



## Impact of zinc oxide nanoparticles on the bio-productivity, seed quality and biochemical properties of *Arachis hypogaea* (L.)

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### ABSTRACT

Zinc oxide nanoparticles (ZnO-NPs) offer improved nutrient delivery, although their optimum concentrations and impact on groundnut crops remain unclear. This study investigated the impact of zinc oxide nanoparticle (ZnO-NPs) concentrations on the growth performance, yield, and biochemical properties of *Arachis hypogaea* L. (groundnut). The concentrations of ZnO-NP investigated were 0.00 (control), 0.25, 0.50, 0.75, 1.00 and 1.25 g/L. The ZnO-NPs synthesized by the direct precipitation method had FTIR that peaks at 455.22 cm<sup>-1</sup> and 1384.94 cm<sup>-1</sup>, corresponding to Zn-O stretching and deformation vibration. The rod-flower shape of ZnO-NP had a particle size of < 0.99 with good purity. The synthesized ZnO-NPs significantly increased (p<0.05) percentage seed germination that ranged from 66.67% at 0.25 g/l to 97.67 at 1.25 g/L when compared with the control (56.65%). All ZnO-NP treatments enhanced morphological growth characters and yield attributes when compared with the control. However, chlorophyll content under ZnO-NPs were at par with the control. Nitrate reductase and carbonic anhydrase enzymes increased with the increase in the concentrations of nanoparticles. Seed quality in terms of mineral and proximate compositions showed inconsistent trends. However, Zn and other mineral elements such as K, Mg, P and Na were significantly higher under ZnO-NPs when compared with the control. Similarly, ZnO-NPs limit fat accumulation in seeds but showed marked influence on ash, fibre, protein, carbohydrate and energy values. It can be concluded that groundnut bio-productivity, biochemical attributes and seed quality were generally enhanced by zinc oxide nanoparticles with doses between 0.75 and 1.25 g/L showing the most profound effects.

### 1.0 Introduction

Nanotechnology is an emerging and fascinating field of science that enables advanced research across numerous disciplines, including agriculture and biotechnology. It also offers promising

applications in energy, medicine, electronics and life sciences [1, 2]. In agriculture, nanotechnology presents expanding opportunities in areas such as reproductive science, conversion of agricultural wastes to energy and other valuable by-products through enzymatic nano-bioprocessing, water

purification, chemical sensing, disease prevention, and plant treatment using various types of functionalized nanoparticles [2].

Although conventional fertilizers are essential for plant growth and development, a significant portion of the applied nutrients often becomes unavailable to plants due to factors such as hydrolysis, leaching, photolytic degradation, and microbial decomposition. Therefore, there is a critical need to reduce nutrient loss during fertilizer application and improve crop yield by leveraging nanotechnology and nanomaterials [2, 3].

Nano-fertilizers or nano-encapsulated nutrients possess unique properties that can positively impact plant growth. These advanced formulations allow controlled on-demand release of nutrients, regulate plant growth, and enhance targeted delivery of agrochemicals [4].

Nanoparticles, have shown a great deal of promise in boosting plant metabolic processes [5]. The delivery of materials like DNA and other biomolecules is made possible by engineered ability of the nanoparticles to penetrate plant tissues, like leaves and cells [6]. This characteristic opens up new possibilities for plant biotechnology, especially for focused expression within particular plant cells and precise gene modification. By increasing the range of light wavelengths absorbed, such as ultraviolet, near-infrared, and green light, the addition of carbon nanotubes to chloroplasts has been demonstrated to increase photosynthetic efficiency [7]. Furthermore, it has been noted that carbon nanotubes encourage seed germination, growth and general development of plants [8]. Research on the beneficial effects of nanoparticles in plant systems is still scarce, despite these promising developments. To investigate their possible uses in the targeted administration of chemicals, proteins, and nucleic acids for crop genetic improvement, more research is needed [9]. The choice of ZnO nanoparticles for groundnut (*Arachis hypogaea*) is to improve plant metabolism, nutritional efficiency, and stress tolerance through specific molecular pathways in addition to enhancing zinc content [10, 11].

This study therefore investigated the effects of zinc nanoparticles on the growth, seed quality and some biochemical changes in *Arachis hypogaea*.

## 2.0 Materials and Methods

### 2.1 Description of the study area and soil characteristics

This study was conducted at the Botanical Garden of the University of Ilorin, Ilorin, Kwara State, Nigeria. The experimental site is located within the Guinea Savannah vegetation zone of Nigeria, between latitude 8.500°N and longitude 4.500°E. The area experiences distinct wet and dry

seasons, each lasting approximately six months, with an annual rainfall of 600 to 1200 mm [12], high temperatures of 21°C to 35°C and significant humidity (30% to 85%). The physicochemical properties of the soil at the experimental site included sandy loam soil with very low organic matter content (0.09%). The soil had a slightly acidic pH of 6.30 and moderately low available nitrogen of 0.12% [13]. The mineral element concentrations were low, including potassium (K) at 0.28cmol/kg, magnesium (Mg) at 0.58 cmol/kg, and sodium (Na) at 0.04 cmol/kg. In contrast, the soil exhibited a high calcium (Ca) content of 11.40 cmol/kg. Both the zinc (Zn) concentration (0.30 mg/kg) and the effective cation exchange capacity (ECEC) (4.58 cmol/kg) were low, indicating limited nutrient retention potential [13, 14].

