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IMPACT OF ZINC OXIDE NANOPRIMING ON GERMINATION AND GROWTH OF SEEDLINGS IN CHICKPEA (*CICER ARIETINUM* L.)

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ABSTRACT:
Zn is a crucial micronutrient involved in various metabolic processes, enzyme activities and enhancing nodulation in leguminous plants. Its deficiency leads to various yield losses. Hence, there is need to use zinc fertilizers to improve growth and productivity. Recently, nanofertilizers are gaining importance due to controlled release of nutrients on demand at a targeted site. Even though lot of work has been undertaken in this area, still there is uncertainty on inhibitory or stimulatory effect of NPs. Therefore, this study was carried out to investigate the influence of zinc oxide nanoparticles (ZnO NPs) on Chickpea germination and seedling growth. FESEM was used to define the shape and size of ZnO NPs produced by hydrothermal method. Seeds were primed in varied concentrations (4-16 µg ml^{-1} of ZnO NPs prepared in 10% starch) and kept for germination in laboratory conditions and sown in soil. Seeds primed with lower concentrations of ZnO NPs (8 µg ml^{-1}) in laboratory conditions and (12 µg ml^{-1}) in pot experiments accelerated growth parameters (shoot length, root length, root to shoot ratio, fresh and dry weight) whereas higher concentration (16 µg ml^{-1}) negatively affected growth compared with control. Enhanced growth at lower concentration enables use of NPs as a potent nanofertilizer in future; also evidence of deleterious effect at higher concentration is observed, indicating concentration dependent response of NPs to plant growth.

Keywords: -*Chickpea, seedling parameters, priming, zinc oxide nanoparticles.*

INTRODUCTION:

Zinc (Zn) is a crucial micronutrient involved in various metabolic processes, enzyme activities and overall growth in plants (Narendhran *et al.* 2016). It is the second most abundant transition metal and twenty third most abundant elements on earth (Laware and Raskar, 2014). Amongst different metals available, Zn is known to play an important role in biochemical, anatomical and physiological responses although not exceeding the threshold level (Zafar *et al.* 2016). It is an important component of various classes of enzymes such as oxidoreductases, transferases, hydrolases, lyases, isomerases and ligases, which are responsible for driving many metabolic reactions in plants (Prasad *et al.* 2012; Korishettar *et al.* 2016). These metabolic functions are based on the ability of Zn to form tetrahedral complexes with N, O and predominantly S-ligands, thus confirming its catalytic and structural role in enzymatic reactions (Panwar *et al.* 2012). Zn is also necessary for the synthesis of PGRs (plant growth regulators) such as auxin mainly IAA (indole acetic acid) from tryptophan and various biochemical reactions involving chlorophyll, carbohydrate, protein and nucleic acids synthesis as well as its degradation (Arif *et al.* 2007). It is involved in photosynthesis, regulating the functions of stomata, cell division, sexual fertilization and maintaining cell membrane integrity and its functions (Chamani *et al.* 2016). Ozturk *et al.* (2006) has also highlighted the involvement of Zn in physiological processes mainly protein synthesis, cell elongation and resistance to abiotic stresses during early seedling development. Even though this micronutrient is

required in minute quantity but its unavailability can affect enzyme activities, metabolic processes and cause physiological imbalances (Korishettar *et al.* 2016).

Zn is considered the fourth most important yield limiting nutrient after N, P and K and its deficiency can hamper plant growth and cause considerable yield loss (Prasad *et al.* 2012). Widespread Zn deficiency which is common throughout developed and developing countries, not only reduces the grain nutritional quality but also causes huge financial losses to the farmers. Zn deficiency is particularly seen in alkaline (high pH) soils as it forms zinc hydroxide and oxides becoming unavailable to the plants (Arif *et al.* 2007). Zn deficiency leads to interveinal chlorosis, development of white necrotic spots and leaf mottling in plants (Valenciano *et al.* 2010). It has been well established that Zn deficiency in soils is positively correlated to Zn deficiency in human beings (Panwar *et al.* 2012).Thus, there is need of appropriate technology to enrich crops with Zn by making it available for uptake by plants and solving the problems associated with oxide formation and run off with water.

