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# Exploring *Bacillus thuringiensis* coated zinc oxide nanoparticles for improving chili seed germination

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## Abstract

Agriculture plays a pivotal role in global food security, and the use of agrochemicals like pesticides and fertilizers is essential for enhancing crop yield. However, the overuse of these chemicals leads to environmental pollution and health risks. In this context, nanotechnology offers a promising solution to mitigate these adverse effects while improving agricultural productivity. This study focuses on the synthesis and application of *Bacillus thuringiensis* coated zinc oxide nanoparticles (*Bt*-ZnO NPs) to enhance the germination and growth of chili pepper seeds (*Capsicum annuum*). The successful synthesis was confirmed by characterization of the nanoparticles, with an average size of 130 nm. The combination of *Bt* and ZnO nanoparticles could provide dual benefits such as boosting plant development while minimizing environmental impact and may show insecticidal effect. The optimal concentration of 500 ppm of *Bt*-ZnO nanoparticles resulted in a notable enhancement in germination indices compared to the control. The values for germination percent (88.3%), root length (2.3 cm), shoot length (4.9 cm), seedling length (7.2 cm), seedling vigor index I (636.4), fresh weight (140.7 mg), and dry weight (13.3 mg) were all higher than those observed in the control group. Higher concentrations showed inhibitory effects, likely due to zinc toxicity. This study suggests that *Bt*-ZnO NPs, at appropriate concentrations, can be an effective tool for improving seed germination and promoting early plant growth, offering a sustainable alternative to conventional agrochemicals.

**Keywords:** *Bacillus thuringiensis*, zinc oxide, vigor

## 1. Introduction

Agriculture has always been a vital and stable sector because it plays a key role in producing raw materials for the food and feed industries. The development of agriculture is essential for eliminating poverty and hunger so in modern agriculture, agrochemicals like pesticides and fertilizers are crucial for achieving high crop yields and efficiency. However, these chemicals can cause problems, such as water pollution and residues in food, which can harm both human health and the environment. To reduce these risks, it's important to carefully manage and control the use of these chemicals. One promising solution is the use of advanced technology, such as smart nanomaterials, in farming. This could help to transform agricultural practices by minimizing the negative effects on the environment and improving both the quality and quantity of crop production (Prasad *et al.*, 2017) [10].

Nanotechnology is a science that deals with the materials having size between 1 and 100 nanometers, with the key characteristic that these materials exhibit different properties from bulk materials due to their nanoscale size (Huang *et al.*, 2007) [5]. Key benefits of nanotechnology in agriculture include its specific uses like nanofertilizers and nanopesticides, which help to manage nutrient levels and boost productivity while protecting soil and water from environmental contamination. Additionally, nanotechnology offers several potential benefits, such as improving food quality and safety, reducing the need for agricultural inputs, and enhancing the absorption of nanoscale nutrients from the soil. These advantages make the application of nanotechnology a valuable and promising approach in agriculture (Prasad *et al.*, 2017) [10].

Zinc is a crucial micronutrient that supports various aspects of plant growth and development. It helps to improve seed viability and promotes root growth during seed germination. Zinc also enhances the plant's ability to take up and transport water, and it can alleviate the damaging effects of heat, drought, or salt stress. Additionally, zinc is involved in the production of important plant hormones such as auxins and gibberellins, which regulate growth. However, when zinc concentrations become too high, it can negatively affect cell function and disrupt several critical processes within the plant (Tymoszuk *et al*., 2020) [14]. The integration of nanotechnology into agriculture, particularly through the use of nanofertilizers and nanopesticides, has shown promising results. Specifically, ZnO NPs enhance seed germination and plant growth, additionally their antimicrobial properties offer potential for biological control, providing a sustainable approach to managing plant diseases and pests. (Gauba *et al*., 2023) [4]. Among several metallic nanoparticles, ZnO NPs have been widely used due to their antibacterial, antifungal, UV filtering properties, and high catalytic and photochemical activity (Meruvu *et al.*, 2011) [8].

