

RESEARCH ARTICLE

Effect of Zinc Oxide Nanoparticle Size on Maize (*Zea mays* L.) Seed Germination and Early Seedling Growth

Hassan Samaila^{1,*}, J. B. Yerima² and Danis S. Balami¹

ABSTRACT: Zinc oxide nanoparticles (ZnO NPs) are wide-band-gap semiconductors (3.37 eV) with high exciton binding energy (60 meV) at room temperature, making them attractive for optoelectronic applications. They are also generally regarded as environmentally friendly and transparent in the visible region of the electromagnetic spectrum. In this study, ZnO nanoparticles were synthesised via a green synthesis route using four zinc salt precursors, while guava (*Psidium guajava*), jatropha (*Jatropha curcas*), and cassava (*Manihot esculenta*) leaf extracts served as reducing, stabilising, and capping agents. A 1 M sodium hydroxide solution was added dropwise to adjust the pH to 11.6, with distilled water used as the solvent. The synthesised nanoparticles exhibited a range of particle sizes, which were subsequently evaluated for their effects on maize (*Zea mays* L.) seed germination and early growth. Germination index results indicated that nanoparticles at both smaller and larger size ranges produced higher germination indices compared to those of intermediate sizes. The germination vigour index showed that particle A recorded the lowest value (11.07), whereas particles G and H exhibited the highest vigour indices of 46.10 and 46.54, respectively. Analysis of seedling dry weight after air-drying for five days revealed that only three out of the twelve nanoparticle sizes enhanced biomass relative to the control, while five sizes showed no significant effect. Nanoparticles with sizes of 18.31 nm and 19.27 nm produced the highest relative dry weights (112.5%), while particles of 24.22 nm also showed a positive effect (106.3%) compared to the untreated control. These findings demonstrate that ZnO nanoparticle effects on maize germination and growth are strongly size-dependent, highlighting the importance of particle size optimisation for agricultural applications. The results suggest that intermediate nanoparticle sizes (18–19 nm) may offer the most promising balance between growth promotion and minimisation of phytotoxic effects, providing valuable insights for the development of optimised ZnO nanofertiliser formulations for sustainable crop production.

Keywords: Zinc Oxide Nanoparticles, Green Synthesis, Maize Germination, Seedling Growth, Particle Size-Dependent Effects, Nanofertiliser.

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1. INTRODUCTION

Zinc stands as an essential micronutrient that plants require for numerous physiological and biochemical processes,

including enzyme activation, protein synthesis, and the regulation of growth hormones. Agricultural soils across the globe widely report zinc deficiency, and this condition has been demonstrated to reduce seed germination, seedling vigor, and overall crop productivity [1, 2]. Conventional zinc fertilizers typically suffer from low use efficiency because of leaching, fixation in soil, and limited uptake by plant roots, and this situation necessitates alternative approaches for efficient micronutrient delivery to crops [3]. Nanotechnology has emerged in recent years as a promising tool for improving nutrient use efficiency in agriculture through the

¹ Department of Physics, University of Maiduguri, P.M.B. 1069, Maiduguri, Borno, Nigeria

² Department of Physics, Modibbo Adama University, P.M.B. 2076, Yola, Adamawa, Nigeria

* Author to whom correspondence should be addressed:
hsmhya@gmail.com (Hassan Samaila)

development of nano fertilizers and nano-enabled agro-inputs that can deliver nutrients more effectively to plants. Among the various metal oxide nanoparticles, zinc oxide nanoparticles have attracted considerable attention from researchers because of their semiconducting properties, relatively low toxicity compared to other metal nanoparticles, and their potential to act as a controlled zinc source for plants [4, 5].

Several studies have reported that zinc oxide nanoparticles can enhance germination rates, improve photosynthetic efficiency, boost antioxidant activity, and increase yield in various crops such as wheat, rice, sorghum, soybean, tomato, peanut, and eggplant [6-10]. Field and pot experiments have further demonstrated improvements in physiological traits and grain quality following foliar or soil application of zinc oxide nanoparticles [11, 12]. However, plant responses to these nanoparticles are highly variable and depend strongly on several factors including nanoparticle concentration, exposure pathway, and physicochemical properties such as size, morphology, and surface chemistry. While low concentrations and suitable particle sizes may stimulate plant growth, higher doses or inappropriate sizes can induce oxidative stress, inhibit root elongation, and cause phytotoxic effects that damage the plant tissues [13-15]. Studies on seed germination have shown both stimulatory and inhibitory effects depending on plant species and nanoparticle characteristics, and this indicates the need for careful optimization of nanoparticle properties for safe agricultural use [16-18].

The green synthesis of nanoparticles using biological resources has gained increasing attention as an environmentally friendly and cost-effective alternative to conventional chemical and physical synthesis methods [19, 20]. Plant extracts contain diverse phytochemicals such as phenolics, flavonoids, alkaloids, and proteins that act as reducing, stabilizing, and capping agents during nanoparticle formation [21, 22]. Consequently, the choice of plant species significantly influences nanoparticle size, morphology, surface functionalization, and stability [23, 24]. Several studies have reported enhanced plant growth using green-synthesized zinc oxide nanoparticles, including improvements in germination, chlorophyll content, and biomass accumulation [25-28]. The biological approach to nanoparticle synthesis offers additional advantages such as reduced environmental impact, lower production costs, and the potential for producing nanoparticles with unique surface properties that can enhance their interaction with plant tissues.