Recently, nanoparticles (NPs) are gaining importance in all fields of science including agricultural divisions. These particles show different effects, based on its size, shape, surface area, composition, nature, reactivity etc. (Zafar *et al.* 2016). NPs increase the catalytic activity, rate of penetration, uptake by plants and are highly reactive due to its large surface area and nano size (Korishettar *et al.* 2016). Hence, application of Zn micronutrients in the form of NPs can prove to be beneficial for gradual and controlled release of micronutrient at a specific site compared to the fertilizers. Diverse studies on ZnO NPs were undertaken by different researchers (Shaymurat *et al.*, 2011; Prasad *et al.*, 2012; Panwar *et al.*, 2012; Wang *et al.*, 2013; Laware and Raskar, 2014; Siddiqi and Husen, 2017 and Taheri *et al.*, 2017) on growth of crops and found both stimulatory as well inhibitory results. Also antibacterial (Narendhran *et al.* 2016) and antifungal (Wani and Shah, 2012) effect has been reported of ZnO NPs, proving its inhibitory action against pathogens and beneficial role as a nanofertilizer. Chamani *et al.* (2015) have found that ZnO NPs at 50 mg L^{-1} and 75 mg L^{-1} improved root and leaf length, leaf number, secondary metabolites and concentration and showed symptoms of leaf toxicity. Nano-ZnO NPs enhanced nitrogenase activity in cluster bean, green gram and cowpea roots when dipped in 1.5 µg ml^{-1} solution and negative effect when dipped in 10 µg ml^{-1} NP solutions whereas the nitrogenase activity decreased in moth bean when dipped in either of the solutions (Kumar *et al.* 2015).

Hence, this study was carried out to investigate the influence of zinc oxide nanoparticles (ZnO NPs) on Chickpea var. *Digvijay* germination and seedling growth. Chickpea (*Cicer arietinum* L.) is the major leguminous crop and highly sensitive to Zn deficiency leading towards yield losses (Valenciano *et al.* 2010). Therefore, three aspects were considered for investigation: 1) Synthesis of ZnO NPs 2) Seed priming with different concentrations of ZnO NPs 3) Chickpea germination and growth studies by laboratory and pot experiments. Many trials were undertaken to optimize the nanoparticle concentration and the range between 4 -16 µg ml^{-1} were considered suitable for experiments.

MATERIALS AND METHODS

1. Seeds

Chickpea (*Cicer arietinum* L.) seeds of variety 'Digvijay' were obtained from Mahatma Phule

Krishi Vidyapeeth, Rahuri, Maharashtra, India. This variety is commonly cultivated in Maharashtra. Seeds for the experiments were selected uniformly to reduce the errors during seed germination and growth.

2. Synthesis of ZnO NPs

ZnO NPs were produced using Zinc nitrate hexahydrate [Zn (NO3)2·6H2O] and potassium hydroxide (KOH) by hydrothermal method. Appropriate concentrations of these were dissolved in 100 ml aqueous solution followed by transferring mixture to round bottom flask. Hydrothermal synthesis was carried at different reaction temperatures i.e. 120°C for 2hrs etc. After complete synthesis, the product was washed with sterile distilled water and dried in oven at 70°C. Using field emission scanning electron microscopy (FESEM), shape and size of NPs were determined.

3. Preparation of ZnO NP suspension and Seed priming

Stock solution of ZnO NPs was prepared at concentration of 10 mg ml^{-1} by adding ZnO NPs in sterile distilled water, which was further dispersed by ultrasonic vibration for half an hour to avoid aggregation (Alharby *et al.*, 2016). Different concentrations of ZnO NPs (0, 4, 8, 12 and 16 µg ml^{-1}) were prepared by diluting the stock solution (stirred on magnetic stirrer before use) in appropriate volume of 10% starch solution (solubilised by boiling) to get the desired concentration of solutions. Starch was used as a coating agent which improves seed ability by increasing seed weight and size, and also adds beneficial elements to the seeds (here, ZnO NPs) to improve water imbibition and germination (Felker*et al.*, 1999).

For seed priming, seeds were surface sterilized with 0.1% (w/v) mercuric chloride for 2-3 min and then washed thrice with distilled water thoroughly (Zafar *et al.*, 2016). Seeds were primed (soaked) in different concentrations of ZnO NPs separately for 4-5 mins, then shade dried overnight and used for experiments the next day.