### 2.2 Seed collection and treatment

*Arachis hypogaea* L. (Groundnut) pods of the cultivar SAMNUT 23 were obtained from the Institute of Agricultural Research, Zaria. The pods were surface-sterilized by immersion in a 0.5% sodium hypochlorite solution for 30 seconds, and thereafter, thoroughly rinsed three times with sterile distilled water to ensure complete removal of residual sodium hypochlorite [15]. Five treatment levels of zinc oxide nanoparticles (ZnO-NPs), 0.25, 0.50, 0.75, 1.00, and 1.25 g/L, were prepared by dissolving the respective quantities in one litre of distilled water. The seeds were soaked in each nanoparticle concentration for 3 hours. Control seeds were soaked in distilled water only.

### 2.3 Synthesis of zinc oxide nanoparticles

Zinc oxide nanoparticles (ZnO-NPs) were synthesized using the direct precipitation method with zinc nitrate ( $Zn(NO_3)_2 \cdot 6H_2O$ ) and sodium hydroxide (NaOH) obtained from Sigma Aldrich, as starting materials [16]. An aqueous solution of 0.45 M zinc nitrate and 2 M NaOH was prepared using deionized water. The NaOH solution was gradually added to the zinc nitrate solution at 60°C with constant stirring. This resulted in the formation of a white precipitate. The precipitate was filtered and repeatedly washed (3-5 times) with deionized water until the filtrate was free of nitrate ions. The final product was oven-dried at 60°C for 3 hours.

### 2.4 Characterization of synthesized nanoparticle

The size and morphology of the synthesized ZnO nanoparticles were characterized using a Scanning Electron Microscope (SEM) (S-4100 Hitachi). A small quantity (0.75 - 1.0 mg) of the nanoparticle powder was placed onto one side of a double-sided carbon conductive adhesive tape.

The tape was then fixed to an 8 mm aluminium stub. The sample surface was examined under the scanning electron microscope at magnifications of  $\times 1,000$  and  $\times 50,000$ , and the images were captured for analysis.

### 2.5 Experimental design and treatment

The germination study was set out in a Completely Randomized Design (CRD). A total of 24 Petri dishes were laid out in a 6-by-4 arrangement using 24 Petri dishes inside an ethanol-sterilized chamber. The Petri dishes were labelled according to the ZnO-NP treatment concentration (0 g, 0.25 g, 0.5 g, 0.75 g, and 1.25 g) with four replicates for each treatment. The pot experiment was similarly arranged in a CRD layout, with 6 pots per row and 6 per column. Each pot was filled with 5 kg of homogeneous loamy soil and had drainage holes to allow excess water to escape. Pre-treated groundnut seeds (previously soaked in ZnO-NP solutions; 0, 0.25, 0.50, 0.75, 1.00, and 1.25 g) were planted, with six replicates per treatments, making a total of 72 pots.

### 2.6 Germination studies

Twenty-five seeds from each zinc oxide nanoparticle (ZnO-NP) treatment concentration were placed on a doubled-layered Whatman filter paper inside 9 cm Petri dishes. The filter papers were moistened with 10 mL of sterile distilled water. Exactly 25 treated seeds were selected per replicate and placed in the labelled Petri dishes. Using a sterile syringe, 4 mL of distilled water was added to each dish. The Petri dishes were incubated under a 12-hour light/ dark photoperiod throughout the 10-day germination period. Germination was monitored and recorded based on clear radicle emergence after 10 days. Percentage germination was calculated using the following expression:

$$\text{Percentage germination} = \frac{N}{A} \times 100$$

Where: N = Number of germinated seeds  
A = Total number of seeds tested

### 2.7 Planting and crop management

The seeds were sown at a rate of three seeds per pot after pre-treatment, and later thinned to two plants per pot after 2 weeks. Weeding was carried out at 2-week intervals for 14 weeks, while 500 mL of water was applied fortnightly to maintain normal adequate soil moisture for optimal plant growth.

### 2.8 Growth and yield parameters

Plant growth was monitored using standard morphological indices. Plant height, number of leaves, primary branches, stem girth and leaf areas

( $\text{m}^2$ ) were recorded at harvest. The number of days to 50% flowering was also recorded as the time taken for half of the plants in each treatment to produce at least one flower. At harvest, the yield parameters, including the number of pods per plant, seeds per pod, pod length, and pod circumference, were also measured and noted.

### 2.9 Determination of chlorophyll content

Chlorophyll content was determined following the method described by Chutia and Borah [17]. Leaves were collected from the plants and extracted in 5 mL of 80% acetone (Sigma Aldrich) for 72 hours in the dark. The supernatant was analyzed using a spectrophotometer by placing extract in glass cuvette and the absorbance read at 663nm for chlorophyll a, and 645 nm for chlorophyll b. The concentration of chlorophyll (in mg/g tissue) was calculated using the following equations:

$$\text{Chlorophyll a (mg/g tissue)} = 12.7 (A_{663}) - 2.69 (A_{645}) \times V/1000 \times W$$

$$\text{Chlorophyll b (mg/g tissue)} = 22.9 (A_{665}) - 4.68 (A_{645}) \times V/1000 \times W$$

$$\text{Total chlorophyll (mg/g tissue)} = 20.2 (A_{645}) + 8.02 (A_{663}) \times V/1000 \times W$$

Where:

A is the absorbance at the specified wavelength,  
V is the volume of extract (mL),  
and W is the weight of the tissue sample (g)