The novelty of this paper lies in its systematic investigation of how nanoparticle size, rather than just concentration or exposure method, influences the germination and early growth of maize plants using green-synthesized zinc oxide nanoparticles. While previous research has examined the effects of zinc oxide nanoparticles on various crops, most studies have focused on either concentration-dependent effects or have used commercially available nanoparticles without considering how the synthesis route and precursor materials influence particle size and subsequent biological activity. Our study uniquely

combines the use of four different zinc salt precursors and three different plant leaf extracts to produce a wide range of nanoparticle sizes, allowing for a comprehensive evaluation of size-dependent effects on maize germination and growth. This approach enables us to identify specific nanoparticle size ranges that promote growth while minimizing potential phytotoxicity, providing valuable guidance for the development of optimized nano fertilizer formulations. Furthermore, the simultaneous evaluation of water absorption, germination parameters, seedling vigor, and biomass accumulation provides a holistic understanding of how nanoparticle size influences multiple aspects of early plant development.

Despite extensive studies on zinc oxide nanoparticles in different crops, systematic investigations focusing on the size-dependent effects of green-synthesized zinc oxide nanoparticles on maize germination and early growth remain limited. Moreover, variations arising from different plant extracts and zinc salt precursors may further modify nanoparticle size and biological activity, yet these factors are often not evaluated simultaneously in a single comprehensive study. Given maize's importance as a staple cereal crop that feeds millions of people worldwide and its sensitivity to micronutrient availability, understanding how nanoparticle size influences early developmental stages is essential for designing safe and effective nano-enabled agricultural inputs that can improve crop productivity without causing environmental harm.

In this paper, we report the synthesis of zinc oxide nanoparticles using different plant leaf extracts and zinc salt precursors via a green synthesis approach, the characterization of the resulting particle sizes, and the evaluation of their effects on maize seed germination, seedling vigor, and biomass accumulation. The study seeks to establish whether specific nanoparticle size ranges promote growth while minimizing potential phytotoxicity, thereby contributing to the development of optimized zinc oxide nanoparticle formulations for sustainable agricultural applications that can address the global challenge of zinc deficiency in crops.

2. MATERIALS AND METHODS

2.1. Materials

The synthesis of zinc oxide nanoparticles was carried out using four different zinc salt precursors, namely zinc acetate dihydrate [$\text{Zn}(\text{C}_4\text{H}_6\text{O}_4) \cdot 2\text{H}_2\text{O}$, molecular weight 219 g/mol], zinc nitrate hexahydrate [$\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$], zinc chloride [ZnCl_2 , molecular weight 126 g/mol], and zinc sulphate heptahydrate [$\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, molecular weight 291 g/mol]. Triple distilled water was employed as the solvent throughout the synthesis procedure to ensure the purity of the reaction medium and to prevent any unwanted contamination that could influence nanoparticle formation. All glassware and equipment used in the synthesis were thoroughly cleaned using nitric acid followed by deionized water rinsing, and

subsequently dehydrated by placing them in a hot air oven prior to the commencement of nanoparticle preparation. This rigorous cleaning protocol was essential to eliminate any trace contaminants that could potentially interfere with the nucleation and growth of nanoparticles during the synthesis process. The fresh leaves of three different plant species, namely guava (*Psidium guajava*), jatropha (*Jatropha curcas*),

and cassia (*Cassia* species), were collected from Shaffa Village in Hawul Local Government Area of Borno State, Nigeria. The entire synthesis process was conducted at the Chemistry Advanced Research Laboratory of the Sheda Science and Technology Complex (SHESTCO) in Sheda, Abuja, Nigeria, which provided the necessary infrastructure and facilities for the experimental work.

