which ultimately results in increase in seedling dry weight. The data was also in accordance with previous research reported by Imtiaz *et al.* (2003) they observed that Zn plays an effective role in increasing dry weight in wheat seedlings.

CONCLUSION

In order to understand the possible benefits of applying nanomaterials in agriculture, it is important to analyzethe penetration and transport of nanoparticles in the plants. Size plays an

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important role in behavior, in reactivity, and in toxicity. Considering these aspects, both positive and negative effects of nanoparticles are observed in maize seedling.The results suggest that the positive effects of lower concentrations (80 ppm) of zinc oxide nanoparticles (ZnO NPs) on seed germination and seed vigor. An inhibitory effect of ZnO NPs on seed germination, seedling length, and seed vigor of maize appeared at the highest concentration (5120 ppm Zn) and became more severe with increased concentration increments.

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