

Assessment of nanofertilizers' effect on yield of seedling biomass in vegetable crops

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ABSTRACT

Modern agriculture relies heavily on chemical fertilizers, which can lead to environmental issues. To address these challenges, nanofertilizers offer a potential solution by improving nutrient delivery and reducing environmental impact. This study explores the synthesis and effects of nanoparticles (NPs) on seed germination and seedling growth. The NPs (ZnO, Fe₂O₃, and MnO₂) were green synthesized by using pea peel biomass and were characterized by UV-visible spectroscopy, Fourier transform infrared spectroscopy (FTIR), and field emission scanning electron microscopy (FESEM), confirming their size and shape. The sterilized seeds of tomato, chili, and brinjal were treated with aqueous solutions of NPs at concentrations of 10, 20, and 50 ppm. The seeds were sprayed at 48-hour intervals. The germination rates and growth parameters such as shoot and root length, as well as fresh and dry weight measured. UV-visible spectra revealed absorption peaks at 357 nm for ZnO, 333 nm for Fe₂O₃, and 360 nm for MnO₂. FTIR and FESEM confirmed the successful synthesis and characterized the NPs' sizes and shapes. Notably, MnO₂ and their combinations of NPs enhanced seedling growth and biomass as compared to control. The findings highlight that green-synthesized MnO₂ NPs effectively promote seed germination and seedling growth. These eco-friendly nanofertilizers offer a promising approach to enhancing crop yield and supporting sustainable agriculture.

1. INTRODUCTION

Nanotechnology can be very helpful in improving agricultural fields' productivity and nutrient efficiency. The rapid growth of nanotechnology can be attributed to the distinctive properties of nanoparticles (NPs), including their small size and high surface area-to-volume ratio [1]. To achieve high yields per unit area, the global agricultural system makes extensive use of a wide range of fertilizers, herbicides, and pesticides; nevertheless, these chemicals can cause serious issues such as environmental contamination. Modern agriculture relies heavily on chemical fertilizers, herbicides, and pesticides, which can lead to significant environmental contamination. To address these concerns and move toward sustainable practices, the green synthesis of NPs has gained popularity over the last two decades [2,3]. Phyto-nanotechnology, which involves the environmentally friendly, fast, stable, and cost-effective production of various NPs,

has proven beneficial in agriculture. Nanofertilizers containing trace amounts of manganese oxide (MnO₂), iron oxide (Fe₂O₃), and zinc oxide (ZnO) have been found to improve crop yields. The U.S. Food and Drug Administration has also classified ZnO as a safe and non-toxic material [4].

Due to their speed, economy, and environment friendliness, the biological methods of nanoparticle synthesis have garnered the attention of many researchers in the current age. The application of nanotechnology in plant science and plant production systems is known as *phyto-nanotechnology*. Plant-mediated nanoparticle synthesis is known to accelerate by a number of important phytochemicals, such as alkaloids, flavonoids, polyphenols, saponins, and steroids, which function as capping, reducing, and stabilizing agents [5]. NPs with sizes ranging from 30 to 40 nm are recognized as nanofertilizers due to their high surface area. This large surface area allows them to efficiently load nutrient ions and release them into the soil.

Biological methods of synthesizing ZnO NPs are considered more versatile than physical and chemical methods, due to their simplicity, biocompatibility, and reduced environmental impact [6]. Zinc is a crucial element for plants, playing a vital role in seed germination and growth, it is regarded as an essential element in plants. However, too