### 2.10 Assay of nitrate reductase activity

Nitrate reductase activity was determined based on nitrite production during the enzymatic reaction according to Jaworski [18]. The plant tissue was cleaned, washed thoroughly chopped into small pieces and then transferred into a pre-chilled mortar and ground into fine powder. A 20  $\mu\text{L}$  homogenate was mixed with 20  $\mu\text{L}$  of 20 mM sodium nitrate and incubated at 37°C for 20 minutes. The reaction was stopped by adding 20  $\mu\text{L}$  of 1% sulfanilamide prepared in 10% trichloroacetic acid (TCA), and the mixture was cooled on ice for 2 minutes. The solution was centrifuged at 6,000g for 1 minute (optional if no precipitates formed). Then, 20  $\mu\text{L}$  of the supernatant was transferred into a clean microplate, followed by the addition of 20  $\mu\text{L}$  of 0.1% N-(1-naphthyl) ethylenediamine for colour development. A calibration curve was prepared using 40 and 80  $\mu\text{L}$  of 100  $\mu\text{M}$  standards, plotting absorbance against concentration. The concentration of nitrite formed ( $\mu\text{mol}$ ) was determined from the calibration curve and

multiplied by the dilution factor. Nitrate reductase activity was calculated as:

Nitrate reductase activity ( $\mu\text{mol}\cdot\text{g}^{-1}\cdot\text{min}^{-1}$ ) =

$$\frac{\text{NC} \times \text{VS}}{\text{DWS} \times \text{IT}}$$

Where:

NC = Nitrite Concentration ( $\mu\text{mol}/\text{ml}$ )

VS = Volume of Solution (ml)

DWS = Dry Weight of Sample (g)

IT = Incubation Time (min)

### 2.11 Assay of carbonic anhydrase activity

Carbonic anhydrase (CA) activity was determined using the electrometric method described by Wilbur and Anderson [19]. A 0.5 g sample of fully expanded, light-exposed groundnut leaves were homogenized in 1 mL of ice-cold Tris-HCl extraction buffer (20 mM, pH 8.3, containing 0.1 mM EDTA and 1% (w/v) polyvinylpyrrolidone (PVP) and centrifuged at 4°C for 15 minutes at 5,000 rpm ( $\approx 2,800 \times g$ ). The supernatant was used for the assay. A pH electrode was inserted in a solution of 3 mL of 20 mM Tris buffer (pH 8.3 at 25°C) along with 50  $\mu\text{L}$  of ice-cold purified water, stirred at approximately 300 rpm. When the pH reached above 8.5, ice-cold  $\text{CO}_2$ -saturated water was added. The time taken for the pH to drop from 8.3 to 6.3 was recorded as the blank (T-Blank). The procedure was repeated with the enzyme extract added after  $\text{CO}_2$  addition, and the time for the pH change was recorded as the test (T-Test). Carbonic anhydrase activity was calculated using the following expression:

$$\text{Enzyme Unit/mL} = \frac{t_0 - t_c}{t_c}$$

Where:

$t_0$  is the time (sec) for the pH to drop in the absence of the enzyme, and

$t_c$  is the time (sec) for the pH to drop in the presence of the enzyme.

### 2.12 Mineral analysis

The mineral content was determined using Atomic Absorption Spectroscopy (AAS), following the standard procedure outlined by the Association of Official Analytical Chemists [20]. The dried sample (0.5 g) was transferred to the digestion glass tube. Ten millilitres (10 ml) of  $\text{HNO}_3$  was added to the sample. The resulting mixture was kept overnight at room temperature. Thereafter, 4.0 ml perchloric acid ( $\text{HClO}_4$ ) was added to the Dispersive X-ray (EDX) spectrum ZnO nanoparticle revealed elemental composition of

mixture and kept in a fume cupboard. The temperature was increased gradually, starting from 50°C and increasing up to 300°C in the digestion unit. The digestion was achieved in about 70-80 minutes, as indicated by the appearance of the white fumes. The mixture was allowed to cool and transferred into 100 ml volumetric flasks, and the volume of the contents was made up to 100 ml with distilled water. The resulting solution was filtered. The filtrate was then used for the mineral determination. Nitrogen (N) was determined using the micro Kjeldhal apparatus. Sodium (Na), iron (Fe), magnesium (Mg), and zinc (Zn) were determined with the use of an Atomic Absorption Spectrophotometer (AAS) (Shimadzu, Japan AA-6200). Other mineral elements, such as potassium (K) were determined by Flame Photometer (Bibby Scientific Limited, UK: Model No PFP7) following the method described by Allen *et al.* [21].

### 2.13 Proximate analysis

Percentage moisture, ash, fibre, crude protein, crude fat, and carbohydrate as a measure of proximate composition were determined according to the method of the Association of Official Analytical Chemists [20]. However, the percentage carbohydrate content was calculated by subtracting the sum of moisture, ash, protein, crude fat, and crude fibre percentage from hundred. The calorific value in each variety was estimated by multiplying the percentage protein and carbohydrate by 4 and that of fat by 9.

### 2.14 Data analysis

All collected data were analysed using one-way Analysis of Variance (ANOVA) to determine whether there were significant differences among treatment means. Treatment means were separated using Duncan's Multiple Range Test (DMRT) at  $p < 0.05$  significance level, using SPSS (Statistical Package for Social Sciences), version 21.

## 3.0 Results

The Fourier Transform Infrared (FT-IR) Spectroscopy of the synthesized ZnO nanoparticle is shown in Figure 1. Metal oxides typically exhibit absorption bands within the fingerprint region. In the spectrum, distinct peaks were observed at 455.22  $\text{cm}^{-1}$  and 1384.94  $\text{cm}^{-1}$ , corresponding to Zn-O stretching and deformation vibration, respectively. A broad peak at 3483.53  $\text{cm}^{-1}$  indicates the presence of O-H functional groups due to the absorbed water molecules.

The chemical composition of the synthesized nanoparticle is shown in Figure 2. The Energy-56.42% of zinc and 21.08% of oxygen, along with trace impurities.