*Bacillus thuringiensis* (*Bt*) is a soil bacterium widely recognized for its insecticidal properties due to the production of crystalline proteins (Cry toxins) that target specific insect pests (Kumar *et al*., 2021) [6]. Over the past few decades, *Bt* has gained significant attention not only for its pest control capabilities but also for its potential to promote plant growth. It has been reported that *B. thuringiensis* showed much higher rate of phosphate solubilization apart from its biopesticidal activity. *B. thuringiensis* can promote the growth by increasing fresh and dry weights of plants, root and shoot length, seed germination rate (Bandopadhyay, 2020) [3].

Chili peppers (*Capsicum annuum* and related species) are a globally important crop, appreciated for their culinary, nutritional, and medicinal benefits. The germination of chili seeds is a key phase in the cultivation process, directly impacting the growth, yield, and quality of the crop. As a plant that thrives in warm conditions, chili peppers require specific environmental factors for optimal seed germination, such as appropriate temperature, moisture, and soil conditions. The successful establishment of seedlings and their early growth are particularly sensitive to changes in these variables, highlighting the importance of studying germination to enhance crop production and contribute to food security. (Pardo *et al*., 2020; Ahmad *et al*., 2021) [9, 2].

The present experiment focuses on the synthesis of *Bacillus thuringiensis* coated zinc oxide nanoparticles (*Bt*-ZnO NPs) to investigate the effects of their different concentrations on the germination of chili seeds. The aim is to combine the growth-promoting effects of both *Bt* and ZnO nanoparticles to enhance seedling development.

## 2. Materials and Methods

### 2.1 Materials

In the present study, experimental material consisting chilli seeds of veriety PDKV-Hirkani were obtained from vegetable reserch unit Dr. PDKV Akola. Zinc acetate as metal ion source was ordered from Himedia. Luria Bertani agar and broth were purchased from Himedia.

Pure culture of *Bacillus thuringiensis* required for synthesis of nanoparticles was procured from Microbial Culture Collection, National Centre for Cell Science, Pune and maintained in Nanotechnology and molecular Biology laboratory, Biotechnology centre, Dr. PDKV Akola.

### 2.2 Methods

### 2.2.1 Maintenance of microbial culture

Loopful growth of *Bt* was taken from pure culture with the help of inoculating needle and was streaked by four-way method in the Petri plate under aseptic conditions. Petri plate was properly sealed with parafilm and plates were incubated at 30 °C for 24 hours. After that Petri plate was preserved at 4 °C in refrigerator until the further use.

### 2.2.2 Synthesis of *Bt*-ZnO NPs

The synthesis of nanoparticles was based on the procedure given by Malaikozundan *et al*. (2017) [7] with some modifications,

Pure culture of *B. thuringiensis* inoculated into a flask containing sterile nutrient broth and then incubated in an orbital shaker at 30 °C for 24 hours. The culture was diluted four times and allowed to grow for another 48-72 h, following incubation, the pH of the culture was adjusted to 6 using 0.4 M NaOH to lengthen the process of transformation. Then 0.1 M zinc acetate was added to the culture solution. Obtained culture was heated to 80°C on a water bath for 5 to 10 min. A white precipitate starts to appear at the bottom of the flask indicating the initiation of the transformation process. Culture was removed from the water bath and incubated at 30 °C for 12 h to allow the deposition of particles at the bottom of the flask. Then the particles were filtered and washed with deionised water followed by drying at 40 °C in a hot air oven for 4 hrs.