4. Experiments

Seed priming experiments were carried out in two sets in the laboratory. One set (three replicates per treatment) was used for studying the NP effect on seed germination and seedling growth in lab conditions, whereas another set was used for pot experiments.

4.1. Seed Germination experiment

Seeds were primed with ZnO NP concentrations viz. 4-16 µg ml^{-1} as mentioned above and used in the experiment. The primed seeds were placed in petri plates containing sterilized germination paper and 5 ml of sterile distilled water was added to the plate as per the International Seed Testing Association's (1976) recommendations. Ten seeds per treatment were placed equidistantly in the petri plates and kept for germination. Similar experiments were conducted for control without using NPs. The petri plates were sealed using parafilm and placed in an incubator at 26 ± 2°C and observed after every 2 days and watered regularly. The experiment was halted after 10 days and seedlings were harvested for analysing germination and growth parameters (Narendhran *et al.*, 2016).Three replicates per treatment were maintained.

4.2. Pot experiment

Second set of seeds were sown in pots containing soil and cocoa pit in appropriate concentration. Care was taken to add soil and water uniformly in the pots to maintain homogeneity.

5. Data analysis

5.1. Germination and seedling growth parameters

Percent seed germination was determined based on number of seeds germinated from the total number of seeds inoculated or sown that were recorded after 10 days (Thunugunta *et al.*, 2018)

$$Seed\ germination\ (\%) = \frac{No.\ of\ seeds germinated}{Total no.\ of seeds} \times 100$$

Following 10th day, seedlings were harvested and used for studying different parameters viz. shoot length, root length and seedling length expressed in centimetre (cm) whereas seedling fresh and dry weight expressed in grams (gms). To determine the dry weight, seedlings were dried in oven at 60°C for 48hr and measured (Panwar *et al.*, 2012). For seedlings harvested from pots, they were initially washed with running tap water to drain away the soil and then dried. From the above recorded data, germination indices and percent increase or decrease over control was calculated.

5.2. Germination Indices

Following formulae were used for calculating Promptness index (PI), Germination stress tolerance index (GSI), Plant height stress tolerance index (PHSI), Root length stress tolerance index (RLSI) and Dry matter stress tolerance index (DMSI) (Prasad *et al.*, 2012)

$$PI = 'n'\ d2\ (1.0) + 'n'\ d4\ (0.75) + 'n'\ d6\ (0.5) + 'n'\ d8\ (0.25)$$

[Where 'n' is no. of seeds germinated at day'd']

$$GSI = \frac{PI\ of\ stressed\ seeds}{PI\ of\ control\ seeds} \times 100$$

$$PHSI = \frac{Plant\ height\ of\ stressed\ plant}{Plant\ height\ of\ control\ plant} \times 100$$

$$RLSI = \frac{Root\ length\ of\ stressed\ plant}{Root\ length\ of\ control\ plant} \times 100$$

$$DMSI = \frac{Dry\ matter\ of\ stressed\ plant}{Dry\ matter\ of\ control\ plant} \times 100$$

5.3. Statistical Analysis

To investigate the ZnO NPs' effect on *Cicer arietinum* seed germination and seedling growth characters; each treatment was conducted in triplicates and mean values were recorded. The results are shown as mean ± standard deviation (SD). The experimental data was compared to its corresponding control and percent increase or decrease over control was determined. Statistical significance of difference among treatments was measured using one way analysis of variance (ANOVA) and critical differences were calculated at significance level of 0.05 ($p < 0.05$).

RESULT AND DISCUSSION:

1. Morphology and size of ZnO nanoparticles

Scanning electron microscopy (SEM) Quanta FEG 450 was used to determine the morphology and size of ZnO NPs. Magnified FESEM images (see Fig. 2), indicate elongated rod shaped structures of ZnO nanoparticles having length of 0.3 – 0.48 µm and width of 40- 65 nm.

