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# Comparative Efficacy of Zn Supplement and Zinc Oxide Nanoparticles Over the Seed Germination of Lentil and Chick Pea

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## Abstract

**The mineral nutrients are absorb from the soil by the roots, these are either macro or micro nutrients. Zinc is micro nutrients that help in several physiological process of plants and help in phyto hormone synthesis. The present Study includes the synthesis of ZnOPs by using Zinc oxalate decomposing method and these particles are characterized by UV-vis spectroscopy, and size was analyzed by Zeta analyzer. ZnOPs size was analyzed 65 nm. Seed germination was done by paper towel method. Soaking of seed was done in 100ppm, 500ppm and 1000ppm of ZnOPs and 100 ppm of ZnO supplement solution. After 5 day germination, germination percentage and seed vigor index (SVI) was calculated. It was observed that seed germination at 100 ppm is maximum in both the seed of Lentil (*Lens esculentum* Linn) and Chick pea (*Cicer arietinum* Linn). Seed vigor index was found increased at concentration of 100ppm. Presented study inferred that zinc provided as nanoparticle absorb more prominently than the traditional zinc supplement available in market at low concentration as the ZnOPs concentration increases the germination and seed vigor index retarded so it can be proposed that micronutrient in nano particles based formulation at lower concentration can provide better result in agriculture production.**

**Keywords:** Micronutrients, Seed vigor index, ZnO nanoparticles (ZnOPs)

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## INTRODUCTION

By the 2050 world population reached to 9.5 billion In present word micronutrient deficiency is one of the major problem because it is very difficult to find the symptom of mall nutrition in human population[1].

Zn mall nutrition is one of the major problem that is associated basically to child malnutrition. According to WHO Zn is the fifth factor associated with the children diseases in developing countries. Food security is associated with the micronutrient also because food having the lack of trace minerals like, Zn, Fe, Cu, Mn, Se etc. leaded to certain diseases like anemia, diarrhoea, skin diseases, lack of immunity[2]. Zn has the effect on several physiological functions like in regulation of more than 200 enzymes, gene expression under stress condition and detoxify the free radicals and reactive oxygen species.

Significance of Zn was first reported in maize, barley and sunflower[3]. Zn act as antioxidant stimulator that increase the photosynthates and essential oil biosynthesis in growing leaves. It was also reported that fresh weight, dry weight, chlorophyll content also increase in plants supplied with optimum concentration of Zn and toxicity and deficiency also has negate effect on plant growth. Zn is important for the biosynthesis of Auxin and tryptophan[4]. Zn is also required by the carbonic anhydrase enzyme the help in interconversion of carbonic acid and carbon dioxide in C4 pathway of photosynthesis[5]. Zn increase the carbonic anhydrage activity ten folds in algage also[6]. Zn shortage result in many physiological problem of plants[7]. Zn deficiency also affect the yields and productivity of crop plants.

It was observed that Indian soil lack the trace element Zn so that it cannot be present in crop product produced in these soil as a result it is also not present in human[8]. Zn deficiency in plant leads to genetic variation in each generation that result in low productivity, poor grain quality, poor germination rate and poor nutritional value. There are report[9] that Zn deficiency increased 10 fold by 2025. There are several strategies to reduce the effect of Zn disorder that include the biofortification, supplementation and fortification of zinc salt that are cheaper method the other method include the using the zinc supplement in soil during the growth of crops that indirectly reaches to the plant cells and ultimately to human through food chain. Along with fertilizer zinc supplement require specific calculation of dose that depend on the crop, variety, soil type and mineral itself. The major advantages of this method are, it is simple, inexpensive and can be achieved in short period of time. By frequent use of zinc supplement along with fertilizer result in accumulation of Zn in soil that create some harmful toxic effect above certain limit on human and animals depend upon the these crop. So it was proposed to use of nano particles of zinc to enhance its uptake by plants that affect the agronomic effectiveness of zinc fertilizer. The present work was performed to evaluate the comparative effect of ZnO supplement and ZnO nanoparticles (ZnOPs) over the seed germination of Lentil (*Lens esculentum* Linn) and Chick pea (*Cicer arietinum* Linn).

