

Differential Growth -Markers and Nutrient Signaling Molecules Mediated by Zinc Oxide Nanoparticles in Black Nightshade (*Solanum Nigrum* L.)

Anthony Wale Ojewumi

anthony.ojewumi@lasu.edu.ng

Lagos State University

Olawale Lawrence Osifeko

Lagos State University

Nneka Sheily Egonu

University of Nigeria

Maryam Omotola Danjumah

Lagos State University

Favour Oluwatoyosi Solomon

Lagos State University

Temitope Hannah Olawale

Lagos State University

Chionyedua Theresa Onwordi

Lagos State University

Anthony Babajide Ojekale

Lagos State University

Research Article

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Posted Date: November 26th, 2025

DOI: <https://doi.org/10.21203/rs.3.rs-8089997/v1>

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Additional Declarations: No competing interests reported.

**DIFFERENTIAL GROWTH -MARKERS AND NUTRIENT SIGNALING
MOLECULES MEDIATED BY ZINC OXIDE NANOPARTICLES IN BLACK
NIGHTSHADE (*Solanum nigrum* L.)**

Anthony Wale Ojewumi¹, Olawale Lawrence Osifeko², Nneka Sheily Egonu³, Maryam Omotola Danjumah¹, Favour Oluwatoyosi Solomon¹, Temitope Hannah Olawale¹, Chionyedua Theresa Onwordi² and Anthony Babajide Ojekale⁴.

¹Department of Botany, Lagos State University, Badagry Express Way, Ojo Lagos

²Department of chemistry, Lagos State University, Badagry Express Way, Ojo Lagos

³Department of plant science and Biotechnology, University of Nigeria, Nsukka

⁴Department of Biochemistry, Lagos State University, Badagry Express Way, Ojo Lagos

Corresponding author's mail: anthony.ojewumi@lasu.edu.ng

ABSTRACT

A pot experiment was conducted to investigate the effects of zinc oxide nanoparticles (ZnONPs) and beans husk extract (BHE) on Black nightshade. Functional groups, morphological, optical appearance and elemental compositions of ZnONPs and BHE were evaluated. Growth parameters (plant height, number of leaves), reactive oxygen species (ROS) and antioxidant activities were also evaluated. Results revealed triple absorption band between 2076.00–2352.36 cm⁻¹, double bond at 1632.34 cm⁻¹ in ZnONPs and BHE between 2352.00–2072.00 cm⁻¹. Zinc (70.56%, 50.45%) was the major element determined in ZnONPs as well as silica (50.25%, 20.34%) in BHE. BHE and ZnONPs showed normal distribution with an average diameter (9.9 nm) in BHE and (8.64 nm) in ZnONPs. Five (5.0 g/L) ZnONPs produced tallest plant height (62.33 cm) and highest number of leaves (64.67 cm). Malondialdehyde (24.27 μmolg⁻¹FW) was significantly in the leaves as well as hydrogen peroxide (39.46 μmolg⁻¹DW) in the roots. Antioxidant activities were significantly lower in the leaves and roots of the vegetable treated with 10.0g/L BHE compared with control. In conclusion, alcohol, hydroxy, silicone and aliphatic nitro compounds are common functional groups observed in ZnONPs and BHE. Medial alkyne, imino and azo were peculiar to ZnONPs while terminal alkyne and Isothiocyanate were the major functional groups determined in BHE. Five (5.0 g/L) ZnONPs enhanced growth-markers and suppressed ROS production in the vegetable, therefore adoption of the zinc oxide nano-particles as sustainable soil conditioners for plant growth enhancement and regulation of reactive oxygen species..

Keywords: Black nightshade, Beans husk extract, Antioxidant activities, Reactive oxygen species, Soil conditioner, Growth parameters, Functional group

INTRODUCTION

Solanum nigrum is widely recognized as black nightshade. It is a vegetable herb belonging to the family Solanaceae. The ripe berries and leaves of nightshade are commonly consumed as food or vegetables (Wang *et al.*, 2017). It serves as a food source for birds and small mammals which feed on its berries and disperse its seeds, aiding in ecological succession in disturbed areas (Wang *et al.*, 2017). The seeds remain viable after digestion, promoting long-distance dispersal and colonization. Its phenotypic appearance such as varying leaf size and height in response to light enhances its adaptability across a wide range of habitats (Kieloch and Golebiowska, 2018).

One of the major challenges affecting the cultivation of black nightshade is its low yield due to reduced soil nutritional quality. In order to ameliorate these conditions, farmers often rely on chemical fertilizers. The fertilizers though are effective but their excessive usage often reduces soil fertility, causes soil-water pollution and increased production costs. Sustainable alternatives soil conditioners that support plant growth and protect the environment are needed to improve soil (Sharma and Thakur, 2024). As a sustainable alternative, nanotechnology offers a promising solution to the detrimental effects of insufficient nutrients and low crop production. Nano-fertilisers offer controlled and slow release of nutrients to plants, making them efficient and cost-effective (Yadav *et al.*, 2023). Due to the high surface to volume ratio property of Nano -fertilizers, they characterize high water holding capacity and slow release of nutrients (Gade *et al.*, 2023) and reduce nutrients leaching (Khatri and Bhateria, 2023).

Zinc oxide nanoparticles (ZnONPs) is an example of nano-fertilizer which is one of the most produced nano-materials and reported to be less toxic and inexpensive. The unique nano-characteristics and nano-structures of ZnONPs enhance development of an innovative approach for enhancing plant growth and improve stress tolerance through targeted delivery

and slow- nutrient release mechanism (Hanif *et al.*, 2024). The mechanisms often enhance nutrient use efficiency, regulate water-use efficiency and increase antioxidant activities (Hanif *et al.*, 2024). Green synthesis of nanoparticles through biological materials such as agricultural wastes, as an economically viable and sustainable method of soil enhancement (Awan *et al.*, 2021). The wastes such as bean husks provide an eco-friendly approach toward nutrient recycling for crop production.

Additionally, most agricultural soils suffer nutrient depletion incurred due to unfavorable environmental conditions such as soil degradation. Plants have several mechanisms used to express the conditions, one of which is production of reactive oxygen species (ROS) and antioxidants as control measured against the challenge (Sachdev *et al.*, 2021). The reactive species possess the capacity to induce oxidative stress (Mittler, 2017). Specifically, ROS are generated when the concentrations of singlet oxygen ($^1\text{O}_2$), superoxide anion ($\text{O}_2^{\cdot-}$), hydrogen peroxide (H_2O_2), and hydroxyl radical ($\cdot\text{OH}$) surpass the plant's cellular tolerance level Mittler, 2017. Free radicals are highly reactive due to having one or more unpaired electrons formed through redox reactions and plant metabolism (Dumanovic *et al.*, 2021). Hydroxyl radicals ($\cdot\text{OH}$ s), are the indicators of unfavorable water or nutrient conditions (Yang *et al.*, 2017). Nutrient imbalances, particularly zinc deficiency, can lead to excessive accumulation of ROS in plants, causing oxidative stress and cellular damage (Blasco *et al.*, 2018). Superoxide radicals and hydrogen peroxide are forms of ROS generated naturally as byproducts of metabolism. Consequences of ROS accumulation include reduced plant growth, chlorophyll degradation, poor flowering, seed set and overall yield losses (Sharma *et al.*, 2017). Zinc (Zn) plays a critical role in maintaining the plant's redox balance by supporting the activity of antioxidant enzymes against the situations (Hanif *et al.*, 2013). This study, therefore, focused on the differential growth, and nutritional composition of Black night shade mediated by zinc

oxide nanoparticles and beans husks. It aims to explore their potential as sustainable bio-fertilizers to provide to provide rich soil quality for black nightshade.

Materials and Methods

This study was conducted at the greenhouse, Lagos State University, Ojo, Nigeria.