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much zinc can lead to morphological physiological, and biochemical problems [7]. Plants with zinc enrichment have traditionally been accomplished by adding it as a chelated compound in ionic form to solid or liquid fertilizers; more recently, tests have been conducted using zinc applied to agriculture in the form of NPs or nanomaterials [8]. Iron, essential for processes such as respiration, photosynthesis, and cell metabolism, is often deficient in plants due to its poor solubility in soil, leading to problems like chlorosis and reduced biomass [9]. As a result, crop production requires an adequate iron supplement, particularly for crops grown in alkaline soils. It is critically necessary to find low-cost and environmentally friendly ways to use NPs as fertilizer to treat iron deficiency-induced chlorosis in plants [10–12]. Plant growth has already benefited somewhat from iron oxide NPs. For instance, it has been demonstrated that iron oxide NPs can control the amounts of antioxidant enzymes and phytohormones in peanut plants (*Arachis hypogaea*), which in turn enhances plant development. Rui *et al.* [10] demonstrated the potential for iron oxide NPs to function as fertilizer [10]. Manganese oxide NPs can also serve as valuable components in fertilizers, because of their special chemical, biological, physical, and photocatalytic characteristics [13]. Manganese oxide NPss can also be utilized as fertilizer components [9] as well as the production of bactericides [14]. The manganese-based nanomaterials have found widespread application in agriculture [15].

The tomato, (*Solanum lycopersicum* L.), belongs to the *Solanaceae* family. Because of its nutritional and therapeutic properties, it is grown all over the world. In addition to being high in antioxidants such as tocopherols and lycopene, it is also high in potassium, carbs, and ascorbic acid. One of the many health advantages of tomato fruit is that it lowers the chance of developing heart disease [16]. The issue of climate change and its numerous consequences is having a negative impact on tomato production overall. As a result, demand for tomatoes is rising while supply is falling. As a result, there is a need for strategies and tactics to boost tomato output using the current soil and land resources. In the tropics, chillies are commonly grown as a spice, vegetable, or cash crop [17]. Around the world, 7.18 million tonnes of chilli are generated on 1.7 million hectares of cropland [18]. One of the causes of the low output of chillies is the uneven and delayed germination of chilli seeds. The irregular and delayed germination of chilli seeds can be attributed to a variety of reasons. Out of the different reasons, illnesses are the most common. Numerous illnesses that affect chillies are brought on by nematodes, bacteria, viruses, fungus, and abiotic stressors. The eggplant (*Solanum melongena* L.), a member of the *Solanaceae* family, is a prominent crop in tropical and subtropical countries [19]. Fruits from eggplants are known for their mineral and vitamin content, which includes vitamins such as A and B. They also have low protein and carbohydrate content and high iron content. The health benefits of eggplant stem from their low-calorie content, which helps prevent obesity and reduces arteriosclerosis by preventing cholesterol from being transmitted and lowering body fat [20].

In this study, pea peel extract was used to biologically synthesize and characterize green NPss of zinc, iron, and manganese oxides. The objective was to assess the impact of these bio-fabricated NPss, both individually and in combination, on the growth and biomass production of tomato, chili, and brinjal seedlings.

2. MATERIALS AND METHODS

The research was carried out in the Department of the Bio-Sciences and Technology; in February 2023 (30.2753°N, 77.0476°E). The seeds (brinjal, tomato, and chilli) were purchased from a neighborhood market in Barara, Ambala. The seeds were washed with distilled water

to get rid of any small dust particles before being utilized in additional experiments.

2.1. Pea Peel Collection and Extract

The plant biomass used is pea (*Pisum sativum* L.) peel. To get rid of the dust particles repeatedly cleaned in double-distilled water. Moreover, it was dried after washing by paper towelling and it was cut into small pieces and finally ground mechanically. Additionally, 25 g of pea peel was added to a 100 ml beaker that had 50 ml of double-distilled water and then heated to 60°C for 10 minutes while being constantly stirred with a hot stirrer plate. After the stirring period was over, the mixture was allowed to cool to ambient temperature before being filtered using Whatman No. 1 filter paper and kept for later use at 4°C [21].

2.2. Synthesis of ZnO, Fe₂O₃ and MnO₂ NPs

For the synthesis of ZnO, Fe₂O₃, and MnO₂ NPss, zinc acetate dihydrate (Zn(CH₃COO)₂·2H₂O), ferric chloride (FeCl₃·6H₂O), and manganese acetate ((CH₃COO)₂Mn·6H₂O) were used as the respective precursors. The process involved preparing three separate solutions by mixing 9 ml, 40 ml, and 180 ml of pea peel extract with 0.01 M of zinc, iron, and manganese salts, respectively. These mixtures were stirred on a magnetic hot plate at 70°C to ensure uniform agitation.