The surface morphology and particle size of the synthesized ZnO nanoparticles are shown in the SEM micrographs (Figure 3). The analysis confirms a generally uniform morphology, with particle sizes ranging from 50-80 nm. These images showed well-defined structures with relatively consistent distribution, confirming the successful formation of nanoparticles within the nanoscale range.

Treatment of the seeds with ZnO nanoparticles at varying concentrations significantly ( $p < 0.05$ ) increased germination percentage compared to untreated control (Table 1). The highest germination rate (96.73%) was recorded at 1.25 g/L, followed by 1.00 g/L (90.00%), 0.75 g/L (83.33%), 0.50 g/L (76.36%), 0.50 g/L (76.36%), and 0.25 g/L (66.67%) as against the control with the lowest germination percentage.

*Arachis hypogaea* plants treated with ZnO-NP generally exhibited improved growth parameters compared to the control, although not all effects were statistically significant (Table 2). The *A. hypogaea* plants treated with 1.25 g/L ZnO-NP consistently showed the most favourable performance across measured parameters. The tallest plants (13.97 cm) were recorded at the 1.25 g/L, followed in decreasing order by other treated groups, as against the least height of 11.67 cm in the control group. A similar trend was observed in the number of leaves. The number of primary branches was also higher in plants treated with 1.25 g/L (6.67) and 1.0 g/L ZnO-NP (6.67), though these differences were not statistically significant ( $p < 0.05$ ). However, there were significant ( $p < 0.05$ ) increases in the stem girth and leaf area as the concentrations of ZnO-NP increased. The highest stem girth (0.91 mm) and largest leaf area (5541.87 cm<sup>2</sup>) were obtained at the 1.25 g/L. Other treatments also revealed values that were significantly ( $p < 0.05$ ) than the control, except for the 0.25 g/L, which was not significantly ( $p > 0.05$ ) different from the control in stem girth (0.25 mm). Furthermore, the chlorophyll contents were not significantly ( $p > 0.05$ ) different across treatments.

The application of ZnO-NP significantly influenced *A. hypogaea* seed yield-contributing parameters (Table 3). Treated seeds exhibited earlier flowering compared to the control, with earliness increasing as ZnO-NP concentration increased. All ZnO-NP treatments outperformed the control across the measured yield parameters. Higher values of yield traits were noted at 1.00 g/L and 1.25 g/L, which include number of seeds per plant (38.00 and 48.67 respectively), pod length (4.10 cm and 4.17 cm respectively), pod circumference (4.17 cm and 4.17 cm respectively), and the number of pods per plant (19.00 and 24.30 respectively) (Table 3). However, a significant difference ( $p < 0.05$ ) was observed only in the number of pods per plant, while other yield

parameters such as pod length, number of seed per plant, and days to 50% flowering showed no significant differences among treatments.

The ZnO-NP application also enhanced enzymatic activities. Nitrate reductase activity increased progressively and dose dependently from the group of *A. hypogaea* treated with the least concentration of ZnO-NP to the highest concentration (1.25 g/L). Similarly, carbonic anhydrase (CA) activity was significantly influenced ( $p < 0.05$ ) by ZnO-NP treatments, with higher values recorded at 1.0 g/L and 1.25 g/L treatment with ZnO-NP when compared to other treatments and the control.

The ZnO nanoparticle application significantly influenced the mineral composition of the harvested *A. hypogaea* seeds, with all determined elements showing statistically significant differences ( $p < 0.05$ ) across treatments (Table 4). The 0.75 g/L of ZnO-NP treatment produced the highest concentrations of calcium (Ca), sodium (Na), nitrogen (N), and phosphorus (P) when compared to other treatments and the control. Potassium (K) content was highest at 1.00 g/L (71.13 mg/L), followed by 0.75 g/L (68.70 mg/L), as against the control group of 53.80 mg/L of K. In contrast, the highest ZnO-NP concentration (1.25 g/L) resulted in the lowest manganese (Mn) content (0.15 mg/L). The treatment of ZnO-NP enhanced mineral accumulation in groundnut seeds, although the response varied depending on the element and concentration.

The proximate composition of groundnut (SAMNUT-23) seeds is presented in Table 5. The mean values indicated moisture content of 7.40%, fat content of 40.99%, ash level of 3.18%, protein content of 32.17%, fibre level of 3.18%, carbohydrate content of 12.66% and energy content value of 541.66 kcal. The ZnO-NP treatment influences the proximate composition of the seeds. The highest moisture content (8.37%) was recorded at 0.75 g/L, while the control had the highest fat content (43.28%). Protein content ranged from 29.90% to 34.25%, and carbohydrate content ranged from 12.02% to 13.42%, across treatments.

#### 4.0 Discussion

The FTIR spectrum of the synthesized ZnO nanoparticles showed characteristic peaks confirming ZnO formation. The band at 455.22 cm<sup>-1</sup> corresponds to Zn-O stretching vibrations, while the peak at 1384.94 cm<sup>-1</sup> indicates bending or deformation vibrations, likely from residual precursor species [22]. The broad peak at 3483.53 cm<sup>-1</sup> is attributed to O-H stretching from adsorbed water or surface hydroxyl groups, commonly observed in ZnO nanoparticles [23, 24]. These FTIR data confirmed successful synthesis and characteristic functional surface groups of ZnO