Soil Collection and Nursery Preparation

Honey Beans husks were collected from a beans seller at Council Market, Egbe, Lagos. Seeds of Black nightshade were obtained from local farmers at Saki, Oyo State. Top soil was randomly collected at intervals from the Botanical Garden, Department of Botany, Lagos State University, Ojo, Lagos State according to the method of Ojewumi *et al.*, (2023) but with minor modifications. The collected soil was sieved and filled in thirty (30) perforated planting buckets (Ojewumi *et al.*; 2025). Black nightshade seedlings were grown in the university greenhouse for three (3) weeks. At the end of this period, one healthy seedling, each measuring about 10-12cm in height, was carefully transplanted into each bucket. The buckets were arranged in a Randomize Complete Block Design. After transplanting, the seedlings were further watered for one week to maintain soil moisture and proper establishment of the seedlings.

Synthesis of Zinc Oxide Nanoparticles

Bean husks (BH) collected were air-dried, blended into a powder in a high speed and multi-functional mill (Model-300) and sifted with a sieve of mesh size 100. Above fifty (50 g) of the sieved powdered BH was transferred into a 1000 mL round bottom flask, and 500 mL distilled water was added and stirred for 3 hours on a Stuart magnetic stirrer. After cooling, the slurry was filtered to obtain a clear brown filtrate and thick dark brown residue. The residue was discarded and the filtrate was used for synthesis of ZnONPs (Ondijo *et al.*, 2022).

Approximately, 50 mL of zinc solution was added in 500 mL of the filtrate of the bean husk extract (BHE), acidified with 2M hydrochloric acid (HCl). The mixture was stirred vigorously

until the zinc oxide dissolved at reduced temperature. The temperature was increased to 80°C for another 2 hours to allow the reduction and encapsulation of nanoparticles. 2 M solution of sodium hydroxide (NaOH) was added dropwise to the mixture while monitoring the pH using indicator paper. A visible colour changed from brown to a darker shade of brown. The pH was intermittently monitored using a paper indicator, and was found to be between 10 -11 for nanoparticle precipitation. The nanoparticles were harvested and centrifuged at 3500 rpm for 30 minutes. The supernatants were discarded, and the residue was suspended in distilled water and vortexed until a clear, colourless, neutralized supernatant was obtained. The residue was re-suspended in acetone, centrifuged for another 30 minutes to remove traces of water, filtered, and dried in the oven at 105°C for 1 hour (Ramesh *et al.*, 2021, Ojewumi *et al.*, 2025).

Characterization of Zinc Oxide Nanoparticles

The synthesized zinc oxide nanoparticles (ZnONPs) were characterized to ascertain their functional groups using Fourier Transform Infrared Spectroscopy (FTIR). Also, morphological properties, optical appearance, and biochemical attributes of the ZnONPs and BHE were determined according to the method of Almessiere *et al.*, (2020). Scanning Electron Microscopy (SEM, SIGMA Model MIRA3; TESCAN Brno) and Transmission Electron Microscopy (TEM, JEM-2200FS) were employed to collect micrographic images (Sohail *et al.*, 2019). The crystalline structure of the ZnONPs was determined using X-ray diffraction (XRD, Bruker D2 Phaser) analysis with monochromatic Cu-K α 1 radiation (Sohail *et al.*, 2020).

Preparation of Zinc Oxide Nanoparticles Concentrations and Beans Husk Extract, and Experimental Design

The ZnONPs concentrations were prepared in five replicates by dissolving separately 2.5 g, 5.0 g, 7.5 g, 10.0 g of ZnONPs and BHE in 500mL of distilled water. The concentrations included 2.5 g/L, 5.0 g/L, 7.5 g/L and 10.0 g/L. The 0.0 g concentration, which contained only

distilled water, served as the absolute control. Each concentration was thoroughly stirred to ensure homogeneity before application.

10 mL of the treatment was applied through foliar spray to the Black nightshade seedlings using a hand sprayer once every three days over six weeks. Throughout the experimental period, the plants were maintained under greenhouse conditions. Proper drainage was ensured by placing four glass petri dishes containers below each pot to prevent water-logging. Weeds growths were managed manually.

The experiment was a 4 by 2 factorial.

Mathematically, there are two factors(k) and four levels(l) of each. The total number of experimental runs was as shown below;

$$\text{Total runs} = l^k \dots\dots\dots \text{Equation 1}$$

Where;

l = Levels of inclusion of the factors

k =factors

The factorial design of this study involved four levels (2.5, 5.0, 7.5 and 10.0 g/L) of two independent variables (factors) (ZnONPs and BHE).

The model equation of the design is:

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{12} X_1 X_2 + \epsilon \dots\dots\dots \text{Equation 2}$$

Where;

Y = Response variable

X_1 and X_2 = The input variables (factors)

β_0 = Intercept

β_1 and β_2 = Coefficients representing the effect of each factor

$\beta_{12} X_1 X_2$ = Interaction effect of coefficient between factor ZnONPs and factor BHE,

ϵ = Random error term.

Analysis of agro morphological traits

Agro morphological traits viz. plant height (cm) and number of leaves were measured before treatment application as base line and once in a week after treatment application (WATA) within six weeks of the study. Plant height was measured using a ruler calibrated in centimeter while number of leaves was counted. At the end of the experiment, leaves and roots of the plant were collected and used to assess selected reactive oxygen species (ROS) and antioxidant activities.

Analysis of reactive oxygen species

Malondialdehyde (MDA) content

Level of lipid peroxidation in the leaf and root tissues of the vegetable by estimating malondialdehyde content using thiobarbituric acid (TBA) reaction. Based on MDA's molar extinction value (ϵ) of $155 \text{ mM}^{-1} \text{ cm}^{-1}$, the MDA was evaluated using the formula stated in Rai-Kalal and Jajoo (2021):

$$MDA(\mu\text{mol/g,FW})=1000 \times \frac{A_{532} - A_{600} \times V}{n} \times W \dots\dots\dots \text{Equation 3}$$

where,

ϵ = molar extinction value ($155 \text{ mM}^{-1} \text{ cm}^{-1}$),

V= volume of crushing medium,

W=Fresh weight of leaf and root tissues,

A532 =Absorbance of the TBA-MDA complex;

A600 =Correction for non-specific turbidity

Analysis of Hydrogen Peroxide (H₂O₂)

Hydrogen peroxide (H₂O₂) was determined using 5 mL of potassium phosphate buffer which extracted supernatant from 500 mg fresh leaf and root tissues of the vegetables. After centrifugation at 10000x for 15 mins, 0.5 mL of supernatant was collected. 5 mL tricarboxylic

acid cycle (TCA) (0.1% w/v) was used to make an aliquot was mixed with 1 mL KI in 10 mM K-P buffer, pH 7 and the H₂O₂ was determined in spectrophotometer at 390 nm.

Analysis of enzymatic antioxidant activities

Fresh leaf and root tissues from the control and treated vegetables were homogenized in a pre-chilled mortar and pestle with liquid nitrogen until suspension was produced, suspended in 5 mL of sodium phosphate buffer (0.2 M, pH 7.2), and centrifuged at 8000 × g for 15 min at 4 °C. The supernatant was used to calculate the activity of the superoxide dismutase (SOD) and catalase (CAT). Inhibition of the photochemical nitroblue tetrazolium (NBT) technique was used to quantify SOD activity. The SOD enzyme unit was quantified using the formula

$$SODactivity_{\mu g/mg} = \frac{Percent\ inhibition \times Dilution\ factor}{50\% \times enzyme\ extract\ taken \times FW\ of\ sample\ in\ mg} \dots\dots\dots Equation\ 4$$

H₂O₂ (10 mL) and phosphate buffer (50 mM; 1.9 mL) were added to 100 µL of fresh enzyme extract to measure CAT activity. The degree of H₂O₂-dependent oxidation was determined by measuring absorbance at 240 nm for 180 seconds. The CAT enzyme unit was quantified using the formula:

$$Enzyme\ activity = \frac{Change\ in\ OD/min}{Fresh\ weight\ of\ sample\ present\ in\ enzyme\ extract} \dots\dots\dots Equation\ 5$$

Ascorbate peroxidase (APX): The concentration of APX was calculated using the method described by Miyake and Asada (1996). The reaction mixture for APX measurement was prepared with 0.5 mL supernatant, 0.5 mM AsA, 0.1 mM H₂O₂, sodium phosphate buffer (pH 7), and 0.25 mL of EDTA. The reaction started after the addition of H₂O₂ and absorbance change at 290 nm was recorded. Specific activity was calculated with an extraction coefficient of 40 mM⁻¹cm⁻¹.