To adjust the pH of the solutions, 2 M sodium hydroxide was gradually added until the pH reached 12 for the zinc solution, 11 for the iron solution, and 8 for the manganese solution. Following this, the solutions were repeatedly washed to neutralize the pH to 7. The NPss suspensions were then centrifuged at 10,000 rpm for 10 minutes to separate the precipitate. The resulting precipitates were filtered using Whatman filter paper No. 1 and subsequently dried overnight at 40°C for ZnO and 80°C for Fe₂O₃ and MnO₂ [22].

2.3. Characterization of ZnO, Fe₂O₃, and MnO₂ NPs

The synthesis of NPs was tracked using a UV-Visible spectrophotometer (Shimadzu 2600) and color change. Fourier transform infrared spectroscopy (FTIR) was used to detect the presence of ZnO, Fe₂O₃, and MnO₂ NPs made from pea peel extract. Numerous phyto constituents that are present in the pea peel extract may be responsible for the synthesis of metal NPs and the reduction of metal ions. For the preparation of pellets for FTIR analysis using the Shimadzu 8400 spectrometer, approximately 2 mg of synthesized NPs were thoroughly mixed with potassium bromide (KBr) powder. The mixture was then pressed into a pellet using a hydraulic press to obtain a transparent disc suitable for FTIR measurements. The KBr served as a matrix to ensure optimal transparency in the infrared region, allowing for accurate analysis of the functional groups present in the NPss. The FTIR spectra were collected at 400–4,000 cm⁻¹ wavelengths with a resolution of 1 cm⁻¹. By using an X-ray diffraction (XRD) diffractometer (XRD-Bruker), the crystalline size and purity of these NPs were determined. For both qualitative and quantitative analysis of NPs, XRD is a powerful characterization technique. XRD analysis is used to determine the crystal structure of NPs. Field emission scanning electron microscopy (FESEM-Zeiss Sigma 3) was used to examine the size, shape, and surface characteristics of these synthesized NPs. The diameters of the synthesized NPs were determined by measuring the particles using a WCIF Image J image analyzer.

2.4. Seed Germination

To assess germination, 20 seeds each of tomato, chili, and brinjal were placed separately in Petri dishes lined with blotting paper.

The blotting paper was moistened with distilled water to maintain a suitable environment for germination. The seeds were incubated under controlled conditions for a period of 7 days, with regular monitoring to ensure adequate moisture levels. After the incubation period, the germination rate was recorded by counting the number of seeds that successfully sprouted in each dish [23]. The plate had 5 ml of water supplied on the second, fourth, and sixth days of germination. The seeds were sprayed three times (every 48 hours) with stickers and varying quantities of NPss 10, 20, and 50 ppm concentration of NPs suspensions singly as well as in combinations (doubled and triples) were added to each plate of seeds. An analogous experiment was carried out as a control, but without NPss. Following a 7-day period of treatment, the seedlings were taken out and their roots and shoots were divided. The effects of NPss on seed germination and early seedling growth were examined by comparing the data on seedling growth in terms of root and shoot length, and fresh and dry weight [24].

2.5. Statistical Analysis

The impact of various nanofertilizers on vegetable seedlings was evaluated through one-way ANOVA tests, focusing on key growth parameters: shoot length (SL), root length (RL), shoot fresh weight (SFW), shoot dry weight (SDW), root fresh weight (RFW), and root dry weight (RDW).

3. RESULTS

3.1. Green Synthesis

The production of NPs suggested by the color change of the reaction mixture, from pale yellow to pale white precipitate, verified the presence of zinc [25]. The reaction mixtures changed from brown to dark brown, confirming the presence of iron [22]. The change from pale white to brown indicated the presence of manganese [26].