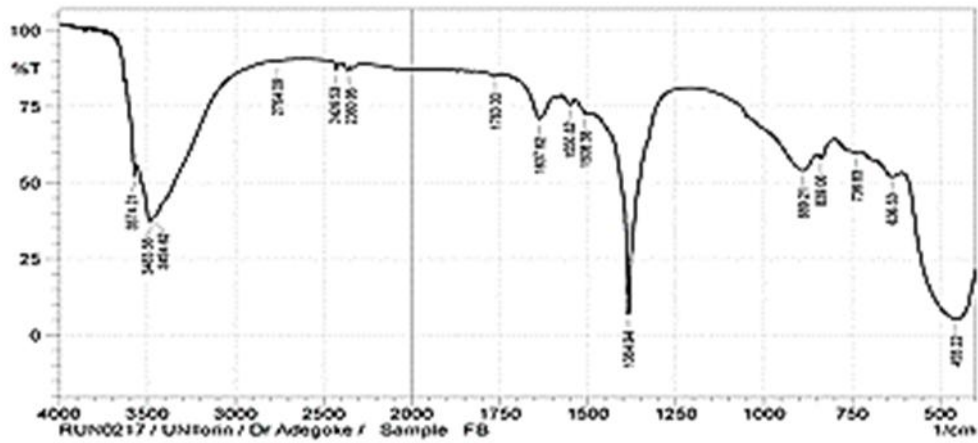


Figure 1: Fourier Transform Infrared (FTIR) transition spectrum of the synthesized ZnO-NP

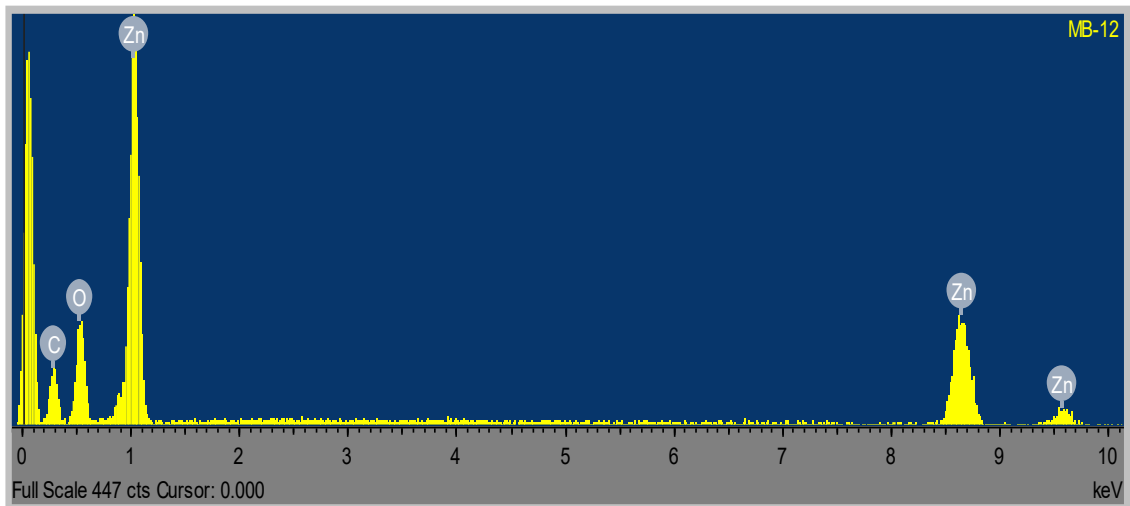


Figure 2: Energy diffraction X-Ray (EDX) of ZnO nanoparticle

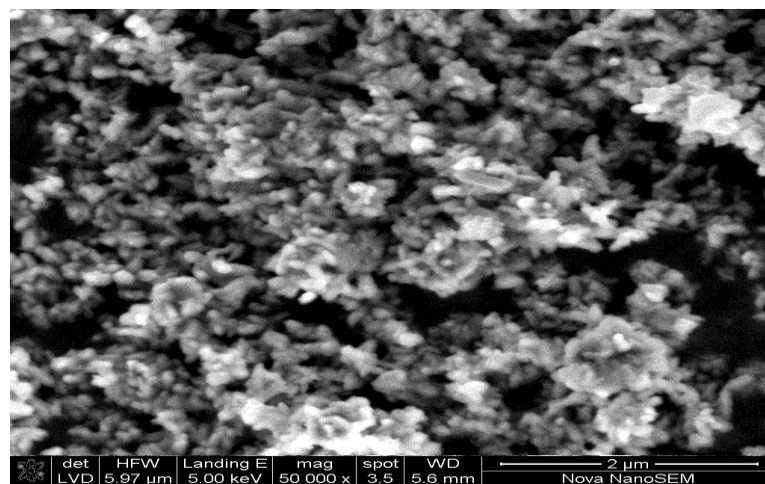


Figure 3: Scanning electron microscope (SEM) of the synthesized ZnO nanoparticle

**Table 1: Influence of zinc oxide nanoparticle on the percentage germination of *Arachis hypogae***

| ZnO nanoparticle (g/L) | Germination (%)     |
|------------------------|---------------------|
| 0 (control)            | 56.65 <sup>c</sup>  |
| 0.25                   | 66.67 <sup>d</sup>  |
| 0.50                   | 76.36 <sup>cd</sup> |
| 0.75                   | 83.33 <sup>bc</sup> |
| 1.00                   | 90.00 <sup>ab</sup> |
| 1.25                   | 96.73 <sup>a</sup>  |
| Mean                   | 78.33               |
| SE                     | 0.35                |
| P-value                | <0.001              |

Values with the same superscript (s) across columns are not significant ( $p < 0.05$ ); ZnO = Zinc oxide