### 2.2.3 Effect of *Bt*-ZnO NPs on seed germination

The germination potential of *Bt*-ZnO nanoparticles was assessed by following the protocol provided by Afrayeen *et al*., 2019. Chili seeds of the PDKV Hirkani variety were selected for the experiment. Healthy seeds were sterilized using a 2% sodium hypochlorite solution to eliminate any contaminants. Stock solutions of *Bt*-ZnO nanoparticles at concentrations of 250, 500, 750, 1000, and 1500 ppm were prepared by adding the appropriate amount of nanoparticles in distilled water and sonicating the mixture for 10-15 minutes to ensure proper dispersion. The seeds were then soaked for 6 hours in each concentration, with separate replications for each, and allowed to dry afterward. Moistened germination paper was used to place 20 seeds per replication, which were then rolled. Water was sprayed as needed throughout the process. After 14 days, the germination percentage, root length, shoot length, total length, fresh weight, and dry weight of the seedlings were recorded.

### 2.3 Characterization of *Bt*-ZnO nanoparticles

### 2.3.1 UV-vis spectrophotometry analysis

UV-Visible spectroscopy is an effective preliminary technique for characterizing nanoparticles, particularly to confirm the formation and presence of nanoparticles in suspension. The *Bt*-ZnO NPs synthesized using *B. thuringiensis* (1 mg) were dissolved in 1 ml distilled water, and the resulting solution was sonicated for 10 minutes to prepare the sample for UV-visible spectroscopy analysis. The UV-visible spectrum of the solution was obtained by scanning within the 200-800 nm range using a UV-visible double beam spectrophotometer, distilled water used as the solvent, was placed in the reference slot of the spectrophotometer and the maximum absorbance (λmax) was recorded.

### 2.3.2 Particle size analysis

Particle size is analyzed by dynamic light scattering to confirm the nano size of synthesized particles. A 12 mm cell was used for dynamic light scattering (DLS) measurements, where 1 ml of distilled water and a small amount of stock dispersion were mixed. The sample was sonicated for some time and the particle size distribution of the nanoparticles was measured. The viscosity, absorption, and refractive index of the water were recorded, and the pH was adjusted using sodium hydroxide or hydrochloric acid before recording the particle sizes.

## 3. Results and Discussion

### 3.1 Synthesis of *Bt*-ZnO NPs

During synthesis, the addition of a 0.1M zinc acetate solution resulted in clump formation, which became visibly evident in the culture solution. The mixture was incubated at 80 °C for 5-10 minutes, during which a white precipitate began to form at the bottom of the flask. After 12 hours, the deposited particles were filtered and washed with deionized water. The final product, which appeared as white powder was *Bt*-ZnO NPs. Similar study by Malaikozundan *et al*. (2017) [7] reported the formation of white powder as a final product that indicate the successful synthesis of *Bt*-ZnO NPs. Similarly, Zhou *et al*., (2021) [15] reported that the use of *Acinetobacter johnsonii* strain RTN1 for the biosynthesis of ZnO NPs resulted in the formation of white precipitates at the bottom of the flask, indicating the successful synthesis of ZnO NPs.

### 3.2 Characterization of *Bt*-ZnO NPs

### 3.2.1 UV-vis spectrophotometry analysis

The absorbance spectrum of *Bt*-ZnO nanoparticles was recorded in the range of 200-800 nm, and the peak absorbance was noted. A characteristic absorption peak for ZnO nanoparticles was observed at 381 nm in the *Bt*-ZnO NPs spectrum, zoom image between 300-500 nm is shown in Fig. 1. Similar results were reported by Ahmed *et al.*, (2021) [15], who confirmed the biosynthesis of ZnO nanoparticles (ZnO NPs) using *Bacillus cereus* through a UV-Vis absorption peak observed at 382 nm.