$$Percent\ inhibition\ (\%) = Control\ OD - treatment \dots\dots\dots Equation\ 6$$

$$OD/ Control\ OD \times 100 \dots\dots\dots Equation\ 7$$

Where, Control OD represents the optical density of blank and treatment OD represent the optical density (OD) of the samples

Glutathione Reductase (GR)

Glutathione reductase (GR) activity was assayed according to Carlberg and Mannervik (1985) with slight modifications. Fresh tissue (300 mg) was homogenized in 1.5 mL of ice-cold 200 mM potassium phosphate buffer (pH 7.0) containing 2 mM EDTA and centrifuged at $12,000 \times g$ for 15 min at 4 °C. The assay mixture contained 750 μ L of 200 mM phosphate buffer (pH 7.0) with 2 mM EDTA, 75 μ L of 2 mM NADPH, and 75 μ L of 20 mM oxidized glutathione (GSSG). The reaction was initiated by adding 100 μ L of enzyme extract, and the decrease in absorbance was monitored at 340 nm for 3 min. Enzyme activity was calculated using the extinction coefficient of NADPH ($\epsilon = 6.22 \text{ mM}^{-1} \text{ cm}^{-1}$) and expressed as units mg^{-1} protein.

Glutathione S-transferase (GST)

Glutathione S-transferase (GST) activity was determined using the method of Habig *et al.* (1974). Fresh tissue (300 mg) was homogenized in 1.5 mL of 50 mM potassium phosphate buffer (pH 6.5) and centrifuged at $12,000 \times g$ for 15 min at 4 °C. The assay mixture contained 1.0 mL of 100 mM phosphate buffer (pH 6.5), 1 mM reduced glutathione (GSH), and 1 mM 1-chloro-2,4-dinitrobenzene (CDNB). The reaction was initiated by adding 100 μ L of enzyme extract, and the increase in absorbance was monitored at 340 nm. Enzyme activity was calculated using the extinction coefficient ($\epsilon = 9.6 \text{ mM}^{-1} \text{ cm}^{-1}$) and expressed as units mg^{-1} protein.

Statistical Analysis

A two-way analysis of variance (ANOVA) was performed using statistical analysis software SAS 9.3 (SAS Institute Inc., Cary, NC., USA). Data were represented using the mean $\pm$ standard error (SE). A p-value ≤ 0.05 was considered statistically significant using Tukey's HSD test. Line graph and histogram were drawn using Origin pro8 software

RESULTS

Surface Functional Groups of Zinc Oxide Nanoparticles and Beans Husk Extract

Figure 1 showed analysis of FTIR of the ZnONPs and BHE in the mid-IR spectrum. Both the samples were found to be in various regions with different bonds. The ZnONPs characterized single bond (O-H, C-N) at wavelength (3394.00 cm^{-1}), triple bond ($\text{C}\equiv\text{C}$) between 2352.36 cm^{-1} and 2076.00 cm^{-1} , double (Alkenyl $\text{C}=\text{C}$ stretch) at 1632.34 cm^{-1} , Silicone, (1032.23 cm^{-1}) and aliphatic nitro compounds at wave number 1385.25 cm^{-1} . In the similar order, BHE also characterized single bond (3384.00 cm^{-1}), double bonds (2352.50 , 2072.00 cm^{-1}) and finger print across (1032.35 cm^{-1} , 1386.00 cm^{-1}) and 631.52 cm^{-1} wavelengths.

Elemental Compositions, Sizes and Morphological Analysis of Zinc Oxide Nanoparticles and Beans Husk Extract

The major functional groups present in ZnONPs are hydroxyl group, medial alkyne, isothiocyanate, amino and azo aliphatic compound, silicone and aliphatic nitro compound. Similar functional groups were noticed in BHE except terminal alkyne observed at 2352.36 cm^{-1} . On the other hand, the results demonstrated that the samples are complex molecules as their FTIR spectrum has more than five absorption bands. The energy dispersion spectrum (EDS) of BHE and ZnONPs showed various elements out of which silica with molecular weight (50.25%) and atomic (20.34%) weight, followed immediately by carbon and oxygen are the major elements in BHE. Zinc with molecular weight molecular (70.56%) and atomic weight (50.45%) was the major element determined in ZnONPs as shown in Figure 2a and Figure 2b. The TEM of both ZnONPs and BHE showed normal distribution of particles present in the samples. The two samples characterized particles of various sizes with an average size of 8.64 nm ZnONPs and 9.19 nm BHE as seen in Figure 3ab and Figure 4. The SEM image of ZnONPs appeared small, spherical and uniformly distributed particles at the nanometer scale, while the BHE showed rough and irregular surfaces as demonstrated in Figure 5.

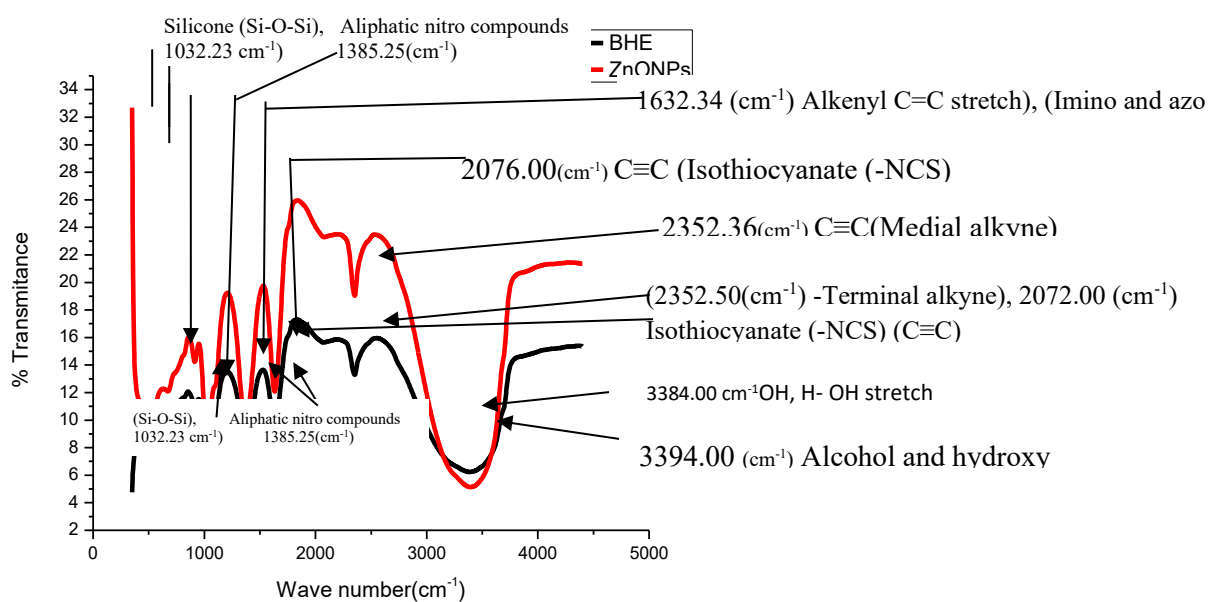


Figure 1. Fourier Transform Infrared spectrum of zinc oxide nanoparticles and beans husk extract.