3.2. UV-Visible Spectrum of NPss

The green synthesis of ZnO, Fe₂O₃, and MnO₂ NPs from the plant extract was investigated using UV-vis spectroscopy (Fig. 1a, b, and c). The wavelengths at which the NPss formation observed are 357, 333, and 360 nm, respectively.

3.3. Field Emission Scanning Electron Microscopy (FESEM)

The FESEM analysis of ZnO, Fe₂O₃, and MnO₂ NPs ranged in size from 40 to 120, 43–51, and 50–100 nm, with spherical to irregular,

agglomerated, and multiform and globular dispersion forms (Fig. 2a, b and c).

3.4. X-ray Diffraction

The face-centered cubic (fcc) structure shows the diffraction angles at 2θ range of 20–80° of 31.8, 34.4, 36.3, 47.5, 56.6, 62.8, 67.98, and 69.1 degrees. ZnO NPs exhibit a distinctive XRD pattern with three strong peaks at the (100), (100), (101), (102), (110), (110), (103), (200), and (201) planes, respectively. These outcomes closely resemble those of earlier research on the chemical and environmentally friendly synthesis of ZnO-NPs [27]. The XRD spectra of MnO₂ with a slit width of 6.0 mm and a scanning rate of 1°/minute, NPs were captured in the 2θ range of 20–60° of 28 and 29 degrees corresponded to (012) and (117). The characteristic diffraction peaks of Fe₂O₃ NPs at 2θ range of 20–60° of 28, 29, and 36 corresponded to (012), (117), and (220) diffraction planes of hexagonal α -Fe₂O₃ (Fig. 3a, b, and c).

3.5. Fourier-Transform Infrared Spectroscopy

The synthesis of ZnO NPs was revealed by the FTIR spectra of ZnO NPs, which showed a considerable peak at 3,435 cm⁻¹. The two primary absorbance bands in the resultant spectrum zinc oxide NPss, which are displayed in the figure, are connected to the hydroxide bond (3,425 cm⁻¹) and (475 cm⁻¹). FTIR analysis was conducted using infrared wave frequencies of 400–4,000 cm⁻¹. The FTIR spectrum of manganese NPss spectrum showed the presence of bands at 613, 860, 1,030, 1,415, 1,589, 2,927, and 3,415 cm⁻¹. The bands at 613cm⁻¹ correspond to amines (C-N-C bend), 860 cm⁻¹ to ketones (C-CO-C bend), 1030 was assigned to vinyl compounds (CH deformation), the band at 1,415cm⁻¹ was assigned to primary aliphatic amines (C-N stretch), 1,030cm⁻¹ to thiocarbonyl compounds (C=S stretch), 1,415cm⁻¹ corresponds to sulfonyl chlorides (SO₂ antisym stretch), 1,589cm⁻¹ to secondary amides (NH deformation), 1,589cm⁻¹ corresponds to primary amides (NH deformation), 2,927cm⁻¹ to aliphatic compounds (CH stretch), 3,415cm⁻¹ to amino acids (NH₃ stretch), and 3,515.97cm⁻¹ to aromatic amines (NH stretch) [28]. The FTIR spectrum of synthesized Fe₂O₃ NPs absorption bands located at 3,415, 1,625, 1,383, 1,052, and 618 cm⁻¹. Fe-O-Fe stretching vibrations of Fe₂O₃ are indicated by the high peak at 618 cm⁻¹. The hydrogen-bonded O-H stretching (str) and C=O stretching vibrations are attributed to the peaks at 3,387 cm⁻¹ and 1,634 cm⁻¹, respectively (Fig. 4a, b, and c).