2. Seed germination

Seeds were germinated in all the treatments showing that there was no any adverse effect on chickpea seed germination. Seeds germinated on germination paper by paper towel method proved to be better compared to whatmann no. 1 filter paper and gave 100 % seed germination in all concentrations of ZnO NPs. In case of potexperiments, where seeds were sown in soil for germination, the percent germination in higher concentration of ZnO NPs i.e. 12 and 16 µg ml^{-1} decreased to 90 % compared with lower concentration (4 and 8 µg ml^{-1}) and control. Similar results of seed germination were seen by Narendhran *et al.* (2016) in sesamum seeds and Boonyanitipong *et al.* (2011) in rice seeds indicating no adverse effect of ZnO NPs on seed germination. It has been known that seed priming improves germination rate uniformly and synchronously in all the seeds seeds (Singh *et al.* 2015), increases fresh weight, dry weight, leaf no., grain yield etc. in many crops (Hussain

et al. 2014). Probably, enhanced and synchronised germination in chickpea seeds might be due to the small size of ZnO NPs having ability to penetrate through the seed coat, thereby getting absorbed and utilised by the seeds. Zn plays an important role as a precursor in various enzymes carrying out different metabolic reactions in many crops. So, ZnO NPs might have enhanced production of antioxidant enzymes and reduced oxidative stress by reducing reactive oxygen species (ROS) and malonyldialdehyde content, promoting seed germination and seedling growth. Korishettar *et al.* (2016) have also recorded highest seed germination i.e. 96% at 750 ppm Zn NP concentration compared to 500 ppm showing 95.30% germination and lowest abnormal seedlings.

3. Seedling growth

Seeds treated with different concentrations of ZnO NPs responded variably in terms of root length, shoot length, seedling length, fresh weight (FW) and dry weight (DW). Root length was significantly increased in lower concentration of ZnO NPs as compared to higher concentrations and control in case of laboratory experiments (see Table 1). There was 15% increase in root length at 4 µg ml^{-1} ZnO NP concentration (21.4 ± 3.13 cm) and 18 % increase at 8 µg ml^{-1} (22 ± 1.87 cm) compared to control (18.6 ± 2.19 cm) whereas root length was decreased at higher concentrations i.e. by 2% (18.2 ± 1.64 cm) and 3% (18 ± 2.74 cm) at 12 µg ml^{-1} and 16 µg ml^{-1} respectively. Along with increased root length, there were prominent root hairs grown. Shoot length was also increased significantly at lower concentrations by 20% (19 ± 2.45 cm), 54% (24.4 ± 1.82 cm) and 35 % (21.4 ± 4.20) at 4µg/ml, 8 µg ml^{-1} and 12 µg ml^{-1} ZnO NP concentration respectively compared to control (15.8 ± 3.83 cm) and decreased by 13 % (13.6 ± 2.07cm) at 16 µg ml^{-1}. Similar trend was observed in seedling length increasing at lower concentrations by 17%, 34% and 15% in seeds treated with 4 µg ml^{-1}, 8 µg ml^{-1} and 12 µg ml^{-1} ZnO NP concentration and decreasing by 8% at 16µg/ml compared with the control. These results were in accordance with Prasad *et al.* (2012) and Raskar and Laware (2014) where ZnO NPs at lower and higher concentrations showed positive and negative effects respectively in peanut seeds and onion seed germination and seedling growth. Kisan *et al.* (2015) have studied the effect of ZnO NPs on the physical properties of Spinach leaves. They observed maximum increase in leaf length and leaf surface area along with slight improvement in leaf width at 1000 ppm concentration followed by 500 ppm and control. There was also significant increase in the nutritional quality of leaves in terms of protein, fat, ash, fiber and carbohydrate content in the above mentioned concentration of nanoparticles. This improvement in physical and nutritional quality may be due to the enhanced root capacity of cation exchange leading to absorption of essential nutrients thus, increasing the protein content, regulating carbohydrate and protein metabolism, boosting processes driven by enzymes such as starch and plant growth hormone synthesis. The probable reason for increase in shoot and root length may be due to mechanism related to zinc regulation. Zinc enhances synthesis of auxin which in turn regulates synthesis of cytokinin. The main gene responsible for cytokinin synthesis i.e. *adenosine phosphate – isopentenyltransferase (PsIPT)* is governed by presence of auxin, thus enhancing shoot and root production (Zafar *et al.* 2016).