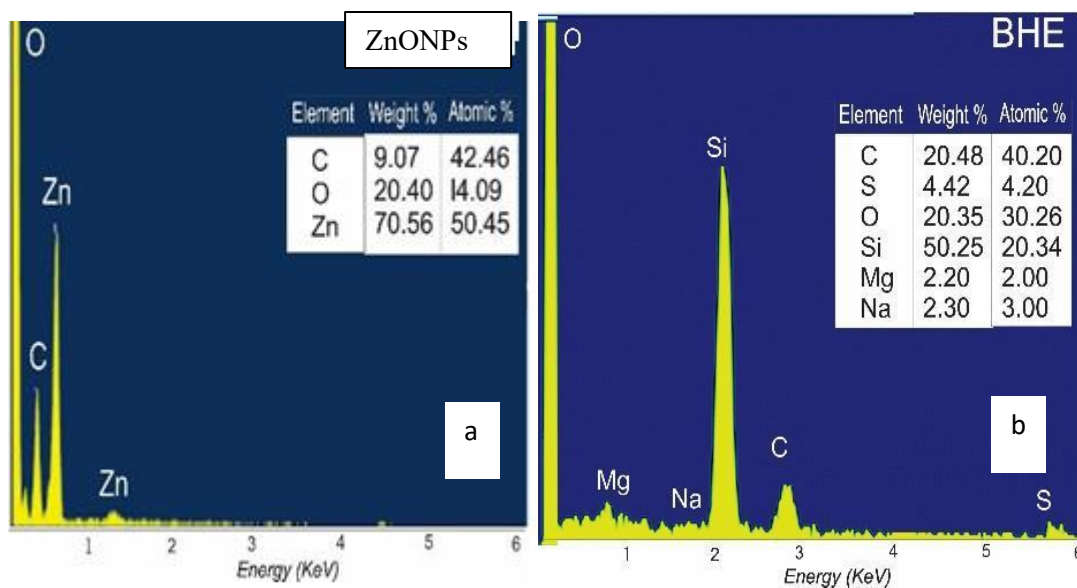


Figure 2 a and b. Elemental compositions of zinc oxide nanoparticles and beans husk extract under energy dispersion spectrum (EDS) image legends

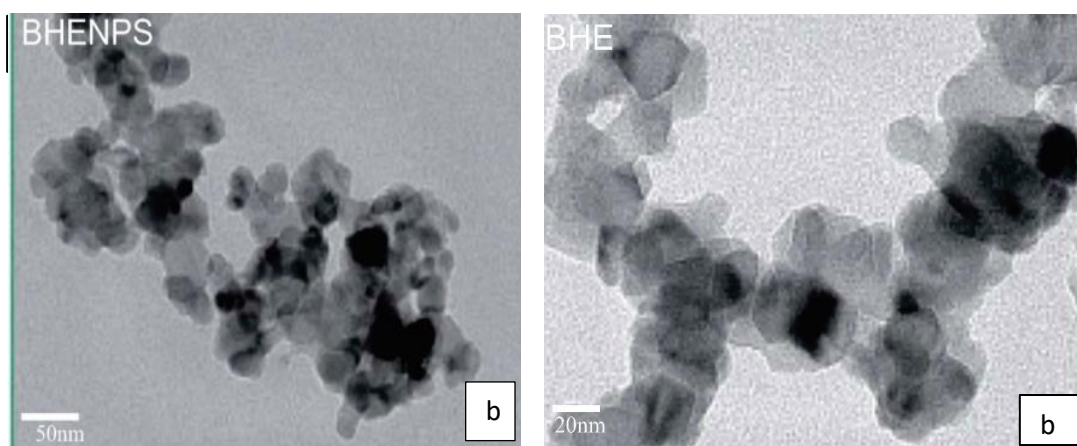


Figure 3 a and b: Zinc oxide nanoparticles and beans husk extract images under transmission electron microscope (TEM) Image legends

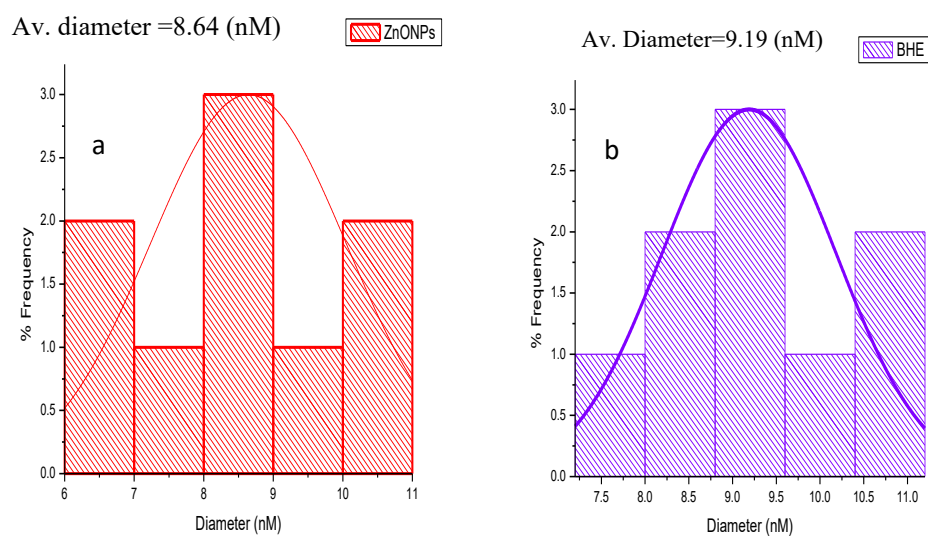


Figure 4a and b: Size and distribution of particles image of zinc oxide nanoparticles and beans husk extract images under transmission electron microscope (TEM)

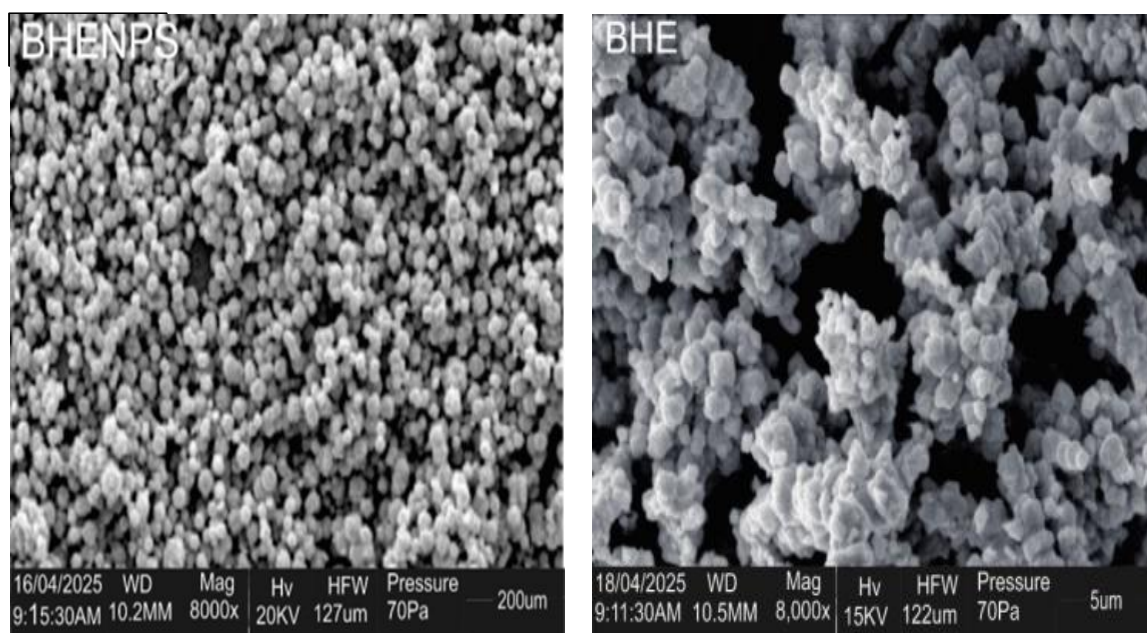


Figure 5a and b: Morphological appearance of beans husk extract and zinc oxide nanoparticles under scanning electron microscope (SEM).

Thermogravimetric and X-ray Diffraction Patterns of Zinc Oxide Nanoparticles and Beans Husk Extract

The thermogravimetric analysis (TGA) of ZnONPs and BHE revealed distinct thermal decomposition patterns. Both samples exhibited significant initial weight loss at around 99°C, indicating moisture evaporation as indicated in Figure 6a and b. The ZnONPs undergoes a major decomposition at 305°C, losing 65.4% of its weight and minimal residue was left afterward as detailed in Figure 6a. BHE also exhibited decomposition, with 68.8% weight loss at 395°C, indicating the breakdown of various organic compounds in the extract as shown in Figure 6b.