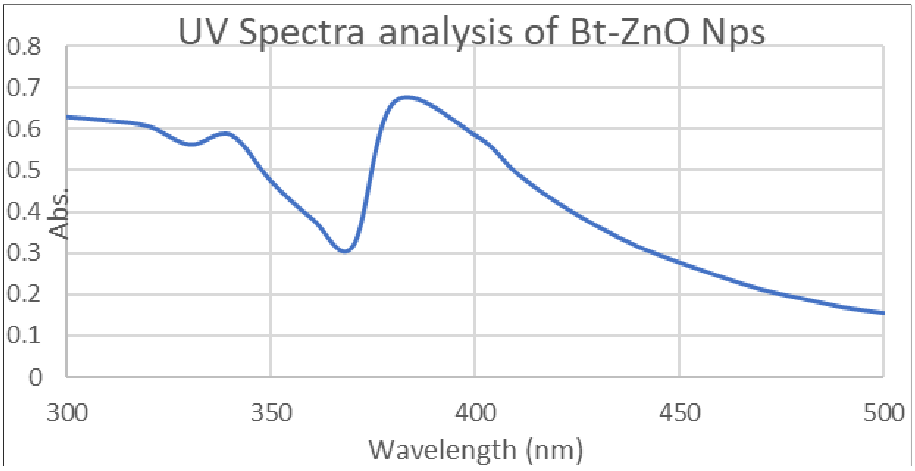

**Fig 1:** UV-Visible spectrophotometry of *Bt*-ZnO NPs

### 3.2.2 Particle size analysis

The preliminary analysis of nanoparticle size using Dynamic Light Scattering (DLS) revealed a hydrodynamic diameter of 130.41 nm, with a peak observed at 146.19 nm, as shown in Fig. 2. Similar results were reported by Shaaban and El-Mahdy (2018) [13], who synthesized Ag, Se, and ZnO nanoparticles using *Streptomyces enissocaesilis* and found that the DLS analysis of nano-ZnO indicated a particle size of 135 nm.

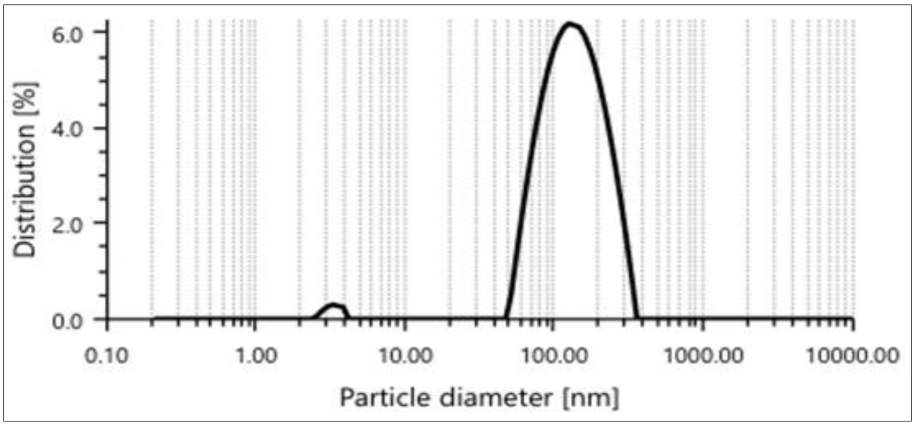

**Fig 2:** Particle size analysis of *Bt*-ZnO NPs

### 3.3. Effect of *Bt*-ZnO NPs on seed germination

The results of the *in vitro* study demonstrated a significant improvement in seed germination after treatment with nanoparticles, compared to the control. and germination was recorded after 7 and 14 days. Various parameters including shoot length, root length, total length, fresh weight, dry weight, and Vigour Index I of the seedlings were measured, as shown in Table 1 and Fig. 3.

**Germination percentage:** The germination percentage of the chili variety PDKV Hirkani was notably influenced by the different treatments, ranging from 71.7% to 88.3%. The highest germination percentage (88.3%) was observed with the 500 ppm nanoparticle treatment (T3), which outperformed the control, which had a germination percentage of 78.3%.

**Shoot Length:** The shoot length of chilli seedlings treated with nanoparticles ranged from 2.1 cm to 4.9 cm. Among the treatments, the 500 ppm (T3) nanoparticles resulted in the longest shoot length of 4.9 cm. This treatment was significantly superior to all other treatments and the control group, which had a shoot length of 3.3 cm.