In case of pot experiments, same parameters as that in laboratory experiments were studied (see Table. 2). Root length was significantly increased

in seeds primed in ZnO NPs at 12 µg ml^{-1} by 19.8% (27.2 ± 3.63 cm) compared to the control unprimed seeds (22.7 ± 2.56 cm) and decreased by 30% in seeds primed with higher concentration of ZnO NPs i.e. 16 µg ml^{-1} (15.88 ± 4.65 cm). Shoot length was increased in all seeds primed with different concentrations of ZnO NPs. The trend was seen to increase from lowest concentration till 8µg/ml and decreased there after compared with lower concentrations but being more than the control. The maximum shoot length was 18.8 ± 2.93 cm at 8 µg ml^{-1} followed by 18.5 ± 2.45cm at 12 µg ml^{-1}, then 18.38 ± 2.91cm at 4 µg ml^{-1} and 16.4 ± 1.67 cm at 16 µg ml^{-1} compared to the control 15.3 ± 1.20cm. The shoot length was increased by 22 % over control. The seedling length also increased from 4 to 12 µg ml^{-1} by 9%, 8% and 20% respectively and declined at 16 µg ml^{-1} by 15%. Similar results were reported by Arif *et al.* (2007) and Valenciano *et al.* (2010) in yield of chickpea and by Panwar *et al*, (2012) in tomato plants. Also nitrogenase activity in leguminous plants was foundto increase when exposed to nano ZnO, whereas decreased when exposed to bulk ZnO. It was observed that nitrogenase activity is dependent on exposure time and concentration of NPs, where long time exposure may allow nanoparticles to get attached to cell membranes, distracting its intact membrane and ultimately producing ROS (reactive oxygen species), damaging DNA and cell division (Kumar *et al.*, 2015). In case of seedling length, maximum seedling length was observed at 12 µg ml^{-1} (20.26%) followed by 4 µg ml^{-1} (9.42%), then 8 µg ml^{-1} (8.95%) and decreased by 15.05% at 16 µg ml^{-1} compared to the control. This result goes in accordance with many results seen above. Increased growth in terms of root, shoot and seedling length at lower concentration may be the result of ZnO NPs entering the seeds, regulating enzymatic and free radical scavenging activities thereby reducing oxidative damage and encouraging growth.

Root to shoot ratio is used to indicate healthier plants, which was slightly decreased in all the treatments compared with control. The decrease was not due to decrease in root size but due to increase in shoot size decreasing the ratio, still indicating plants to be healthier. There were no significant results in case of fresh weight and dry weight in seeds grown in laboratory and pot conditions, but compared with control FW increased in all the treatments in both the conditions. In pot conditions, maximum FW was seen at 4 µg ml^{-1} to be increased by 46.28% with slight decreased followed by 8 µg ml^{-1} (36.57 %), then 12 µg ml^{-1} (35.21%), 16 µg ml^{-1} (18.51%) compared with the control whereas in lab conditions grown on germination paper, maximum FW was seen at 8, 12, 16 and 4 µg ml^{-1} than control. DW was increased of plants grown in pot conditions whereas decreased in those grown in laboratory conditions as shown in table 3 & 4. Similarly, both positive as well as negative effects have been shown by ZnO NPs, which varies based on concentration of NPs and exposure time (Shaymurat *et al.*, 2011; Taheri *et al.*, 2015). Overall, giving an insight that ZnO NPs help in growth and development at lower concentrations whereas becomes toxic to cells at higher concentrations (Siddiqi and Husen, 2017).

4. Germination indices

Data from the results of pot conditions indicated maximum PI and GSI at 8 µg ml^{-1} followed by 12 µg ml^{-1}, 4 µg ml^{-1} and decreased at higher concentration 16 µg ml^{-1} (see Table. 4) whereas PHSI, RLSI and DMSI was maximum at 12 µg ml^{-1} and minimum at 16 µg ml^{-1}. Increasing trend was seen in all the three lower concentration of ZnO NPs. In case of seeds

germinated on germination paper and grown in laboratory conditions, Promptness index (PI) and Germination stress tolerance index (GSI) was higher at 12 µg ml^{-1} followed by 4 µg ml^{-1}, 8 µg ml^{-1} and decreased at higher concentration 16 µg ml^{-1} (see Table. 3). PHSI, RLSI and DMSI increased in lower concentration viz. 4 µg ml^{-1} and 8 µg ml^{-1} and decreased at higher concentrations namely 12 and 16 µg ml^{-1}, indicating toxicity at higher concentration. Likewise, Tang *et al.* (2012) studied effect of five different NPs' on germination and root elongation in 3 different seeds viz. cucumber, radish and lettuce seeds. They have explained that positive or negative effect of NPs is not only dependent on size, shape, structure, reactivity and composition of NPs but also on the size of seeds (surface area to volume ratio). Copper oxide (CuO) NPs had detrimental effect on lettuce seeds being smaller in size compared to cucumber and radish seeds.