X-ray diffraction patterns of ZnONPs and BHE exhibited diverse peaks against 2theta micrograph. ZnONPs displayed peaks at 8.50°, 16.50°, 28.00°, 39.50°, 49.50°, and 68.0°, corresponding to lattice planes (100), (002), (101), (200), (220), and (300), respectively in Figure 7. Furthermore, BHE displayed peaks at 20.50°, 26.50°, 27.50°, 36.50°, 50.00°, 60.5° and 68.50°, corresponding to lattice planes (100), (002), (101), (200), (220), (300) and (311), respectively in Figure 7.

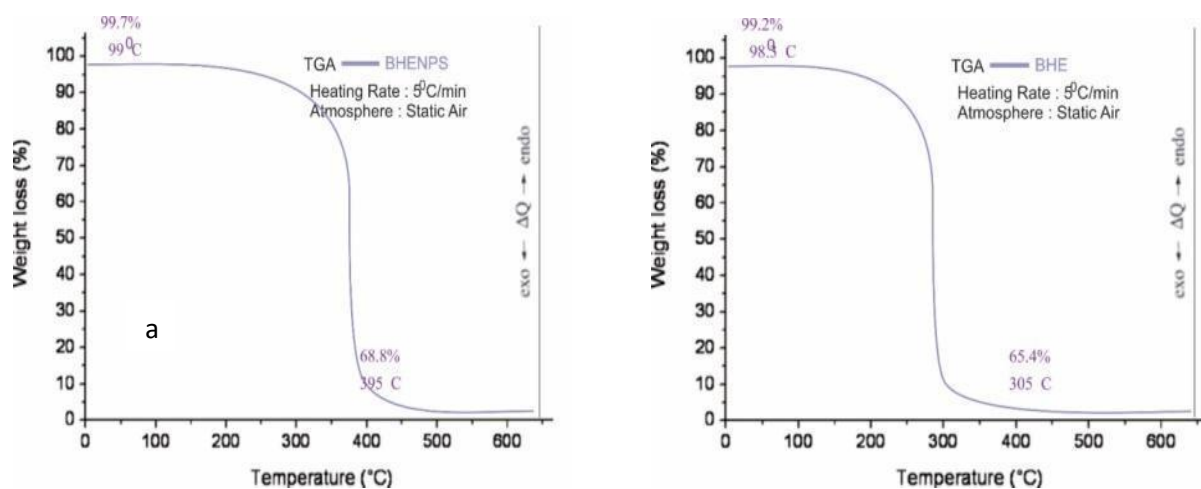


Figure 6a and b: Thermal decomposition of zinc oxide nanoparticles and beans husk extract using thermogravimetric analysis

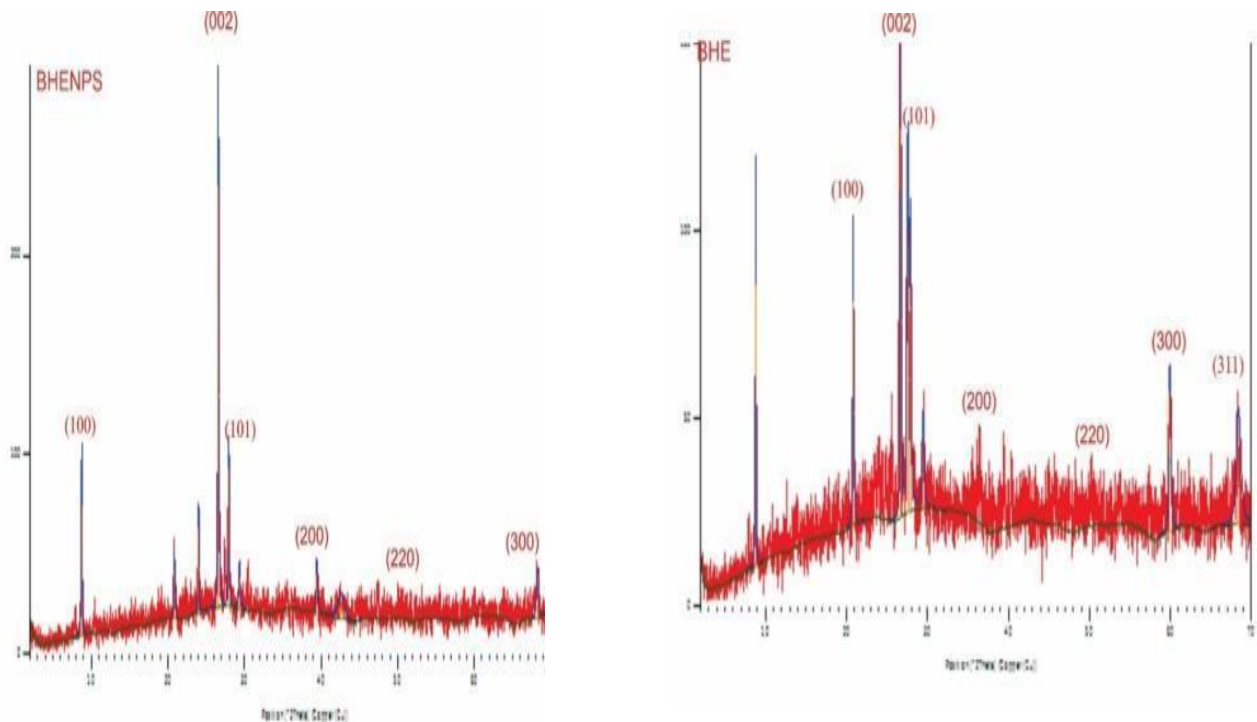


Figure 7a and b: X-ray diffraction (XRD) patterns of Zinc oxide nanoparticles and beans husk extract

Effects of ZnONPs and BHE on the morphological characters

ZnONPs and BHE produced non-significant effects ($P>0.05$) on the height of black nightshade at 2 Weeks after treatment application (WATA). However, the observation showed substantial increased between 4-6 WATA with appreciable plant height (57.50cm) recorded in the vegetable sprayed with 5.0 g/L of the treatments (Figure 8). Further observation on the application of ZnONPs and BHE at varying concentrations revealed tallest plant (62.33cm) in the vegetable sprayed with ZnONPs at 5.0 g/L compared with other concentrations of the two factors as illustrated in Table 1. Also, there was a significant interaction between ZnONPs and BHE as well as their concentrations on the parameter most especially at 4 and 6 WATA in Table 2.

Additionally, responses of number of leaves of the vegetable to varying concentrations of either ZnONPs or BHE revealed that the concentrations did not influence any significant change on

the number of leaves at 2WATA. However, the concentrations produced significant increase at 4 and 6WATA as represented by higher number of leaves (56.83) recorded in 10 g/L compared with other concentrations including control which is seen in Figure 9. In addition, 5.0 g/L of ZnONPs produced highest number of leaves (64.67) compared with the concentrations of BHE shown in Table 3. The concentrations of the factors and their interactions showed positive significant effects ($P \leq 0.05$) on the number of the leaves of the plant presented in Table 4.

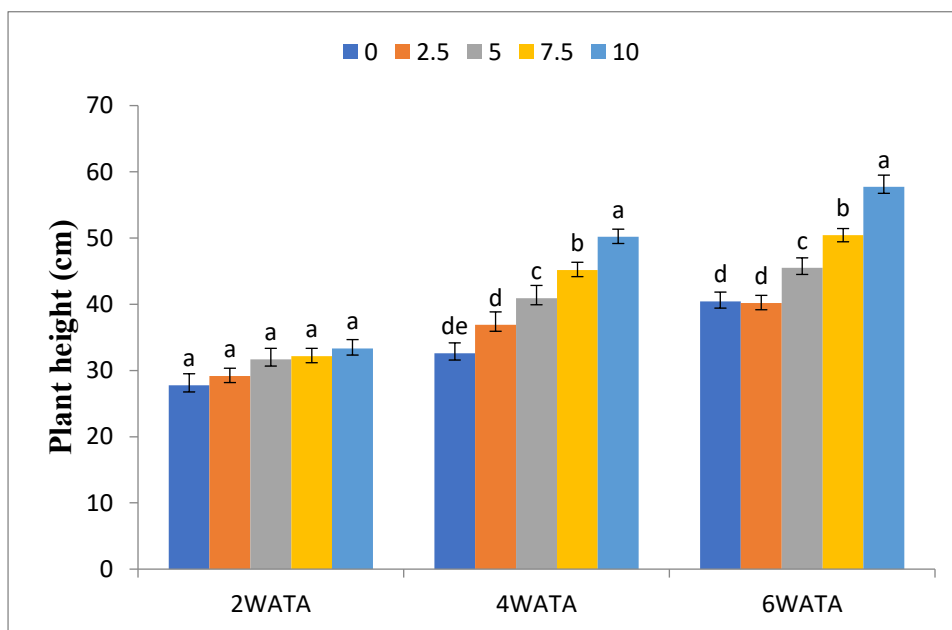


Figure 8: Effects of varying concentrations of ZnONPs and BHE on height of Black Nightshade at various weeks after treatment application. Means $\pm$ stand errors ($n=5$) followed by different superscripts on the same columns are significantly different at $P \leq 0.05$ using Turkey's HSD test. WATA = Weeks after treatment application