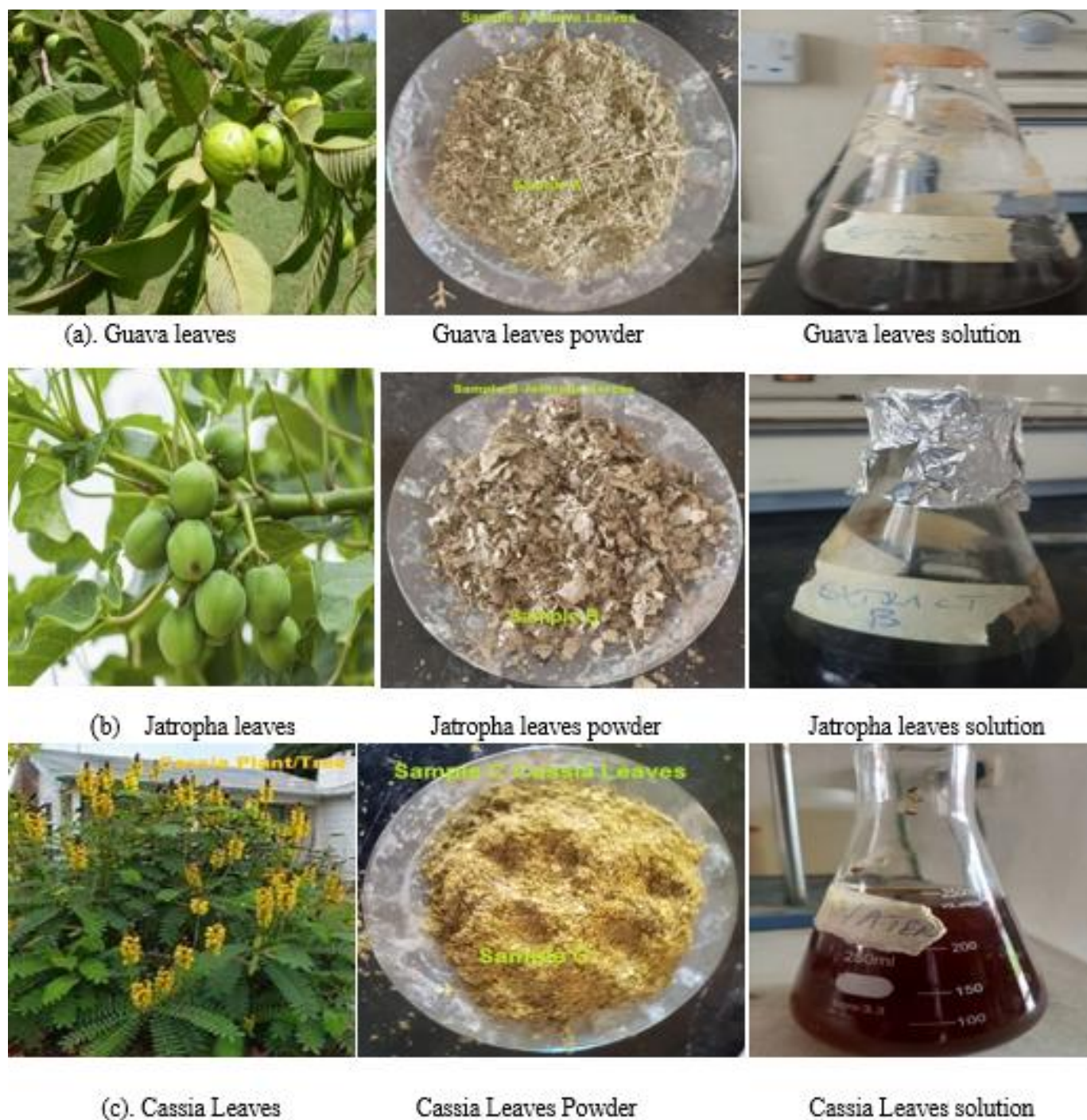


Fig. 1. Visual documentation of plant materials and aqueous extracts used for green synthesis of zinc oxide nanoparticles. (a) Fresh guava leaves, powdered guava leaves, and guava leaf extract solution; (b) Fresh jatropha leaves, powdered jatropha leaves, and jatropha leaf extract solution; (c) Fresh cassia leaves, powdered cassia leaves, and cassia leaf extract solution. The colour variations among the extracts indicate differences in phytochemical composition, which may influence nanoparticle formation and properties.

2.2. Leaves Extract Preparations

The fresh leaves collected from guava, jatropha, and cassia plants were initially washed thoroughly with running tap water to remove any surface impurities such as dust, soil particles, and other adhering contaminants. The cleaned leaf samples were subsequently dried away from direct sunlight for a period of five days to preserve the phytochemical constituents that could be degraded by ultraviolet radiation and to prevent any photochemical reactions that might alter the composition of the bioactive compounds present in the leaves. Following the drying process, the leaves were ground into a fine powder using an electric grinder to increase the surface area for efficient extraction of phytochemicals. For each plant species, 20 g of the powdered leaf material was added to 200 ml of distilled water in a 250 ml beaker, and the mixture was maintained at 60°C on a temperature-controlled hot plate until the color of the water changed completely to the characteristic color of each respective plant extract, indicating the successful extraction of phytochemicals. The extracts were then cooled to room temperature and filtered using Whatman number 1 filter paper to remove any particulate matter and undissolved leaf debris, ensuring a clear and homogeneous extract solution. The filtered extracts were stored at a temperature of 7°C in a refrigerator to preserve their phytochemical activity and prevent microbial growth for further experimental analysis. The three sample extracts were labelled as Sample A (guava leaves extract), Sample B (jatropha leaves extract), and Sample C (cassia leaves extract). Figure 1 presents the visual documentation of the leaves, powdered leaf materials, and the resulting aqueous extracts for each of the three plant species used in this study.

The visual representation provided in Figure 1a shows the guava leaves in their fresh state, the powdered form after drying and grinding, and the characteristic greenish-brown aqueous extract obtained after the extraction process. Figure 1b depicts the jatropha leaves, their powdered form, and the resulting extract solution. Figure 1c illustrates the cassia leaves, the corresponding powder, and the aqueous extract. These images demonstrate the color variations among the different plant extracts, which indicate the presence of diverse phytochemical compositions that could influence nanoparticle synthesis and properties.

2.3. Preparation of Zinc Precursors

The preparation of zinc precursor solutions was carried out by dissolving varying quantities of each zinc salt in distilled water to achieve different concentrations for the synthesis of nanoparticles. Specifically, 30 g, 40 g, and 50 g of zinc acetate dihydrate [$\text{Zn}(\text{CH}_3\text{COO})_2 \cdot 2\text{H}_2\text{O}$] were each dissolved in 1000 ml of distilled water to obtain precursor solutions with different zinc ion concentrations. Similarly, 30 g, 40 g, and 50 g of zinc nitrate hexahydrate [$\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$] were dissolved separately in 1000 ml of distilled water to prepare the nitrate precursor solutions. Following the same procedure,

30 g, 40 g, and 50 g of zinc chloride [ZnCl_2] and zinc sulphate heptahydrate [$\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$] were also dissolved in 1000 ml of distilled water to prepare their respective precursor solutions. These precursor solutions served as the source of zinc ions for the subsequent green synthesis of zinc oxide nanoparticles at the SHESTCO facility.

2.4. Green Synthesis of Zinc Oxide Nanoparticles Using the Four Precursors

The green synthesis of zinc oxide nanoparticles was performed using each of the four zinc precursors in combination with the three different plant extracts. The synthesis protocol followed a systematic procedure that was consistently applied across all precursor-extract combinations to ensure reproducibility and comparability of the resulting nanoparticles.