CONCLUSION:

The effect of ZnO nanoparticles on Chickpea germination and seedling growth under both laboratory and pot conditions was studied. ZnO NPs enhanced seed germination, SL, RL, seedling length, FW and DW at (8 µg ml^{-1}) in laboratory conditions and (12 µg ml^{-1}) in pot conditions. It was found that these nanoparticles showed positive effect at lower concentrations and negative effect at higher concentrations on plants, indicating ZnO NPs to be toxic at higher concentration. Thus, concluding ZnO NPs to be detrimental on growth due to accumulation, whereas stimulatory at lower concentration. This gives an insight that size and concentration of NPs need to be optimised to reduce its detrimental effect on plants. Size of NPs and size of seeds play an important role in action, reactivity and showing toxic nature. Chickpea being the major leguminous crop, its yield is highly hampered due to Zn deficiency. So, above priming method can be considered useful in improving yield and productivity in Chickpea. Hence, the result proves beneficial role of ZnO NPs to be used as a nanofertilizer in future for enhancing growth and productivity in plants.

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Table. 1: Effect of ZnO NPs on Root length (RL), Shoot length (SL), Fresh weight (FW) & Dry weight (DW) of Chickpea (Laboratory conditions)

ZnO NPs (µg ml^{-1})	RL (cm)	PI/PDOC in RL	SL (cm)	PI/PDOC in SL	Seedling length (cm)	PI/PDOC in Seedling length	Root to Shoot ratio	FW (g)	PI/PDOC FW	DW (g)	PI/PDOC DW
0	18.6 ± 2.19	0.00	15.8 ± 3.83	0.00	34 ± 4.58	0.00	1.17 ± 0.14	4.13 ± 0.74	0.00	1.61 ± 0.55	0.00
4	21.4 ± 3.13	15.05	19 ± 2.45	20.25	41.34 ± 1.52	17.44	1.13 ± 0.29	4.49 ± 0.95	8.72	1.47 ± 0.76	-8.70
8	22 ± 1.87	18.28	24.4 ± 1.82	54.43	46.66 ± 1.15	34.88	0.91 ± 0.02	5.61 ± 1.45	37.31	1.66 ± 0.80	3.13
12	18.2 ± 1.64	-2.15	21.4 ± 4.20	35.44	39 ± 2.29	15.12	0.85 ± 0.23	5.51 ± 1.54	33.41	1.50 ± 0.76	-6.83
16	18 ± 2.74	-3.23	13.6 ± 2.07	-13.92	32.66 ± 2.30	-8.14	1.39 ± 0.05	5.04 ± 0.89	22.03	1.51 ± 0.77	-6.21
CD 5%	4.30		4.49		5.03		0.35	1.08		0.20	

CD= Critical difference; PI/PDOC= Percent increase or decrease over control

Table. 2: Effect of ZnO NPs on Root length, Shoot length, Fresh & Dry weight of Chickpea (Pot trials)

ZnO NPs (µg/ml)	RL (cm)	PI/PDOC in RL	SL (cm)	PI/PDOC in SL	Seedling length (cm)	PI/PDOC in Seedling length	Root to Shoot ratio	FW (g)	PI/PDOC FW	DW (g)	PI/PDOC DW
0	22.7 ± 2.56	0.00	15.3 ± 1.20	0.00	38.16 ± 3.21	0.00	1.43 ± 0.09	4.44± 0.48	0.00	1.56 ± 0.25	0.00
4	23.2 ± 2.59	2.20	18.38 ± 2.91	20.13	41.96 ± 2.68	9.42	1.24 ± 0.14	6.18 ± 1.29	46.28	1.90 ±0.03	18.63
8	22.8 ± 2.88	0.44	18.8 ± 2.93	22.88	41.5 ± 0.5	8.95	1.22 ± 0.33	6.04 ± 0.53	36.57	1.65 ± 0.01	2.48
12	27.2 ± 3.63	19.82	18.5 ± 2.45	20.92	45 ± 3.60	20.26	1.46 ± 0.23	6.20 ± 0.62	35.21	1.94 ± 0.34	19.44
16	15.88 ± 4.65	-30.04	16.4 ± 1.67	7.19	31.33 ± 4.53	-15.05	0.93 ± 0.17	5.01 ± 1.32	18.51	1.79 ± 0.1	11.18
CD 5%	4.96		3.49		5.29		0.39	0.93		0.28	