Table 1: Effects of ZnONPs and BHE and their varying concentrations on height of Black Nightshade at various weeks after treatment application

Factors	Concentration(g/L)	2WATA (cm)	4WATA (cm)	6WATA (cm)
ZnONPs	0	32.33	37.67	52.50
	2.5	32.67	37.67	40.67
	5.0	29.67	51.17	62.33
	7.5	34.00	40.67	45.00
	10.0	34.00	37.33	42.67
BHE	2.5	25.67	36.17	39.67
	5.0	41.67	50.67	52.67
	7.5	26.33	27.67	33.00
	10.0	32.67	41.00	42.83
	Std. Error	4.20	6.34	7.18

ZnONP= Zinc oxide nanoparticles, BHE = Beans husk extract, WATA = Weeks after treatment application

Table 2: Interaction between ZnONPs and BHE and their varying concentrations on height of *nigrum* (Black Nightshade)

Interactions	2WATA	4WATA	6WATA
Factors	ns	*	*
Concentration	ns	*	*
Factors * Concentration	ns	*	*

ZnONPs= Zinc oxide nanoparticles, BHE = Beans husk extract, WATA = Weeks after treatments* = significant, ns = non-significant at ≤ 0.05

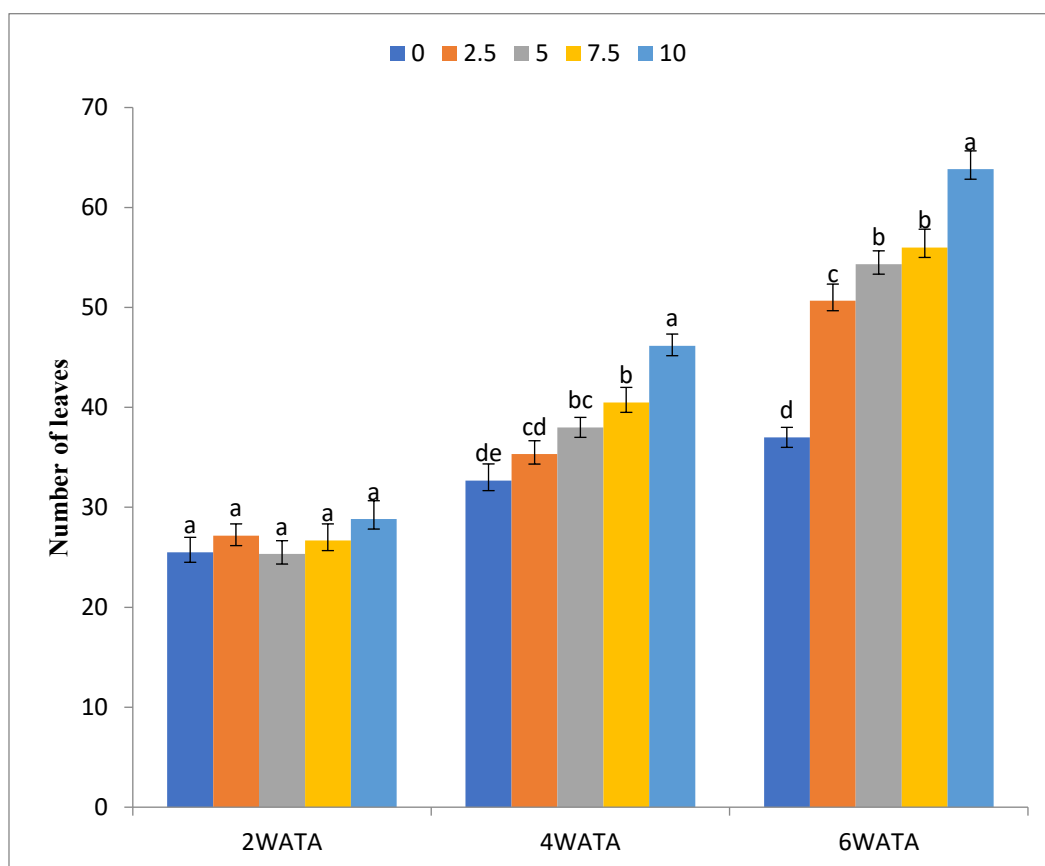


Figure 9: Effects of varying concentrations of ZnONPs and BHE on number of leaves of Black Nightshade

ZnONPs= Zinc oxide nanoparticles, BHE = Beans husk extract, WATA = Weeks after treatments, * = significant, ns = non-significant at ≤ 0.05 WATA = Weeks after treatment application

Table 3: Effects of ZnONPs and BHE and their concentrations on number of leaves of Black Nightshade

Factors	Concentration(g/L)	2WATA	4WATA	6WATA
ZnONPs	0	27.67	55.00	67.33
	2.5	36.67	45.67	58.00
	5.0	24.67	40.67	64.67
	7.5	28.67	35.00	40.33
	10.0	27.67	36.67	42.00
BHE	2.5	17.67	39.00	43.33
	5.0	20.00	29.33	44.00
	7.5	24.67	30.00	35.33
	10.0	32.00	45.67	71.67
	Std. Error	3.32	2.56	3.51

ZnONP= Zinc oxide nanoparticles, BHE = Beans husk extract, WATA = Weeks after treatments

Table 4: Interaction between ZnONPs and BHE and their concentrations on number of leaves of Black Nightshade

Interactions	2WATA	4WATA	6WATA
Factors	ns	ns	*
Concentrations	ns	ns	*
Factors* Concentrations	ns	ns	*

ZnONPs= Zinc oxide nanoparticles, BHE = Beans husk extract, WATA = Weeks after treatments* = significant, ns = non-significant at ≤ 0.05

Effects of ZnONPs and BHE on Production of Reactive Oxygen Species and Antioxidant Activities

Effects of ZnONPs and BHE on reactive species showed that leaves ($22.67\mu\text{molg}^{-1}\text{FW}$) and root ($21.55\mu\text{molg}^{-1}\text{FW}$) of control significantly generated MDA compared with lowered quantities of the parameter recorded in the plant sprayed with varying concentrations of the factors. Significant reduction was noticed in H_2O_2 in the root ($29.52\mu\text{molg}^{-1}\text{DW}$) and leaves ($22.26\mu\text{molg}^{-1}\text{DW}$) of the vegetable generated the lowest values of the parameter shown in Table 5.

Similar observation was noticed in the parameter recorded in the leaves ($24.27\mu\text{molg}^{-1}\text{FW}$) and roots ($39.46\mu\text{molg}^{-1}\text{FW}$) of control compared with concentrations of ZnONPs and BHE. Least of the parameters were produced between 7.5-10 g/L ZnONPs. Least antioxidant activities were observed in the vegetable treated with ZnONPs compared with BHE seen in Table 6. Interaction was noticed in 7.50 g/L of ZnONPs and BHE concentrations in Table 6. Similar trend was noticed between ZnONPs and BHE at varying concentration showed significant effects on the prediction of the production of MDA presented in Table 7. Interaction between the parameters in relation to their concentrations showed significant reduction on H_2O_2 seen in Table 7.