For the zinc acetate dihydrate precursor, 20 ml of guava leaf extract (Extract A) was added to 200 ml of zinc acetate dihydrate solution at a concentration of 30 g/dm³. The pH of the resulting mixture was adjusted to 11.6 through the dropwise addition of a 1M sodium hydroxide (NaOH) solution, which facilitated the precipitation of zinc hydroxide and subsequent formation of zinc oxide nanoparticles. The mixture was then placed on an electric heater equipped with a magnetic stirrer and maintained at 60°C for two hours with continuous stirring to ensure complete reaction and uniform nanoparticle formation. Upon completion of the reaction, the mixture was allowed to cool to room temperature (25°C) and then left to settle for three hours, allowing the nanoparticles to sediment at the bottom of the container. The clear supernatant solution above the settled nanoparticles was carefully decanted and discarded, while the remaining precipitate was filtered and washed three times with distilled water to remove any unreacted precursors and by-products. The washed precipitate was poured into a clean Petri dish and oven-dried at 100°C for one hour to remove residual moisture. The dried material was subsequently ground into a fine powder using a pestle and mortar to obtain a homogeneous powder, and then calcined at 550°C for two hours in a muffle furnace to remove any organic impurities and to promote the crystallization of zinc oxide nanoparticles. The annealed powder was finally stored in an airtight glass vial for further characterization and subsequent experiments. This entire procedure was systematically repeated for the 40 g and 50 g concentrations of zinc acetate dihydrate precursor, as well as for the jatropha leaf extract (Extract B) and cassia leaf extract (Extract C), resulting in the synthesis of nine different nanoparticles from the zinc acetate precursor, which were labelled as A1, B1, C1, AA1, BB1, CC1, AAA1, BBB1, and CCC1, where the letters indicate the plant extract used and the numerical designation indicates the precursor concentration and specific synthesis conditions.

Figure 2 illustrates the various stages involved in the nanoparticle synthesis process. Figure 2a shows the combination of plant extract and zinc precursor solution before the reaction. Figure 2b depicts the reaction mixture

being heated with constant stirring on a magnetic stirrer-hot plate for two hours. Figure 2c shows the reaction mixture after cooling and allowing overnight settling, where the nanoparticles have precipitated at the bottom of the container. Figure 2d presents the final stage where the residue after filtration was dried in an oven and subsequently calcined at 550°C for two hours to obtain the pure zinc oxide nanoparticles.

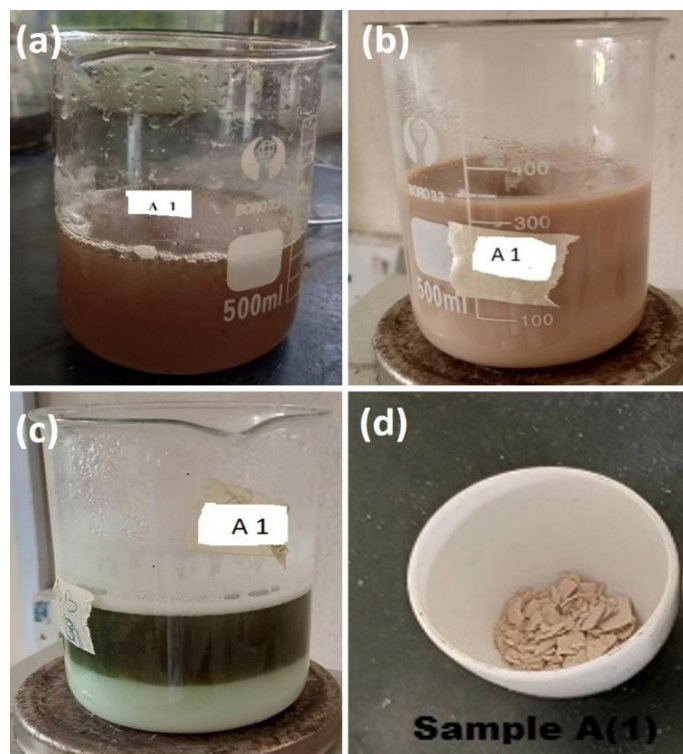


Fig. 2. Stages involved in the green synthesis of zinc oxide nanoparticles. (a) Initial mixture of plant extract and zinc precursor solution before reaction; (b) Reaction mixture being heated at 60°C with constant magnetic stirring for two hours; (c) Reaction mixture after cooling and overnight settling, showing precipitated nanoparticles at the bottom; (d) Final product after filtration, oven-drying at 100°C, and calcination at 550°C for two hours, yielding pure zinc oxide nanopowder.

The identical synthesis protocol was repeated for the zinc nitrate hexahydrate precursor, which similarly produced nine zinc oxide nanoparticles labelled as A2, B2, C2, AA2, BB2, CC2, AAA2, BBB2, and CCC2 respectively. Likewise, the zinc chloride precursor yielded nine nanoparticles labelled as A3, B3, C3, AA3, BB3, CC3, AAA3, BBB3, and CCC3. Finally, the zinc sulphate heptahydrate precursor produced nine nanoparticles labelled as A4, B4, C4, AA4, BB4, CC4, AAA4, BBB4, and CCC4. In total, thirty-six different zinc oxide nanoparticle samples were synthesized using the combination of four zinc precursors and three plant extracts, with three different precursor concentrations for each combination.

2.5. Seed Germination and Growth Measurements

Untreated and unprocessed maize (*Zea mays* L.) seeds were procured from a local market in Hawul Local Government Area of Borno State, Nigeria, ensuring that all seeds were obtained from the same stalk to minimise genetic variability and ensure uniform seed quality for the germination experiments. The seed germination assay was performed on tissue paper using light-transparent trays in the absence of light to simulate optimal germination conditions and to eliminate the influence of light on the germination process.