CD= Critical difference; PI/PDOC= Percent increase or decrease over control

Table. 3: Effect of ZnO NPs on PI, GSI, PHSI, RLSI and DMSI of Chickpea (Laboratory conditions)

ZnO NPs (µg ml^{-1})	PI	GSI	PHSI	RLSI	DMSI
0	89	100.00	100.00	100.00	100.00
4	88	98.88	117.44	115.05	91.28
8	82	92.13	134.88	118.28	103.10
12	94	105.62	115.12	97.85	93.24
16	80	89.89	91.86	96.77	93.64

Table. 4 Effect of ZnO NPs on PI, GSI, PHSI, RLSI and DMSI of Chickpea (Pot conditions)

ZnO NPs (µg ml^{-1})	PI	GSI	PHSI	RLSI	DMSI
0	72	100.00	100.00	100.00	100.00
4	65	90.28	109.42	102.20	118.56
8	84	116.67	108.95	99.56	102.42
12	76	105.56	120.26	119.82	119.37
16	63	87.50	84.95	69.96	111.58

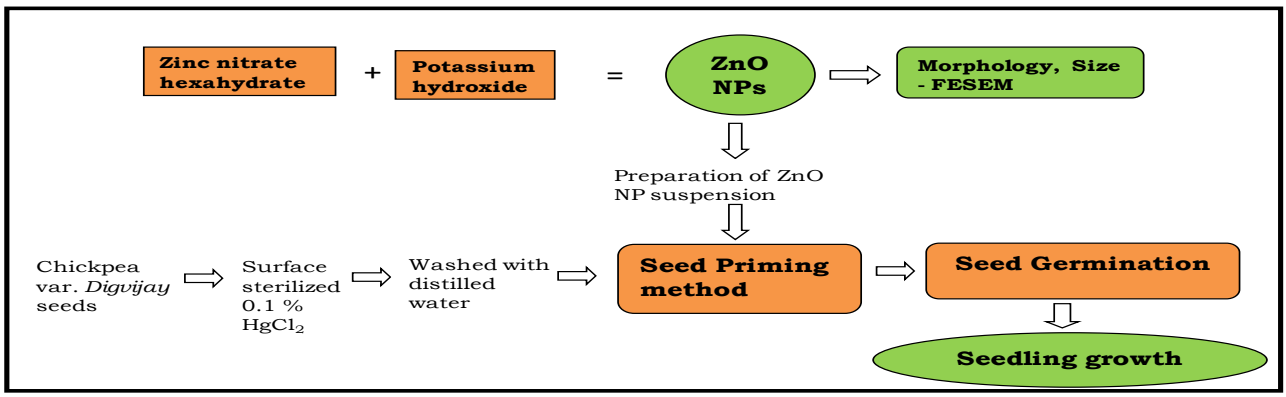


Fig. 1 Schematic representation of ZnO NP priming study on germination and growth studies