Effects of ZnONPs and BHE and Antioxidant Activities in Black Nightshade

Effects of ZnONPs and BHE on antioxidants showed that leaves and roots of Black nightshade presented in Table 8 showed higher GST ($6.76\mu\text{molmin}^{-1}\text{mg}^{-1}$), APX ($2.64\mu\text{molmin}^{-1}\text{mg}^{-1}$) and GR ($2.51\mu\text{molmin}^{-1}\text{mg}^{-1}$) in the leaves of Black nightshade control compared with other treatments. Similar effects were noticed on roots of the plants. Effects of between ZnONPs and BHE at varying concentration showed significant effects on the prediction of the production of

GST, APX and GR as shown in Table 9. Interaction between the parameters in relation to their concentrations showed significant reduction on SOD illustrated in Table 10.

Table 5: Effects of different concentrations of ZnONPs and BHE on ROS content in Black Nightshade

Concentration (g/L)	Leaves	Roots		
	MDA ($\mu\text{molg}^{-1}\text{FW}$)	H ₂ O ₂ ($\mu\text{molg}^{-1}\text{DW}$)	MDA ($\mu\text{molg}^{-1}\text{FW}$)	H ₂ O ₂ ($\mu\text{molg}^{-1}\text{DW}$)
Control (0)	22.67 ^a	30.94 ^a	21.55 ^a	39.90 ^a
2.50	18.30 ^b	28.63 ^b	19.87 ^b	38.18 ^b
5.00	18.40 ^b	27.42 ^c	19.83 ^b	34.66 ^c
7.50	17.40 ^c	22.26 ^c	17.93 ^c	29.52 ^d
10.0	16.53 ^d	23.85 ^d	17.71 ^c	29.51 ^d
Std. error	0.14	0.19	0.19	0.24

Mean $\pm$ stand errors (n=5) followed by different superscripts on the same columns are significantly different at $P \leq 0.05$ using Turkey's HSD test
MDA = Malondialdehyde, H₂O₂= Hydrogen peroxide

Table 6: Effects of ZnONPs and BHE at different concentrations on ROS levels in Black Nightshade

Factors	Concentration (g/L)	Leaves		Roots			
		MDA	(μmolg^{-1}	H ₂ O ₂	(μmolg^{-1}	MDA	(μmolg^{-1}
		¹ FW)	¹ DW)		¹ FW)	¹ DW)	
ZnONPs	Control	24.27	29.91		21.47	39.46	
	2.50	16.70	27.35		18.27	36.90	
	5.00	21.07	26.14		18.23	33.38	
	7.50	15.80	20.98		16.33	28.24	
	10.0	14.93	22.57		16.11	28.43	
BHE	2.50	19.90	29.66		19.95	38.62	
	5.00	16.80	28.70		21.43	35.94	
	7.50	18.99	25.13		19.53	30.80	
	10.0	18.13	23.54		19.31	30.99	
	Std. error	0.20	0.26		0.27	0.34	

ZnONP= Zinc oxide nanoparticles, BHE = Beans husk extract, MDA = Malondialdehyde, H₂O₂= Hydrogen peroxide, WATA = Weeks after treatments

Table 7: Interaction between ZnONPs and BHE at different concentrations on ROS levels in Black Nightshade

	Leaves		Roots	
Interactions	MDA (nmol/g)	H ₂ O ₂ (nmol/g)	MDA (nmol/g)	H ₂ O ₂ (nmol/g)
Factors	*	*	*	*
Concentration	*	*	*	*
Factors * Concentration (g/L)	*	*	*	*

ZnONP= Zinc oxide nanoparticles, BHE = Beans husk extract, MDA = Malondialdehyde, H₂O₂= Hydrogen peroxide, WATA = Weeks after treatments, * = significant at ≤ 0.05 , ns = non-significant

Table 8: Effects of different concentrations of ZnONPs and BHE on antioxidants content in Black Nightshade

Concentration (g/L)	Leaves					Roots					
	APX	CAT	GR	GST	GST	SOD	APX	CAT	GR	GST	
	(μmolmin ⁻¹ mg ⁻¹)	(μmolmin ⁻¹ mg ⁻¹)	(μmolmin ⁻¹ mg ⁻¹)	(μmolmin ⁻¹ mg ⁻¹)	(μmolmin ⁻¹ mg ⁻¹)	(μmolmin ⁻¹ mg ⁻¹)	(μmolmin ⁻¹ mg ⁻¹)	(μmolmin ⁻¹ mg ⁻¹)	(μmolmin ⁻¹ mg ⁻¹)	(μmolmin ⁻¹ mg ⁻¹)	
0	2.16 ^a	2.64 ^a	2.49 ^a	2.51 ^a	6.76 ^a	3.99 ^a	4.00 ^a	3.83 ^a	3.93 ^a	8.58 ^a	
2.5	1.68 ^b	2.25 ^b	2.25 ^b	2.15 ^b	4.64 ^b	3.18 ^b	3.35 ^b	3.52 ^b	3.48 ^b	8.08 ^b	
5.0	1.62 ^{bc}	2.22 ^b	2.04 ^c	2.11 ^c	4.52 ^c	3.02 ^c	2.61 ^c	2.99 ^c	2.70 ^c	7.57 ^c	
7.5	1.60 ^c	2.21 ^b	2.04 ^c	1.94 ^d	4.17 ^d	2.76 ^d	2.34 ^d	2.73 ^d	2.64 ^c	7.37 ^d	
10.0	1.60 ^c	2.18 ^c	1.96 ^d	1.92 ^d	3.13 ^e	2.55 ^e	2.19 ^e	2.39 ^e	2.34 ^d	5.44 ^e	
Std. error	0.02	0.01	0.01	0.01	0.04	0.01	0.02	0.02	0.02	0.02	

Mean $\pm$ stand errors (n=5) followed by different superscripts on the same columns are significantly different at $P \leq 0.05$ using Turkey's HSD test
SOD = Superoxide Dimutase, APX = Ascorbate Peroxidase, CAT = Catalase, GR= Glutathione Reductase, GST= Glutathione S-Transferase,
WATA = Weeks after treatments

Table 9: Interaction between ZnONPs and BHE at different concentrations on Antioxidants levels in Black Nightshade

Factors	Concentration (g/L)	Leaves					Roots				
		SOD	APX	CAT	GR	GST	SOD	APX	CAT	GR	GST
ZnONPs	0	2.31	2.90	2.80	2.95	8.09	4.14	4.27	4.14	4.38	9.91
	2.5	1.84	2.51	2.56	2.60	5.98	3.34	3.61	3.83	3.93	9.41
	5.0	1.77	2.48	2.35	2.55	5.85	3.18	2.87	3.30	3.15	8.91
	7.5	1.75	2.47	2.35	2.38	5.50	2.91	2.60	3.04	3.09	8.71
	10.0	1.76	2.44	2.27	2.37	4.47	2.71	2.45	2.70	2.78	6.77
BHE	2.5	1.53	1.99	1.94	1.71	3.31	3.03	3.09	3.21	3.04	6.74
	5.0	1.46	1.96	1.73	1.66	3.18	2.87	2.35	2.68	2.26	6.24
	7.5	1.45	1.95	1.73	1.49	2.83	2.60	2.08	2.42	2.20	6.04
	10.0	1.44	1.92	1.65	1.48	1.80	2.40	1.93	2.08	1.89	4.10
	Std. error	0.03	0.02	0.02	0.01	0.05	0.02	0.02	0.03	0.04	0.03

SOD = Superoxide Dimutase, APX = Ascorbate Peroxidase, CAT = Catalase, GR= Glutathione Reductase, GST= Glutathione S-Transferase,
WATA = Weeks after treatments

Table 10: Interaction between ZnONPs and BHE at different concentrations on Antioxidants levels in Black Nightshade

Concentration(g/L)	Leaves					Roots				
	SOD	APX	CAT	GR	GST	SOD	APX	CAT	GR	GST
Factors	*	*	*	*	*	*	*	*	*	*
Concentration(g/L)	*	*	*	*	*	*	*	*	*	*
Factors * Concentration(g/L)	*	*	*	*	*	*	*	*	*	*

SOD = Superoxide Dimutase, APX = Ascorbate Peroxidase, CAT = Catalase, GR= Glutathione Reductase, GST= Glutathione S-Transferase,
WATA = Weeks after treatments, * = significant at ≤ 0.05 , ns = non-significant

DISCUSSION

The use of flora waste-enhanced soil conditioners for improvement of crops and achievement of food security is one of the major concerns of livelihood of people in Nigeria. The FTIR analysis revealed several surface functional groups. These functional groups are important because they confirm the presence of biomolecules from the BHE that acted as reducing and stabilizing agents during nanoparticle formation (Ibrahim *et al.*, 2024). In particular, the hydroxyl and carbonyl groups likely capped the nanoparticles, making them stable and bioactive. Similar observations were reported in earlier studies where plant extracts supplied phytochemicals that mediated nano-particle stability (Yadav *et al.*, 2024).