**Root Length:** The root length of chilli seedlings treated with nanoparticles varied between 1 cm and 2.3 cm. The highest root length of 2.3 cm was observed with the 500 ppm (T3) nanoparticles. This treatment was statistically superior to the other treatments and the control, which had a root length of 1.3 cm.

**Seedling Length:** Seedling length for nanoparticle-treated chilli plants ranged from 3.1 cm to 7.2 cm. The 500 ppm (T3) nanoparticles produced the greatest seedling length of 7.2 cm. This treatment was found to be statistically superior to all other treatments and the control, where seedling length was 4.6 cm.

**Seedling Vigour Index I:** The seedling vigour index I was significantly influenced by nanoparticle treatments, with values ranging from 221.4 to 636.4. The 500 ppm (T3) nanoparticles resulted in the highest vigour index of 636.4, which was statistically superior to all other treatments and the control, where the vigour index was 360.3.

**Fresh Weight:** The fresh weight of chilli seedlings treated with nanoparticles ranged from 72.1 mg to 140.7 mg. The highest fresh weight, 140.7 mg, was recorded with the 500 ppm (T3) nanoparticle treatment, which was statistically superior to the other treatments and the control, which had a fresh weight of 107.2 mg.

**Dry Weight:** The dry weight of nanoparticle-treated chilli seedlings ranged from 7.9 mg to 13.3 mg. The 500 ppm (T3) nanoparticles recorded the highest dry weight of 13.3 mg, which was significantly superior to all other treatments and the control, which had a dry weight of 10.1 mg.

However, higher nanoparticle concentrations, particularly 1500 ppm, exhibited inhibitory effects, resulting in reduced germination percentage (71.7%) even than the control and stunted growth across all parameters.

Similar results were obtained by Raskar and Laware (2014) [12] in onion (*Allium cepa* L.), showed that ZnO NPs at a lower concentration enhanced cell division, seed germination, and seedling growth. On the other hand, at a higher concentration, a decreased mitotic index, a decreased seed germination ratio. Raja *et al.* (2019) [11] observed results as same as present study.

**Table 1:** Effect of *Bt*-ZnO NPs on germination parameters of chill

| Sr. No. | Treatments | Concentrations (ppm) | Germination % | Shoot length (cm) | Root length (cm) | seedling length (cm) | Vigour index I | Fresh weight (mg) | Dry weight (mg) |
|---|---|---|---|---|---|---|---|---|---|
| 1 | $T_1$ | Control | 78.3 | 3.3 | 1.3 | 4.6 | 360.3 | 107.2 | 10.1 |
| 2 | $T_2$ | 250 | 83.3 | 3.7 | 1.5 | 5.3 | 442.0 | 125.3 | 11.7 |
| 3 | $T_3$ | 500 | 88.3 | 4.9 | 2.3 | 7.2 | 636.4 | 140.7 | 13.3 |
| 4 | $T_4$ | 750 | 86.6 | 3.8 | 1.9 | 5.7 | 494.0 | 127.2 | 11.7 |
| 5 | $T_5$ | 1000 | 83.3 | 3.2 | 1.5 | 4.7 | 400.5 | 100.4 | 9.7 |
| 6 | $T_6$ | 1500 | 71.7 | 2.1 | 1.0 | 3.1 | 221.4 | 72.1 | 7.9 |
|  |  | F Test | Sig | Sig | Sig | Sig | Sig | Sig | Sig |
|  |  | SE(m)± | 2.04 | 0.046 | 0.0.043 | 0.048 | 13.02 | 2.57 | 0.302 |
|  |  | CD at 1% | 8.81 | 0.2 | 0.185 | 0.20 | 56.26 | 11.082 | 1.303 |

**Fig 3:** Effect of *Bt*-ZnO NPs on germination parameters of Chilli

## 4. Conclusion

These findings highlight the optimal concentration of *Bt*-ZnO NPs for promoting seedling growth. The result shows that *Bt*-ZnO NPs at lower concentration enhances germination indices such as germination percentage, shoot length, root length, vigour index I, fresh weight and dry weight while avoiding the negative impact of excessive nanoparticle concentrations which may be result of zinc toxicity at higher concentrations.