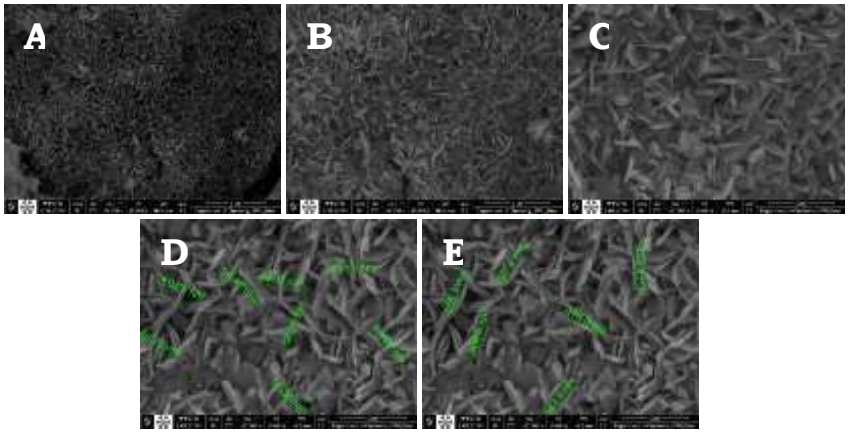
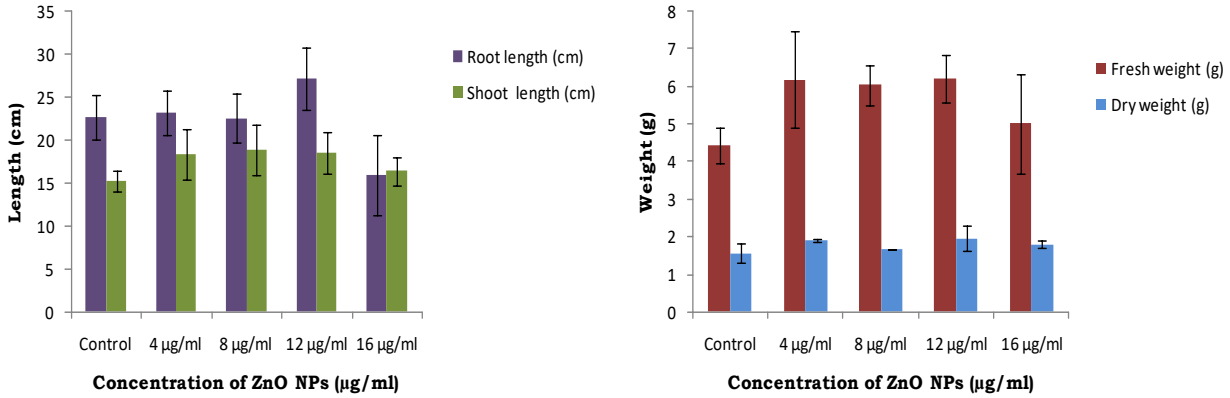
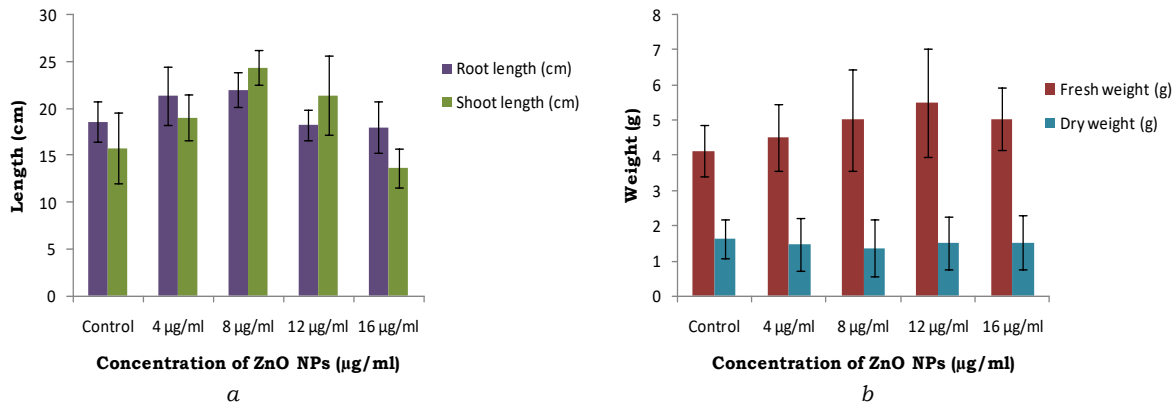


Fig. 2: FESEM images of ZnO nanoparticles: A, B, C- Magnfied images, D- Image showing width, E- Image showing length of nanoparticles



a *b*

Fig. 3. Effect of ZnO nanoparticles on seedling growth under laboratory conditions: **(a)** root and shoot length, **(b)** fresh weight and dry weight



a *b*

Fig. 4. Effect of ZnO nanoparticles on seedling growth in pot conditions: **(a)** root and shoot length, **(b)** fresh weight and dry weight