## METHODOLOGY

### Synthesis of Zinc oxide nano particles (ZnOPs)

Zinc oxide nano particles was synthesized by using Zinc oxalate decomposing method as describe by Prasad *et al.* (2012). Zinc oxalate was prepared by mixing the Zinc acetate (0.1M) and oxalic acid (0.1M) in equal amount as result zinc oxalate was precipitated that was rinsed by distilled water and allow in air for decomposition dried in oven at 280°C and these particles are characterized by UV-vis spectroscopy and size was analyzed by Zeta analyzer. ZnOPs size was analyzed 65 nm. Suspension of ZnO of 100 ppm and ZnOPs of 100, 500 and 1000 ppm was prepared by dissolving 100, 500 and 1000 mg/L in distilled water.

### Germination of Seed

Fifty seeds each of Lentil (*Lens esculentum* Linn) and Chick pea (*Cicer arietinum* Linn) were soaked in 100 ppm of ZnO supplement solution that was taken as control and in100ppm, 500ppm and 1000ppm of ZnOPs solution for 12 hours. After soaking seeds were placed on paper towel for germination at 37°C under moist and dark condition for five days. Germination was done After five days shoot length, root length was measured in centimeter, germination percentage and Seedling Vigour Index (SVI) was calculated.

Seedling Vigour Index (SVI) was calculated by measuring the length of root and shoot length by measuring scale in centimeter using the following formula described by

Seed Vigour Index = Germination%× (root length + shoot length) cm

## RESULTS

Ten seeds (S1 to S10) of Lentil and Chick pea were soaked in ZnO and ZnONp as described in methodology and their root (radical) length and shoot (Plumule) length were measured in centimeter (cm) by measuring scale Table 1, 2, 3 and 4. Fifty seeds was soaked in ZnO and ZnONp for the germination and germination percentage was calculated Table 5 and 6. Seed vigour index was calculated in cm it show that at 100 ppm concentration of ZnONp it is more than the control while at 500ppm and 1000ppm concentration of ZnONp it is less than control Table 7 and 8.

## DISCUSSION

Efficacy of seed germination in bulk ZnO and ZnONp was evaluated by soaking the seeds of *Lens esculentum* Linn and *Cicer arietinum* in suspension of 100 ppm of ZnO supplement solution that was selected as control and in100ppm, 500ppm and 1000ppm solution of ZnONp and it was inferred that percentage germination of seeds increased 90 to 98 percentage for *Lens esculentum Linn* and 86 to 96 percent for *Cicer arietinum* in 100 ppm solution of ZnONp takes place while percentage seed germination decreases at higher concentration as compare to control. The reason

**Table 1.** Effect of ZnONp on shoot Length(cm) of Lentil (*Lens esculentum*)

| | S1 | S2 | S3 | S4 | S5 | S6 | S7 | S8 | S9 | S10 | Average (cm) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| (Control ZnO) 100 PPM | 2.1 | 3.2 | 3.6 | 3.9 | 4.5 | 4.5 | 4.2 | 3.2 | 3.2 | 2.5 | 3.49 |
| ZnONp 100 PPM | 6.2 | 8.1 | 7.3 | 5.4 | 6.2 | 7.2 | 8.3 | 11.2 | 6.2 | 3.1 | 6.92 |
| ZnONp 500 PPM | 3.2 | 3.1 | 3.3 | 3.2 | 3.1 | 3 | 4.5 | 3.6 | 3.3 | 3 | 3.33 |
| ZnONp 1000 PPM | 3.1 | 3.2 | 2.7 | 2.2 | 2.4 | 3.2 | 4.2 | 3.2 | 4.1 | 5.1 | 3.34 |