**Table 2: Morphological growth attributes and chlorophyll contents of groundnut (SAMNUT 23) as influenced by different concentrations of zinc oxide nanoparticle at harvest**

| ZnO-NP (g/L) | Plant height (cm)       | Number of leaves           | Number of primary branches | Stem girth (mm)         | Leaf area (cm <sup>2</sup> )  | Total Chlorophyll (mg/g fresh weight) |
|--------------|-------------------------|----------------------------|----------------------------|-------------------------|-------------------------------|---------------------------------------|
| 0            | 11.67±3.26 <sup>c</sup> | 168.67±21.19 <sup>c</sup>  | 4.67±1.15 <sup>cd</sup>    | 0.25±0.14 <sup>c</sup>  | 2299.07±1474.31 <sup>d</sup>  | 0.12±0.00026 <sup>a</sup>             |
| 0.25         | 12.17±2.42 <sup>b</sup> | 173.33±82.03 <sup>d</sup>  | 5.00±1.73 <sup>c</sup>     | 0.25±0.14 <sup>c</sup>  | 2895.53±1597.44 <sup>cd</sup> | 0.12±0.00012 <sup>a</sup>             |
| 0.50         | 12.63±0.95 <sup>b</sup> | 195.33±67.42 <sup>cd</sup> | 5.00±1.73 <sup>c</sup>     | 0.28±0.09 <sup>bc</sup> | 2949.20±167.20 <sup>c</sup>   | 0.12±0.00024 <sup>a</sup>             |
| 0.75         | 13.67±1.01 <sup>a</sup> | 202.67±74.22 <sup>c</sup>  | 6.00±2.00 <sup>b</sup>     | 0.32±0.19 <sup>b</sup>  | 4542.47±1425.22 <sup>b</sup>  | 0.12±0.00041 <sup>a</sup>             |
| 1.00         | 13.67±1.01 <sup>a</sup> | 257.33±24.11 <sup>b</sup>  | 6.67±1.15 <sup>a</sup>     | 0.40±0.23 <sup>ab</sup> | 5150.47±669.42 <sup>ab</sup>  | 0.12±0.00030 <sup>a</sup>             |
| 1.25         | 13.97±1.33 <sup>a</sup> | 302.00±161.78 <sup>a</sup> | 6.67±0.58 <sup>a</sup>     | 0.91±0.52 <sup>a</sup>  | 5541.87±2656.62 <sup>a</sup>  | 0.12±0.00032 <sup>a</sup>             |
| P-value      | 0.47                    | 0.40                       | 0.41                       | 0.06                    | 0.11                          | 0.19                                  |

Values are Mean ± standard deviation: Values with the same superscript (s) across columns are not significantly different ( $p > 0.05$ ); ZnO-NP = Zinc oxide nanoparticle

**Table 3: Yield attributes, nitrate reductase and carbonic anhydrase as influenced by different concentrations of zinc oxide particles at harvest**

| ZnO-NP (g/L) | No. of pod per plant     | No of seeds per plant    | Circumference of pod (cm) | Length of pod (cm)     | Days to 50% flowering   | Nitrate reductase (μmol/min) | Carbonic anhydrase (μmol/min) |
|--------------|--------------------------|--------------------------|---------------------------|------------------------|-------------------------|------------------------------|-------------------------------|
| 0            | 13.33±2.03 <sup>c</sup>  | 28.00±3.46 <sup>a</sup>  | 4.13±0.12 <sup>a</sup>    | 3.87±0.07 <sup>a</sup> | 29.00±1.16 <sup>a</sup> | 0.69±0.16 <sup>a</sup>       | 1.07±0.16 <sup>d</sup>        |
| 0.25         | 14.00±1.73 <sup>bc</sup> | 30.00±1.16 <sup>a</sup>  | 4.13±0.17 <sup>a</sup>    | 3.87±0.13 <sup>a</sup> | 30.33±0.67 <sup>a</sup> | 1.01±0.16 <sup>a</sup>       | 2.00±0.48 <sup>c</sup>        |
| 0.50         | 15.00±0.58 <sup>bc</sup> | 32.67±2.91 <sup>a</sup>  | 4.13±0.18 <sup>a</sup>    | 4.00±0.12 <sup>a</sup> | 31.00±1.53 <sup>a</sup> | 1.21±0.24 <sup>a</sup>       | 4.27±0.11 <sup>b</sup>        |
| 0.75         | 16.33±1.45 <sup>bc</sup> | 38.00±15.11 <sup>a</sup> | 4.13±0.12 <sup>a</sup>    | 4.07±0.09 <sup>a</sup> | 33.67±3.67 <sup>a</sup> | 1.31±0.02 <sup>a</sup>       | 5.45±0.53 <sup>ab</sup>       |
| 1.00         | 19.00±1.00 <sup>b</sup>  | 38.00±3.46 <sup>a</sup>  | 4.17±0.03 <sup>a</sup>    | 4.10±0.06 <sup>a</sup> | 35.33±2.96 <sup>a</sup> | 1.41±0.39 <sup>a</sup>       | 5.89±1.30 <sup>a</sup>        |
| 1.25         | 24.30±2.03 <sup>a</sup>  | 48.67±4.06 <sup>a</sup>  | 4.17±0.09                 | 4.17±0.09 <sup>a</sup> | 37.67±4.33 <sup>a</sup> | 1.53±0.35 <sup>a</sup>       | 6.65±0.62 <sup>a</sup>        |
| p-value      | 0.00                     | 0.35                     | 1.00                      | 0.21                   | 0.28                    | 0.05                         | 0.05                          |

Values are Mean ± standard deviation: Values with the same superscript (s) across columns are not significant at  $p > 0.05$ ; ZnO-NP = Zinc oxide nanoparticle;