The water absorption rate of the seeds was initially tested by selecting four seeds, each with a mass of 0.37 g, which were washed thoroughly with distilled water and then soaked in a solution containing 50 mg/L of the different zinc oxide nanoparticles with sizes labelled A through L. After three hours of soaking, the seeds were removed from the solution and carefully dried with tissue paper to remove any surface moisture, and the new masses of the seeds were recorded. A control group was also maintained in which seeds were soaked in distilled water without any nanoparticles to establish a baseline for comparison. For the germination experiments, fifteen seeds, each with a mass of 0.35 g, were placed in a transparent container, and the nanoparticle solution (50 mg/L) was used to wet the tissue paper that served as a germination medium. The rate of seed germination was recorded after an interval of 72 hours to determine the germination percentage and to assess the early germination response to the different nanoparticle treatments. Another set of three seeds per transparent container was established for the evaluation of growth parameters. The seeds were allowed to grow for 25 days, during which they were regularly moistened with the respective nanoparticle solutions to maintain optimal growth conditions. At the end of the 25-day growth period, the germinated seedlings were harvested, and various growth parameters were recorded, including relative root elongation (RE), relative seed germination (SG), root length (RL), shoot length (SL), fresh weight (FW) of roots and shoots, and dry weight (DW) of roots and shoots. The root elongation, seed germination, and germination index were determined using the equations described by Singh et al. [18]. The relative root elongation was calculated as the ratio of the mean root length of the test sample to the mean root length of the control, expressed as a percentage.

$$\text{Root elongation(RE)} = \frac{\text{mean root length with test sample}}{\text{mean root length with control}} \times 100 \quad (1)$$

The seed germination percentage was calculated as the ratio of the number of germinated seeds in the test sample to the number of germinated seeds in the control, expressed as a percentage.

$$\text{Seed Germination (SG)} = \frac{\text{seed germination with test saple}}{\text{seed germination with control}} \times 100 \quad (2)$$

The seed germination index was calculated as the product of the seed germination percentage and the relative root elongation, providing a comprehensive measure of germination performance.

$$\text{seed germination index (SGI)} = \frac{SG}{RE} \times 100 \quad (3)$$

Figure 3 presents the various stages of the plant germination and growth experiment, showing the progression from seed placement in the germination trays to the development of seedlings over the 25-day growth period. The visual documentation captures the morphological differences between the control and nanoparticle-treated plants, which were subsequently analyzed quantitatively using the measured growth parameters. All experiments were conducted under ambient conditions to simulate realistic agricultural environments, and each treatment was performed

in triplicate to ensure statistical reliability of the results.

The experimental design was carefully structured to evaluate the size-dependent effects of green-synthesized zinc oxide nanoparticles on maize germination and early growth, with particular emphasis on identifying nanoparticle size ranges that promote growth while minimizing potential phytotoxicity. The data obtained from these experiments were subjected to statistical analysis to determine the significance of the observed differences between treatments and the control group.

3. RESULTS AND DISCUSSION

The germination and early growth of plants depend on several interrelated factors, including seed quality, seed mass, and the capacity for water absorption, all of which can be significantly influenced by the presence of nanoparticles in the germination medium.



Fig. 3. Stages of maize seed germination and seedling growth experiment. The images show the progression from seed placement in transparent containers with tissue paper germination medium to seedling development over the 25-day growth period. The control group (distilled water) and nanoparticle-treated groups were maintained under identical ambient conditions to evaluate the effects of zinc oxide nanoparticles on germination and early growth parameters.

Table 1. Effect of ZnO nanoparticle size on seed water absorption in maize

S/N	Nanoparticle Sample	Size (nm)	Mave (g)	RWA (%)
1	CC (1)	15.4	0.447	100.40
2	BB (1)	16.62	0.468	105.20
3	BBB (3)	17.54	0.443	99.60
4	CCC (4)	18.31	0.458	102.90
5	B (2)	18.64	0.463	104.00
6	AA (3)	19.08	0.438	98.40
7	CCC (3)	19.27	0.433	97.30
8	A (3)	20.55	0.438	98.40
9	BBB (1)	21.22	0.428	96.20
10	A (4)	22.42	0.443	99.60
11	BBB (2)	23.47	0.453	101.80
12	CCC (1)	24.22	0.448	100.70
13	Control	-	0.445	-

In the present investigation, we systematically evaluated the relative water absorption of maize seeds in the presence of zinc oxide nanoparticles of varying sizes and compared these values with those obtained from control seeds that were not exposed to any nanoparticles. The comprehensive results of these experiments, along with detailed discussions of the observed phenomena, are presented in the following sections

3.1. Effect of Zinc Oxide Nanoparticles on Seed Water Absorption

Table 1 presents the effect of different sizes of zinc oxide nanoparticles on the water absorption capacity of maize seeds. For this experiment, four seeds each of mass 0.37 g were thoroughly washed with distilled water and subsequently soaked in solutions containing 50 mg/L of each nanoparticle formulation for a period of four hours. The average mass of the soaked seeds and the corresponding relative water absorption percentages were calculated and compared against the control group that was soaked in distilled water without any nanoparticles.

The results presented in Table 1 demonstrate that, with the exception of nanoparticles with sizes of 20.55 nm and 19.08 nm, all other nanoparticle formulations exhibited different water absorption rates compared to the control group. The nanoparticle designated as BB (1), with a size of 16.62 nm, showed the highest relative water absorption percentage of 105.2%, followed by particle B (2) with a size of 18.64 nm, which achieved a relative water absorption of 104.0%. The nanoparticle CCC (4), with a size of 18.31 nm, also demonstrated a notable improvement in water absorption, reaching 102.9%. These findings suggest that nanoparticles within the size range of approximately 16 to 19 nm can enhance the water uptake capacity of seeds, potentially by creating microscopic channels or by modifying the surface properties of the seed coat to facilitate greater water penetration.