**Table 2.** Effect of ZnONp on Root Length(cm) of Lentil (*Lens esculentum*)

| | S1 | S2 | S3 | S4 | S5 | S6 | S7 | S8 | S9 | S10 | Average (cm) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| (Control ZnO) 100 PPM | 1.1 | 0.7 | 1.2 | 1.1 | 1.1 | 1.2 | 1.3 | 1.3 | 1.2 | 0.9 | 1.11 |
| ZnONp 100 PPM | 1.2 | 3.3 | 1.8 | 1.2 | 2.2 | 3.2 | 1.1 | 3.3 | 1.2 | 0.9 | 1.94 |
| ZnONp 500 PPM | 0.2 | 0.3 | 0.3 | 0.3 | 0.2 | 0.2 | 0.2 | 0.3 | 0.3 | 0.3 | 0.26 |
| ZnONp 1000 PPM | 0.2 | 0.1 | 0.3 | 0.2 | 0.1 | 0.1 | 0.1 | 0.2 | 0.1 | 0.2 | 0.16 |

**Table 3.** Effect of ZnONp on shoot Length (cm) of Gram (*Cicer arietinum*)

| | S1 | S2 | S3 | S4 | S5 | S6 | S7 | S8 | S9 | S10 | Average(cm) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| (Control ZnO) 100 PPM | 3.1 | 0.8 | 3.3 | 1.1 | 1.6 | 3.1 | 3.5 | 3.1 | 2.9 | 2.2 | 2.47 |
| ZnONp 100 PPM | 3.4 | 3.5 | 3.3 | 1.1 | 3.5 | 3.2 | 2.5 | 1.1 | 1.3 | 0.8 | 2.37 |
| ZnONp 500 PPM | 2.1 | 0.9 | 1 | 1.3 | 0.9 | 0.8 | 0.7 | 1.2 | 1 | 0.2 | 1.01 |
| ZnONp 1000 PPM | 0.8 | 0.5 | 0.8 | 1.1 | 0.9 | 0.8 | 1.2 | 0.8 | 0.7 | 0.3 | 0.79 |

**Table 4.** Effect of ZnONp on root Length (cm) of Gram (*Cicer arietinum*)

| | S1 | S2 | S3 | S4 | S5 | S6 | S7 | S8 | S9 | S10 | Avearge (cm) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| (Control ZnO) 100 PPM | 2.8 | 0.8 | 0.9 | 0.5 | 3.2 | 5.4 | 2.8 | 3.3 | 1.1 | 0.9 | 2.17 |
| ZnONp 100 PPM | 3.4 | 4.2 | 4.8 | 0.3 | 4.5 | 4.1 | 0.2 | 0.3 | 0.1 | 0.2 | 2.21 |
| ZnONp 500 PPM | 0.2 | 0.3 | 0.3 | 0.3 | 0.2 | 0.2 | 0.2 | 0.3 | 0.3 | 0.3 | 0.26 |
| ZnONp 1000 PPM | 0.2 | 0.1 | 0.3 | 0.2 | 0.1 | 0.1 | 0.1 | 0.2 | 0.1 | 0.2 | 0.16 |

behind it that nano particles easily penetrate the seed pore as compare to bulk ZnO[10]. The effect of Zn nano particle also described by fact that it increase the higher precursor activity for producing the higher biomolecules. Similar findings have also reported positive impact of ZnO NPs in different crops[11-16]. Effects of ZnONp was also observed on shoot length, root length and on seed vigor index. In *Lens esculentum* Linn shoot length and root length increased at lower concentration of Zn nano particle solution while at higher concentration it was retarded as compare to control. It was observed that no significant difference was observed on root and shoot length of germinating seed of *Cicer arietinum* while higher concentration of nano solution of Zn retard the growth of root and shoot of gram.