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## 6. References

1. Afrayeem SM, Chaurasia AK. Effect of zinc oxide nanoparticles on seed germination and seed vigour in chilli (*Capsicum annuum* L.). J Pharm Phytochem. 2017;6(5):1564-6.
2. Ahmad M, Nisar M, Hafeez M. Effect of environmental factors on seed germination and early growth of chili (*Capsicum annuum* L.). Pak J Bot. 2021;53(1):123-8.
3. Bandopadhyay S. Application of plant growth promoting *Bacillus thuringiensis* as biofertilizer on *Abelmoschus esculentus* plants under field condition. J Pure Appl Microbiol. 2020;14(2):1287-94.
4. Gauba A, Hari SK, Ramamoorthy V, Vellasamy S, Govindan G, Arasu MV. The versatility of green synthesized zinc oxide nanoparticles in sustainable agriculture: A review on metal-microbe interaction that rewards agriculture. Physiol Mol Plant Pathol. 2023;125:102023.
5. Huang J, Li Q, Sun D, Lu Y, Su Y, Yang X, *et al*. Biosynthesis of silver and gold nanoparticles by novel sundried *Cinnamomum camphora* leaf. Nanotechnology. 2007;18(10):105104.
6. Kumar P, Kamle M, Borah R, Mahato DK, Sharma B. *Bacillus thuringiensis* as microbial biopesticide: uses and application for sustainable agriculture. Egypt J Biol Pest Control. 2021;31(1):1-7.
7. Malaikozhundan B, Vaseeharan B, Vijayakumar S, Thangaraj MP. *Bacillus thuringiensis* coated zinc oxide nanoparticle and its biopesticidal effects on the pulse beetle, *Callosobruchus maculatus*. J Photochem Photobiol; c2017.
8. Meruvu H, Vangalapati M, Chippada SC, Bammidi SR. Synthesis and characterization of zinc oxide nanoparticles and its antimicrobial activity against *Bacillus subtilis* and *Escherichia coli*. J Rasayan Chem. 2011;4(1):217-222.
9. Pardo J, Vázquez M, Martínez J. Seed germination and seedling establishment in chili peppers: A review of environmental and biological factors. Scientia Horticulturae. 2020;258:108832.
10. Prasad R, Bhattacharyya A, Nguyen QD. Nanotechnology in sustainable agriculture: recent developments, challenges, and perspectives. Front Microbiol. 2017;8:1014.
11. Raja K, Sowmya R, Sudhagar R, Moorthy PS, Govindaraju K, Subramanian KS. Biogenic ZnO and Cu nanoparticles to improve seed germination quality in blackgram (*Vigna mungo*). Mater Lett. 2019;235:164-167.
12. Raskar SV, Laware SL. Effect of zinc oxide nanoparticles on cytology and seed germination in onion. Int J Curr Microbiol App Sci. 2014;3(2):467-473.
13. Shaaban M, El-Mahdy AM. Biosynthesis of Ag, Se, and ZnO nanoparticles with antimicrobial activities against resistant pathogens using waste isolate *Streptomyces enissocaesilis*. IET Nanobiotechnol. 2018;12(6):741-747.
14. Tymoszuk A, Wojnarowicz J. Zinc oxide and zinc oxide nanoparticles impact on *in vitro* germination and seedling growth in *Allium cepa* L. Mater. 2020;13(12):2784.
15. Zhou G, Yu R, Ahmed T, Jiang H, Zhang M, Lv L, *et al*. Biosynthesis and characterization of zinc oxide nanoparticles and their impact on the composition of gut microbiota in healthy and attention-deficit hyperactivity disorder children. Front Microbiol. 2021;12:700707.