Conversely, several nanoparticle sizes resulted in reduced water absorption compared to the control group. The most pronounced inhibitory effect was observed with particle BBB (1) at 21.22 nm, which exhibited the lowest relative water absorption of 96.2%. This was followed by particle CCC (3) at 19.27 nm with 97.3%, and particles AA (3) at 19.08 nm and A (3) at 20.55 nm, both showing 98.4% relative water absorption. The variation in water absorption across different nanoparticle sizes indicates that nanoparticle size is a critical factor that can either enhance or inhibit the germination process of maize plants. This size-dependent behavior may be attributed to the ability of smaller nanoparticles to penetrate the seed coat more effectively and facilitate water uptake, while larger nanoparticles may physically block the pores or interact unfavorably with the seed surface.

3.2. Effect of Zinc Oxide Nanoparticles on Seed Germination

Figure 4 presents a bar chart illustrating the number of seeds that germinated after 72 hours of monitoring in the presence of different nanoparticle sizes. The visual representation clearly demonstrates that neither the smallest nor the largest nanoparticle sizes enhanced germination when compared to the control group without nanoparticles. This observation suggests that there exists an optimal size range for promoting germination, and deviations from this range, whether towards smaller or larger sizes, can result in reduced germination performance.

The detailed germination parameters, including relative root elongation (RRE), relative germination percentage (RG), and germination vigor index (GVI), are presented in Table 2. Fifteen seeds were placed in each transparent container with tissue paper moistened with 50 mg/L nanoparticle solution, and the solution was replenished every 24 hours to maintain adequate moisture. After 72 hours, the number of germinated

seeds was recorded, and the seeds were subsequently reduced to three per container for continued growth over 25 days.

The relative germination percentages presented in Table 2 reveal that only the smallest nanoparticle size (15.4 nm) exhibited a relatively low germination rate of 35.71%, while all other sizes demonstrated germination percentages exceeding 50%. Notably, three nanoparticle formulations achieved 100% relative germination, namely BBB (3) at 17.54 nm, AA (3) at 19.08 nm, and A (3) at 20.55 nm. The remaining nanoparticle sizes showed relative germination percentages ranging from 78.57% to 92.86%, indicating that most nanoparticle treatments maintained high germination rates comparable to or exceeding the control group.

The germination vigor index, which provides a

comprehensive measure of germination performance by combining both germination percentage and root elongation, showed an interesting size-dependent trend. The lowest GVI values were observed at the extremes of the size range, with the 15.4 nm particles showing a GVI of 17.86% and the 30.94% recorded for 16.62 nm particles. Similarly, the larger nanoparticles at 23.47 nm and 24.22 nm exhibited GVI values of 44.20% and 48.75%, respectively. The highest GVI values were observed for intermediate nanoparticle sizes, with 19.27 nm particles achieving 70.18% and 21.22 nm particles reaching 78.38%. This pattern strongly suggests that nanoparticle size optimization is essential for achieving maximum germination vigor.

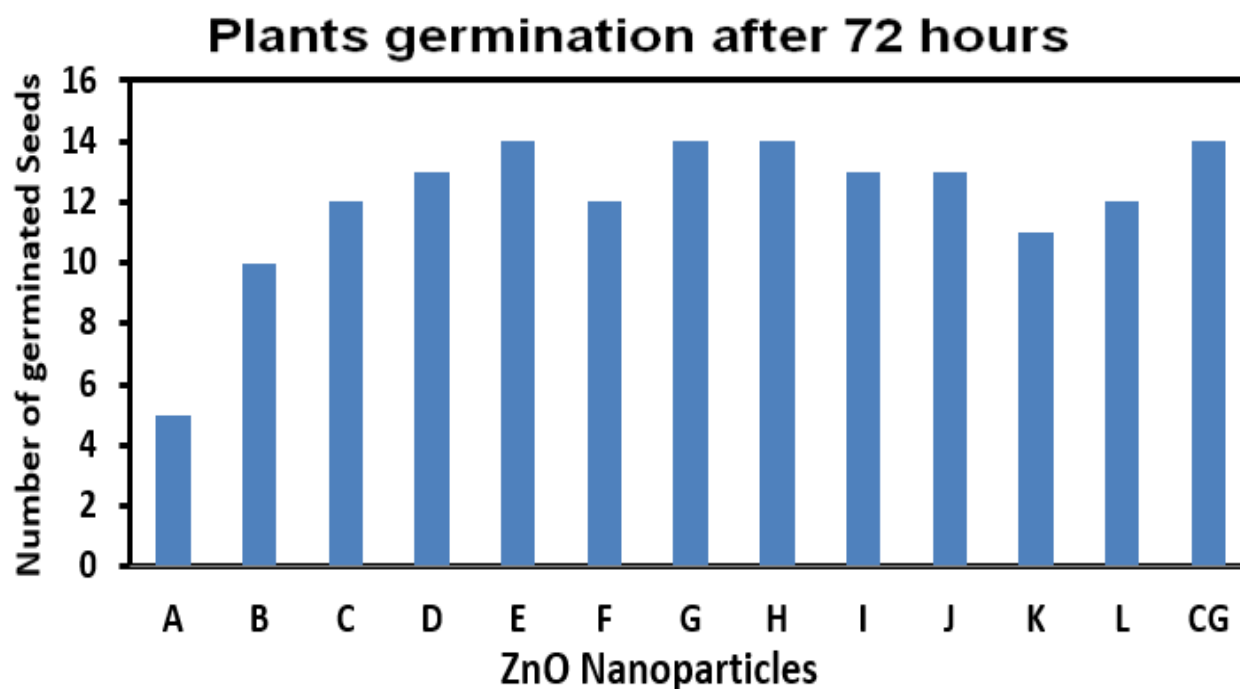


Fig. 4. Bar chart showing the number of germinated maize seeds after 72 hours of exposure to different zinc oxide nanoparticle sizes compared to the control group.