The elemental composition analysis by EDS indicated elemental dominance of the samples, with silica being the predominant element in the BHE and zinc in ZnONPs. This suggests that chemical transformations took place during synthesis (Kumar *et al.*, 2023). The TEM analysis of BHE and ZnONPs revealed a normal distribution of particles with varying sizes, having average diameters of 9.19 nm and 8.64 nm, respectively. This indicates that the synthesis process was effective in producing nanoparticles within the nanoscale range. The observation is essential for their enhanced surface reactivity and potential biological applications and agriculture (Ahmad *et al.*, 2022). The sizes obtained in this study are consistent with those reported by (Selim *et al.*, 2020), who observed ZnONPs in the range of 9.26 to 31.18 nm using a similar method. Slight variations in particle size between studies may be attributed to differences in plant extract composition, synthesis conditions, or stabilization mechanisms. The relatively small particle sizes recorded in this work suggest that both BHE and ZnONPs are suitable for applications where high surface area and bioactivity are required (Mandal *et al.*, 2022).

Although both ZnONPs and BHE characterized small diameter, the small size observed in ZnONPs might enhance faster absorption, entry and translocation of nanoparticles within the

plant for activation of physiological actions in black nightshades (Rani *et al.*, 2023). Studies of Singh *et al.*, 2018 revealed that particles of smaller sizes are more easily metabolized compared with larger ones. The rough and irregular surfaces, observed in BHE as well as ZnONPs appear small, spherical and uniformly distributed particles observed in ZnONPs is relevant in the entry of the particles into the plant and their targeted delivery into the specific organ in the plant (Raha and Ahmaruzzaman, 2022). TGA analysis shows that the synthesized nanoparticles are thermally stable at temperatures above 290°C. The attribute is beneficial to the thermal condition of the agricultural soil (Bhuyan *et al.*, 2015). The thermally stable nanoparticles noticed in ZnONPs may enhance their bioavailability and efficiency of the nano-materials in plant systems (Begum *et al.*, 2022 Munir *et al.*, 2024).

The XRD diffractograms of BHE and ZnONPs confirmed their crystalline nature, which enhances nutrient absorption and plant growth, improving leaf development and overall crop production. These results align with previous studies, that demonstrated that plant-mediated synthesis of zinc oxide nanoparticles yields highly crystalline particles (Bhuyan *et al.*, 2015). The observed improvement observed in growth parameters and reduction in oxidative stress under zinc oxide nanoparticles and bean husk extract treatments suggest significant physiological modulation effects of the treatments on the black nightshade. These effects can also be linked to enhanced- zinc bioavailability, antioxidant regulation and improved nutrient signaling pathways in the vegetable (Costa *et al.*, 2023).

The application of different concentrations of ZnONPs and BHE significantly influenced the growth of Black nightshade. Production of higher plant height and highest number of leaves in plant sprayed with 5.0 g/L ZnONPs indicates that moderate concentrations of the treatments can significantly improve nutrient assimilation and metabolic activity (Singh *et al.*, 2019). As such, the potential of green synthesized ZnONPs has been recognized as a nano-based nutrient source for agricultural applications (Singh *et al.*, 2019). The improved physiological status of

the plant sprayed with ZnONPs and BHE concentrations supports the hypothesis that green-synthesized nano-materials derived from agricultural waste can be sustainable alternatives for crop improvement (Wang *et al.*, 2023).

Furthermore, the control plants produced the highest MDA and H₂O₂ as a clear indication that untreated plants experienced more oxidative damage, hence, application of ZnONPs and BHE can reduce ROS levels, especially at higher concentrations (Khan *et al.*, 2023). This is in agreement with previous findings where nanoparticles alleviated oxidative stress by stabilizing membranes and reducing ROS accumulation (Kumar *et al.*, 2020). This pattern suggests that lower ROS concentrations are associated with enhanced nutrient availability in the soil, thereby supporting improved plant growth and physiological performance (Huang *et al.*, 2019). Hence, treatments that minimized ROS (particularly at moderate concentrations) likely contributed to better growth outcomes by reducing oxidative stress and promoting nutrient assimilation. The observed reduction in ROS markers and lower activities of antioxidant enzymes suggest that these treatments alleviated oxidative stress using antioxidant activities possibly by stabilizing cellular structures and improving membrane integrity (Zandi and Schnug, 2022). This study demonstrates that ZnONPs and BHE have dual roles in improving plant growth and mitigating oxidative stress through their impactful physiological and biochemical processes.

CONCLUSION

The use of organic waste-enhanced soil conditioners for improvement of crops and achievement of food security is one of the major concerns of livelihood of people in Nigeria. This research investigated the green synthesis of ZnONPs using BHE as reducing and stabilizing agents and further applied various concentrations of the ZnONPs and BHE as a nano-fertilizer for the growth of Black nightshade plant. The study monitored the height and number of leaves of the Black nightshade as well as the reactive oxygen species and antioxidant activities of leaves and roots of Black nightshade after the application of the ZnONPs and BHE. The ZnONPs and BHE were characterized. The FTIR analysis revealed presence of key functional groups, while EDS showed silica in BHE and zinc in ZnONPs as the major elements. The two chemicals characterized desirable thermal stability. Five (5.0 g/L) ZnONPs produced tallest plants and highest number of leaves in black nightshade compared with BHE. Furthermore, 7.5-10.0 g/L ZnONPs suppressed ROS and antioxidant production in the leaves and roots of the plant. However, 2.5 g/L BHE produced highest antioxidants in the leaves of the plant. Conclusively, ZnONPs and BHE enhanced growth and nutrient signaling molecules in black nightshade by reducing oxidative stress and improving antioxidant defense in the vegetable, therefore adoption of the substances as sustainable and eco-friendly soil conditioner for enhancement of growth of the vegetable is thereby suggested. This can serve as a promising replacement for chemical fertilizer for the growth of vegetables. This research as addressed the SDG goal 2 which addresses hunger, food security

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Declarations

Acknowledgements

The authors acknowledge the Department of Botany, Lagos State University, for providing greenhouse facilities and research support. We also appreciate the Departments of Chemistry and Biochemistry, Lagos State University, for access to analytical instruments used for nanoparticle characterization. The authors thank the technical staff and research assistants for their valuable help during sample preparation, laboratory analysis, and data collection.

Funding

The authors received no specific funding for this work.

Ethical Approval

This study did not involve human participants or animals. Experimental work on *Solanum nigrum* was conducted in compliance with institutional, national, and international guidelines for the ethical use of plant materials in research.

Author Contributions

A.W.O. conceived and designed the study, supervised the research, and wrote the main manuscript text.

O.L.O. conducted the synthesis and characterization of the zinc oxide nanoparticles (FTIR, SEM, TEM, EDS, XRD) and contributed to data interpretation.

N.S.E. assisted in the greenhouse experiment, plant sampling, and analysis of morphological traits.

M.O.D. performed the reactive oxygen species and antioxidant enzyme assays and assisted with data analysis.

F.O.S. contributed to statistical analysis, figure preparation, and data visualization.

T.H.O. assisted in laboratory work, data organization, and validation of methods.

C.T.O. participated in chemical analysis and verification of nanoparticle synthesis protocols.

A.B.O. provided technical guidance on nanoparticle synthesis, critically reviewed the manuscript, and contributed to the discussion and conclusions.

All authors read and approved the final manuscript.

Competing Interests

The authors declare that they have no competing interests.