3.6. Effect of NPss on Seed Germination

The maximum shoot and root length (7.91 ± 2.63 and 6.34 ± 2.11 cm) of brinjal seeds was observed in the 20 ppm treatment of Mn followed

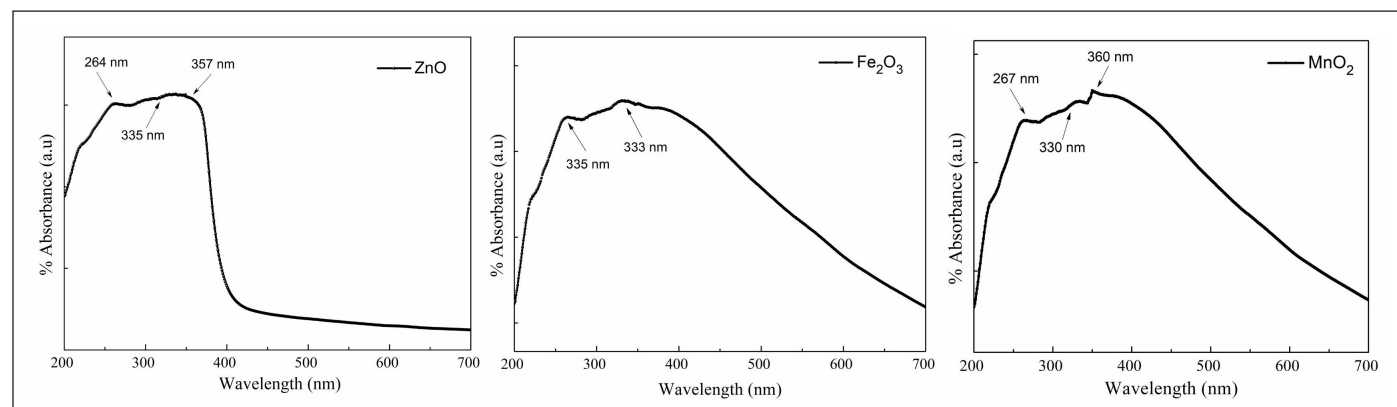


Figure 1. UV spectra of green synthesized a- ZnO, b- Fe₂O₃, c- MnO₂-NPs.

by ZnFeMn, ZnFe, and Zn. The maximum shoot fresh wt. and dry wt. (0.75 ± 0.25 and 0.36 ± 0.12 gm) was found in Mn 20 ppm followed by Mn (0.48 ± 0.16 gm), 10 ppm, MnZn (0.48 ± 0.16 gm), and ZnFeMn (0.36 ± 0.12 gm) in 20 ppm for fresh wt. and dry wt., respectively. More or less similar observations were also found in the case of root fresh wt. and dry wt. (Table 1). Therefore, overall 20 and 10 ppm concentrations of NPs show the best growth for brinjal seed germination.

The maximum shoot and root length (7.55 ± 2.63 cm) and (6.2 ± 2.06 cm) of tomato seeds was observed in the 20 ppm treatment of Mn and ZnFe, respectively, followed by ZnFe, ZnFeMn, and Zn in 20 ppm followed by 10 ppm. The maximum shoot fresh wt. and dry wt. (0.73 ± 0.24 gm) and (0.37 ± 0.12 gm) was found in 20 ppm of Mn and MnZn, respectively, followed by Zn (0.64 ± 0.21 gm) and ZnFeMn (0.63 ± 0.21 gm) in 20 ppm. The maximum root fresh wt. and dry wt. (0.68 ± 0.22 and 0.27 ± 0.09 gm) was observed in Mn followed by ZnFeMn, MnZn, and Zn in 20 ppm followed by 10 ppm (Table 2). Therefore, 20 ppm and 10 ppm concentrations of NPs show the best biomass growth for tomato seedlings.

The maximum shoot and root length (5.26 ± 1.75 cm) and (5.06 ± 1.68 cm) of chilli seeds was observed in the 20 ppm treatment of MnZn and Zn, respectively, followed by Zn, Mn, and FeMn in 20 ppm and 10 ppm. The maximum shoot fresh wt. and dry wt. (0.52 ± 0.17 gm) and (0.21 ± 0.07 gm) was found in 20 ppm of Mn and MnZn, respectively, followed by MnZn (0.51 ± 0.17 gm) and Zn (0.48 ± 0.16 gm) in 20 ppm. The maximum root fresh wt. and dry wt. (0.47 ± 0.15 , 0.45 ± 0.15 and 0.41 ± 0.13 gm) was observed in Mn MnZn and Zn in 20 ppm, respectively, followed by ZnFeMn and FeMn (Table 3). Hence,

20 ppm and 10 ppm concentration of NPs shows the best biomass growth for chilli seedlings.