Table 2. Effect of ZnO nanoparticle size on maize seed germination parameters

S/N	Nanoparticle Sample	Size (nm)	RRE (%)	RG (%)	GVI
1	CC (1)	15.4	50.00	35.71	17.86
2	BB (1)	16.62	39.38	78.57	30.94
3	BBB (3)	17.54	58.13	100.00	58.13
4	CCC (4)	18.31	67.50	85.71	57.86
5	B (2)	18.64	46.88	92.86	43.53
6	AA (3)	19.08	69.38	100.00	69.38
7	CCC (3)	19.27	81.88	85.71	70.18
8	A (3)	20.55	55.63	100.00	55.63
9	BBB (1)	21.22	81.38	92.86	78.38
10	A (4)	22.42	72.50	92.86	67.32
11	BBB (2)	23.47	56.23	78.57	44.20
12	CCC (1)	24.22	52.50	92.86	48.75

13 Control

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These findings are partially consistent with the work of Sencan et al. [29], who reported that zinc oxide nanoparticles improve seed germination, seedling emergence, and growth by playing an important role in the biosynthesis of endogenous hormones such as auxin and gibberellin. Additionally, zinc oxide nanoparticles have been shown to act as essential components in carbohydrate and protein metabolism, effectively breaking seed dormancy to initiate the germination process, as previously reported by Azimi et al. [30].

3.3. Effect of Zinc Oxide Nanoparticles on Root and Shoot Elongation

The effects of different nanoparticle sizes on root and shoot elongation are presented in Table 3. The results indicate that all root lengths were shorter compared to the control group, suggesting that the presence of nanoparticles, regardless of size, exerted some degree of inhibitory effect on root development. However, it is noteworthy that several nanoparticle treatments resulted in shoot lengths that exceeded those of the control group, indicating that certain nanoparticle sizes can selectively promote shoot growth even when root growth is inhibited.

The germination vigor index values presented in Table 3 show that particle A (presumably the smallest size) exhibited the lowest vigor index of 11.07, while the two intermediate particles G and H demonstrated the highest vigor indices of 46.10 and 46.54, respectively. This observation reinforces the conclusion that optimum nanoparticle sizes are required for achieving a reasonably high vigor index. In other words, the optimization of zinc oxide nanoparticle sizes is necessary for obtaining higher germination vigor index values, which ultimately translate to better seedling establishment and growth potential.

3.4. Effect of Zinc Oxide Nanoparticles on Seedling Fresh and Dry Weight

The comparative analysis of relative fresh and dry weights of maize seedlings grown in the presence of different nanoparticle sizes is presented in Table 4. The results demonstrate that certain nanoparticle sizes significantly improved both fresh and dry weights of the seedlings compared to the control group that was grown without any nanoparticles.

In terms of fresh weight, several nanoparticle sizes demonstrated significant positive effects on seedling growth compared to the control group. The nanoparticle with a size of 23.47 nm showed the most pronounced effect, achieving a relative fresh weight of 132.4%, followed by particles of 18.31 nm (117.6%) and 16.62 nm (113.9%). These results indicate that nanoparticles within specific size ranges can substantially enhance the fresh biomass accumulation of maize seedlings, potentially through improved nutrient uptake, enhanced photosynthetic activity, or stimulation of metabolic processes.

Conversely, several nanoparticle sizes exhibited negative effects on fresh weight accumulation. The most detrimental effect was observed with the 20.55 nm nanoparticles, which resulted in a relative fresh weight of only 47.2%, followed by 15.40 nm (52.8%) and 18.64 nm (60.2%). These findings suggest that both very small and certain intermediate nanoparticle sizes can inhibit fresh biomass production, possibly through phytotoxic effects or disruption of normal physiological processes.

Regarding dry weight, the results after air-drying the seedlings under ambient conditions for five days revealed that only three out of the twelve nanoparticle sizes performed better than the control. Nanoparticles with sizes of 18.31 nm and 19.27 nm both achieved relative dry weights of 112.5%, while the 24.22 nm particles showed a positive effect of 106.3% relative dry weight.

Table 3. Effect of ZnO nanoparticle size on maize root and shoot elongation

S/N	Nanoparticle Sample	Size (nm)	RL (cm)	SL (cm)	SDL (cm)	RG (%)	GVI
1	CC (1)	15.4	8.0	23.0	31.0	35.71	17.86
2	BB (1)	16.62	6.3	36.5	42.8	78.57	30.94
3	BBB (3)	17.54	9.3	31.5	40.8	100.00	58.13
4	CCC (4)	18.31	10.8	36.1	46.9	85.71	57.86
5	B (2)	18.64	7.5	31.5	39.0	92.86	43.53
6	AA (3)	19.08	11.1	35.0	46.1	100.00	69.38
7	CCC (3)	19.27	13.1	41.2	54.3	85.71	70.18
8	A (3)	20.55	8.9	33.0	41.9	100.00	55.63
9	BBB (1)	21.22	13.5	33.2	46.7	92.86	78.38
10	A (4)	22.42	11.6	26.0	37.6	92.86	67.32
11	BBB (2)	23.47	9.0	35.0	44.0	78.57	44.20
12	CCC (1)	24.22	8.4	28.6	37.0	92.86	48.75