**Table 4: Mineral composition of the harvested groundnut seed (mg/L)**

| ZnO-NP<br>(g/L) | Concentration of minerals (mg/L) |                         |                        |                         |                        |                        |                         |                         |
|-----------------|----------------------------------|-------------------------|------------------------|-------------------------|------------------------|------------------------|-------------------------|-------------------------|
|                 | Ca                               | K                       | Na                     | Mg                      | Mn                     | Zn                     | N                       | P                       |
| 0               | 2.43±0.03 <sup>c</sup>           | 53.80±0.00 <sup>f</sup> | 2.20±0.00 <sup>f</sup> | 28.13±0.03 <sup>c</sup> | 0.24±0.00 <sup>a</sup> | 0.09±0.00 <sup>c</sup> | 51.81±0.02 <sup>b</sup> | 10.05±0.03 <sup>d</sup> |
| 0.25            | 1.93±0.03 <sup>d</sup>           | 66.57±0.39 <sup>c</sup> | 4.03±0.03 <sup>b</sup> | 43.13±0.03 <sup>b</sup> | 0.24±0.00 <sup>a</sup> | 0.12±0.01 <sup>d</sup> | 50.41±0.01 <sup>d</sup> | 12.32±0.01 <sup>b</sup> |
| 0.50            | 2.80±0.00 <sup>b</sup>           | 63.77±0.03 <sup>c</sup> | 3.07±0.03 <sup>c</sup> | 45.10±0.00 <sup>a</sup> | 0.24±0.00 <sup>a</sup> | 0.14±0.00 <sup>c</sup> | 47.92±0.04 <sup>c</sup> | 12.32±0.02 <sup>b</sup> |
| 0.75            | 3.10±0.00 <sup>a</sup>           | 68.70±0.10 <sup>b</sup> | 6.57±0.03 <sup>a</sup> | 42.17±0.67 <sup>c</sup> | 0.24±0.00 <sup>a</sup> | 0.16±0.00 <sup>b</sup> | 54.81±0.04 <sup>a</sup> | 13.11±0.01 <sup>a</sup> |
| 1.00            | 2.40±0.00 <sup>c</sup>           | 71.13±0.33 <sup>a</sup> | 3.63±0.03 <sup>d</sup> | 41.43±0.33 <sup>d</sup> | 0.24±0.00 <sup>a</sup> | 0.17±0.01 <sup>b</sup> | 51.61±0.03 <sup>b</sup> | 11.88±0.01 <sup>c</sup> |
| 1.25            | 1.83±0.03 <sup>c</sup>           | 65.83±0.03 <sup>d</sup> | 3.83±0.03 <sup>c</sup> | 26.50±0.40 <sup>f</sup> | 0.15±0.01 <sup>b</sup> | 0.19±0.00 <sup>a</sup> | 51.06±0.22 <sup>c</sup> | 11.91±0.01 <sup>c</sup> |
| p-value         | 0.01                             | 0.05                    | 0.04                   | 0.03                    | 0.01                   | 0.05                   | 0.03                    | 0.05                    |

Values are mean ± standard deviation; Values with the same superscript (s) across columns are not significantly different ( $p < 0.05$ ); ZnO-NP = Zinc oxide nanoparticle

**Table 5: Proximate composition of groundnut seeds as influenced by different concentrations of zinc oxide nanoparticles**

| ZnO-NP<br>(g/L) | Moisture<br>(%)        | Fat (%)                 | Ash (%)                | Protein (%)              | Fibre (%)              | Carbohydrate<br>(%)     | Energy Value<br>(Kcal)   |
|-----------------|------------------------|-------------------------|------------------------|--------------------------|------------------------|-------------------------|--------------------------|
| 0               | 6.04±0.04 <sup>d</sup> | 43.28±0.19 <sup>a</sup> | 2.85±0.03 <sup>d</sup> | 32.48±0.09 <sup>bc</sup> | 3.16±0.13 <sup>c</sup> | 12.20±0.07 <sup>d</sup> | 528.72±0.52 <sup>d</sup> |
| 0.25            | 7.66±0.07 <sup>b</sup> | 41.52±0.05 <sup>c</sup> | 2.90±0.06 <sup>d</sup> | 31.45±0.05 <sup>c</sup>  | 3.61±0.01 <sup>b</sup> | 12.86±0.06 <sup>b</sup> | 550.86±0.47 <sup>a</sup> |
| 0.50            | 7.19±0.11 <sup>c</sup> | 42.17±0.13 <sup>b</sup> | 3.65±0.03 <sup>a</sup> | 29.90±0.06 <sup>d</sup>  | 3.78±0.04 <sup>b</sup> | 13.42±0.07 <sup>a</sup> | 552.77±0.53 <sup>a</sup> |
| 0.75            | 8.37±0.07 <sup>a</sup> | 38.65±0.03 <sup>c</sup> | 3.37±0.06 <sup>b</sup> | 34.25±0.09 <sup>a</sup>  | 3.35±0.03 <sup>c</sup> | 12.02±0.06 <sup>d</sup> | 532.91±1.14 <sup>d</sup> |
| 1.00            | 7.81±0.06 <sup>b</sup> | 40.11±0.06 <sup>d</sup> | 3.28±0.03 <sup>b</sup> | 32.28±0.04 <sup>c</sup>  | 4.01±0.07 <sup>a</sup> | 12.51±0.16 <sup>c</sup> | 540.09±4.46 <sup>c</sup> |
| 1.25            | 7.36±0.04 <sup>c</sup> | 40.28±0.16 <sup>d</sup> | 3.12±0.04 <sup>c</sup> | 32.69±0.17 <sup>b</sup>  | 3.57±0.08 <sup>b</sup> | 12.98±0.12 <sup>b</sup> | 545.13±2.31 <sup>b</sup> |
| Mean            | 7.40                   | 40.99                   | 3.18                   | 32.17                    | 3.58                   | 12.66                   | 541.66                   |
| SD              | 0.18                   | 0.37                    | 0.06                   | 0.32                     | 0.07                   | 0.12                    | 2.31                     |

Values are Mean ± standard deviation; Values with the same superscript (s) across columns are not significant at  $P < 0.05$ ; ZnO-NP = Zinc oxide nanoparticle; SD = Standard Deviation

nanoparticles.