The probable reason for decreased germination at higher concentration could be the increased absorption and accumulation of these ZnONPs both in extracellular space and within the cells resulted in reduction in cell division,

**Table 5.** Effect of ZnONp on Seed Germination % of Lentil (*Lens esculentum*)

| Concentration | Sown | Germinated | % |
|---|---|---|---|
| (Control ZnO) 100 PPM | 50 | 45 | 90 |
| ZnONp 100 PPM | 50 | 49 | 98 |
| ZnONp 500 PPM | 50 | 44 | 88 |
| ZnONp 1000 PPM | 50 | 46 | 92 |

**Table 6.** Effect of ZnONp on Seed Germination % of Gram (*Cicer arietinum*)

| Concentration | Sown | Germinated | % |
|---|---|---|---|
| (Control ZnO) 100 PPM | 50 | 43 | 86 |
| ZnONp 100 PPM | 50 | 48 | 96 |
| ZnONp 500 PPM | 50 | 41 | 82 |
| ZnONp 1000 PPM | 50 | 39 | 78 |

**Table 7.** Effect of ZnONp on Seedling Vigour Index of Lentil (*Lens esculentum*)

| Concentration | % | Root Length (cm) | Shoot Length (cm) | SVI (cm) |
|---|---|---|---|---|
| (Control ZnO) 100 PPM | 94 | 1.11 | 3.49 | 432.4 |
| ZnONp 100 PPM | 98 | 1.94 | 6.92 | 868.28 |
| ZnONp 500 PPM | 88 | 0.26 | 3.33 | 315.92 |
| ZnONp 1000 PPM | 92 | 0.16 | 3.34 | 322 |

**Table 8.** Effect of ZnONp on Seedling Vigour Index of Gram (*Cicer arietinum*)

| Concentration | % | Root Length (cm) | Shoot Length (cm) | SVI (cm) |
|---|---|---|---|---|
| (Control ZnO) 100 PPM | 86 | 2.17 | 2.47 | 399.04 |
| ZnONp 100 PPM | 90 | 2.21 | 2.37 | 412.2 |
| ZnONp 500 PPM | 82 | 0.26 | 1.01 | 104.14 |
| ZnONp 1000 PPM | 78 | 0.16 | 0.79 | 74.1 |

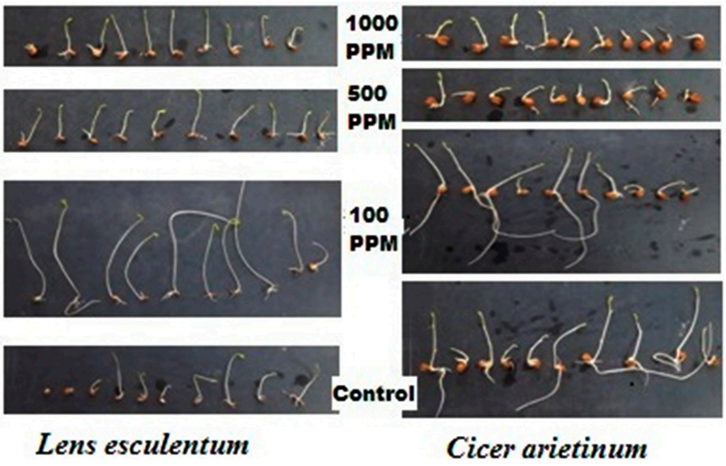


**Fig.1 .** Effect of ZnONp on seed germination of *Lens esculentum Linn* and *Cicer arietinum*

cell elongation and inhibition of the hydrolytic enzymes involved in food mobilization during the process of seed germination. Similar results were noticed by several workers who observed that ZnONPs at higher concentration had inhibitory effect on growth and development in different crops including maize[17,18].

Effect of ZnONp on Seedling Vigour Index of Lentil was observed around two fold increased as compare to control while at higher concentration of ZnONp it was observed slight lower. In the case of gram not much difference was observed on Seedling Vigour Index. Higher seedling vigour under natural condition represent that soil have the optimum concentration of Zn[19].

## FUTURE PROSPECTS

Present study support the role of nano particles in plant development up to certain limit. This study open the future prospect for exploring the Role of different micro nutrient and macronutrient must be evaluated on plant physiology and anatomy by forming their nano particles. It may help reduce the toxicity of elements on plant metabolism.

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## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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None.

## AUTHORS CONTRIBUTION

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

## DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

## ETHICS STATEMENT

Not applicable.

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