13	Control	-	16.0	35.0	51.0	-	-
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Table 4. Effect of ZnO nanoparticle size on maize seedling fresh and dry weight

S/N	Nanoparticle Sample	Size (nm)	SFW (g)	SDW (g)	RSFW (%)	RSDW (%)
1	CC (1)	15.4	0.57	0.16	52.8	100.0
2	BB (1)	16.62	1.23	0.16	113.9	100.0
3	BBB (3)	17.54	0.77	0.15	71.3	93.8
4	CCC (4)	18.31	1.27	0.18	117.6	112.5
5	B (2)	18.64	0.65	0.16	60.2	100.0
6	AA (3)	19.08	1.03	0.15	95.4	93.8
7	CCC (3)	19.27	0.99	0.18	47.2	112.5
8	A (3)	20.55	0.51	0.15	62.0	93.8
9	BBB (1)	21.22	0.67	0.16	62.0	100.0
10	A (4)	22.42	0.78	0.16	72.2	100.0
11	BBB (2)	23.47	1.43	0.15	132.4	93.8
12	CCC (1)	24.22	0.91	0.17	84.3	106.3
13	Control	-	1.08	0.16	-	-

The remaining nanoparticle sizes either showed no significant effect (100%) or exhibited inhibitory effects (93.8%) on dry weight accumulation. It is particularly noteworthy that nanoparticle size 18.31 nm was the only formulation that positively enhanced both relative fresh weight (117.6%) and relative dry weight (112.5%), indicating that this size range may represent an optimal formulation for promoting overall seedling biomass accumulation.

The results of the present study are in agreement with several previous investigations that have examined the effects of nanoparticles on plant growth and development. Sencan et al. [29] reported that silver nanoparticles increased germination percentage and root relative elongation, while iron oxide nanoparticles improved seed water intake and the number of root pores at optimum concentrations. Maity et al. [31] investigated the effects of zinc oxide, titanium dioxide, copper oxide, and silver nanoparticles on sorghum and cowpea, reporting that all nanoparticles improved germination in both plants, with titanium dioxide showing exceptional performance at high doses. Other researchers who have studied the effects of zinc oxide nanoparticles on plant germination and growth include Singh et al. [18] on rice, Dogaroglu et al. [17] on different wheat species, Savassa et al. [15] on beans and maize, Fathi et al. [32] on wheat, Solanki and Laura [33] on wheat, and Rosa et al. [14] on *Cucumis sativus*. All these studies consistently reported that the concentration of zinc oxide nanoparticles needs to be optimized for positive outcomes, which aligns well with our finding that nanoparticle size optimization is crucial for achieving maximum germination and growth benefits in maize plants.

The size-dependent effects observed in this study can be attributed to several mechanisms. Smaller nanoparticles may

penetrate the seed coat more easily and interact directly with embryonic tissues, potentially causing oxidative stress or physical damage when present at certain concentrations. Conversely, larger nanoparticles may be unable to penetrate the seed coat effectively, limiting their beneficial effects or causing physical blockage of seed pores. The intermediate nanoparticle sizes, particularly those in the range of 18 to 19 nm, appear to provide an optimal balance between penetration capability and beneficial interaction with seed tissues, resulting in enhanced water uptake, improved germination, and increased biomass accumulation. These findings have significant implications for the development of zinc oxide nanoparticle-based agricultural formulations, suggesting that careful size control during synthesis is essential for maximizing the beneficial effects while minimizing potential phytotoxicity.

4. CONCLUSION

This study systematically investigated the size-dependent effects of green-synthesized zinc oxide nanoparticles on maize seed germination and early seedling growth using nanoparticles produced from four different zinc precursors and three plant leaf extracts. The findings clearly demonstrate that nanoparticle size plays a critical role in determining the biological responses of maize plants, with specific size ranges exhibiting either stimulatory or inhibitory effects on various germination and growth parameters. The water absorption capacity of seeds was significantly enhanced by nanoparticles in the size range of 16 to 19 nm, while both smaller and larger particles showed reduced or inhibitory effects. Germination vigor index values

were highest for intermediate nanoparticle sizes, particularly at 19.27 nm and 21.22 nm, indicating that optimization of particle size is essential for achieving maximum germination performance. The analysis of seedling biomass revealed that nanoparticles of 18.31 nm uniquely enhanced both fresh and dry weights, achieving 117.6% and 112.5% respectively compared to the control, suggesting this size range represents an optimal formulation for promoting overall seedling development. These results underscore the importance of precise size control during nanoparticle synthesis for agricultural applications. The incorporation of appropriately sized zinc oxide nanoparticles into fertilizer formulations at the production stage could provide a sustainable approach to addressing zinc deficiency in crops while minimizing potential phytotoxic effects. Future research should focus on field-scale evaluations of optimized nanoparticle formulations and their long-term effects on crop productivity and soil health.

DECLARATIONS

Ethical Approval

We affirm that this manuscript is an original work, has not been previously published, and is not currently under consideration for publication in any other journal or conference proceedings. All authors have reviewed and approved the manuscript, and the order of authorship has been mutually agreed upon.

Availability of data and material

All of the data obtained or analyzed during this study is included in the report that was submitted.

Conflicts of Interest

The authors declare that they have no financial or personal interests that could have influenced the research and findings presented in this paper. The authors alone are responsible for the content and writing of this article.

Authors' contributions

All authors contributed equally to this work.

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