The EDX analysis confirmed the elemental composition, showing dominant zinc and oxygen peaks with minimal impurities, and no detectable sodium, suggesting effective removal of precursor residues during synthesis [23]. These results collectively verify the purity, nanoscale size, and characteristic morphology of the ZnO nanoparticles.

The SEM analysis revealed that the synthesized ZnO nanoparticles were generally uniform, with particle sizes ranging from 50-80 nm, consistent with nanoscale morphology reported in similar studies [22, 24]. The particles displayed well-defined surfaces, indicating successful nanoparticle formation.

In this study, application of ZnO nanoparticle had marked influence on of the emergence of the plant. The germination of seeds was further enhanced with increase in concentration of the ZnO nanoparticles. The current results are in agreement with the findings of Acharya *et al.* [25], where the authors reported that seed priming with silver

nanoparticles (Ag-NP) significantly improved seed germination and seedling development in watermelon. Similarly, Mawale *et al.* [26] had also recorded significant increase in seed germination under Ag, Cu and Cu-Ag NPs in Chili plants.

Morphological growth characters such as plant height, number of leave, stem girth and leaf area expansion were enhanced under ZnO-NPs when compared with the control, Groundnut grown under ZnO Nps from 0.75 to 1.25g /L showed greater growth performance as compared to other concentrations and control. The enhancement of growth under these treatments could be traceable to high delivery of ZnO NPs to protoplasmic contents of the cells all culminating in better growth as evident in all the morphological growth attributes that were studied. The results aligned with Al Salama *et al.* [27]. who reported increase in plant height, number of branches and number of spikes in Durum wheat with silver nanoparticle treatment. In groundnut, Olayinka *et al.* [28] and Fayomi *et al.* [29] also reported that ZnO nanoparticles positively influenced the overall growth and morphological

characteristics. With respect to chlorophyll contents, ZnO-NPs did not show statistical difference when compared to the control. The findings here did not align with those of Chen *et al.* [30], where the authors reported that chlorophyll contents were on the increase following ZnO-NPs treatment in tea plant. The variation could be attributed to plant species-specific responses, experimental conditions, or nanoparticles characteristics.

The results of yield attributes such as the number of seeds per plant, number of pods per plant circumference of pod and pod length increased following exposure to ZnONPs. Higher yield attributes with ZnO-NPs at 0.75, 100 and 1.25 g/L implied that higher ZnO-NPs are needed to achieve higher crop productivity in *A. hypogaea* seeds. Earlier findings had shown that nanoparticles of metals such as silver, copper and zinc had the advantage of improving crop productivity and plants respond differently [27, 31]. In groundnut, Suganya *et al.* [32] observed yield enhancement following ZnO-NP application.

The dose-dependent significant increase in the activities of nitrate reductase and carbonic anhydrase after exposure of *A. hypogaea* seeds to ZnO-NPs may be attributed to the co-enzyme role of the micro element, Zn. Zinc, a co-enzyme for carbonic anhydrase, plays a significant role in carbon dioxide fixation for photosynthetic process. As noted in the current study, the activity of this enzyme was further enhanced when the Zn was delivered on nanoscale and this account for the noticeable growth recorded. Earlier researchers had reported similar increase in the activity of carbonic anhydrase and nitrate reductase when ZnO-NPs were applied exogenously to tomatoes [33].

This study has demonstrated that ZnO-NPs had profound influence on seed quality, irrespective of the outcome from the mineral and proximate experiments in the present investigation. The generally higher increases in the concentrations of Zn, K, Mg, P and Na following treatment of the seeds of *A. hypogaea* with the ZnO-NPs aligned with previous study by Yusefi-Tanha *et al.* [34], where it was demonstrated that soil-applied ZnO nanoparticles significantly increased soybean seed qualities.

It should be noted the foregoing mineral elements had been shown to play a significant role in the overall well-being of human and livestock. For instance, K and Mg are essential for movement of nutrient to body cells, nerve signaling, muscle and nerve function and energy production [28]. As observed in this study, application of ZnONPs limited fat accumulation but enhanced ash, fibre, carbohydrate, protein and energy value of the seeds. Diet rich in fibre are good for bowel movement. The higher ash contents after ZnO-NPs treatment are a clear indication that *A. hypogaea*

plant exposed to metal nanoscale will have higher concentration of mineral elements. High carbohydrate and energy contents makes *A. hypogaea* a good source of energy [28]. The protein contents might make *A. hypogaea* seeds a good source of supplement for malnourished children. Again, the moisture contents of the seeds were higher following the application of ZnO-NPs. Samples with higher moisture contents are easily prone to rancidity and low shelf-life [28], hence, might prone to early spoilage and thus cannot be stored for a long time [28].

In conclusion, application of nanoscale zinc oxide (ZnO-NP) improved seed germination, plant growth, yield, biochemical properties, and also altered the mineral elements and some proximate compositions. The study recommended the use of ZnO-Nps at all the tested concentrations for beneficial agronomic outcomes.

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None

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### Authors Contributions

ARL – Conceptualization, methodology, data curation, formal analysis and writing-original draft; BUO – Supervision, Resources and Writing-review and editing; ASA – Methodology and Formal analysis; KAA – Methodology and Formal analysis; RBM – Methodology, Data curation and Formal analysis; LBA – Methodology, Data curation and Writing-original draft; AA – Methodology and Formal analysis; POB – Methodology and Data curation; and BTS – Data curation.

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