3.7. Statistical Assessment of Nanofertilizers' Effects through ANOVA

The results (with 95% confidence) highlight the significant influence of different NPs types on these growth metrics, with some combinations standing out for their efficacy (Fig. 5). For SL, the ANOVA results (look at plot 10a) showed a significant effect of NPs type (f -value = 2.43, p -value = 0.029). MnO_2 NPss had the most substantial impact, with an average shoot length of 5.018 cm, compared to the control group's 2.947 cm. This suggests that MnO_2 can notably enhance shoot growth. Other effective treatments included MnO_2ZnO (4.538 cm) and $\text{ZnOFe}_2\text{O}_3\text{MnO}_2$ (4.327 cm), indicating these combinations also promote shoot elongation.

Similarly, RL was significantly influenced by the type of NPss used (f -value = 2.62, p -value = 0.019). The MnO_2 treatment resulted in the longest roots, with an average length of 4.437 cm, significantly longer than the control's 2.647 cm (see plot 10b). MnO_2ZnO (4.384 cm) and ZnOFe_2O_3 (4.071 cm) were also effective, showing that these combinations can improve root development. When assessing SFW, the ANOVA revealed significant differences (f -value = 3.24, p -value = 0.005). MnO_2 -treated seedlings had the highest mean shoot fresh weight at 0.4233 g, followed by MnO_2ZnO at 0.3622 g and ZnO at 0.3456 g (check plot 10c). These data suggest that MnO_2 -based NPss significantly boost the fresh biomass of shoots. In the case of SDW, significant differences were again observed (f -value = 2.93, p -value =

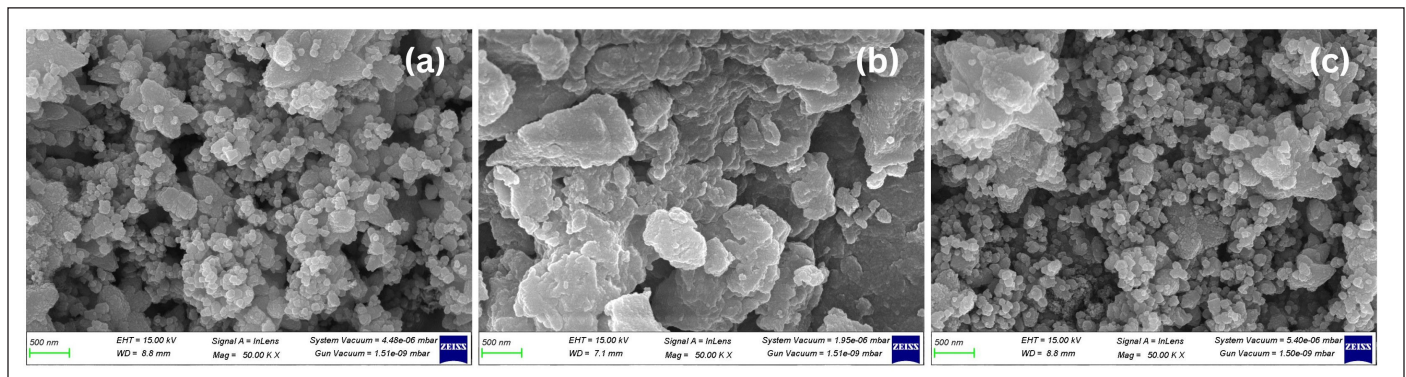


Figure 2. FESEM images of different NPss: a- ZnO, b- Fe_2O_3 , c- MnO_2

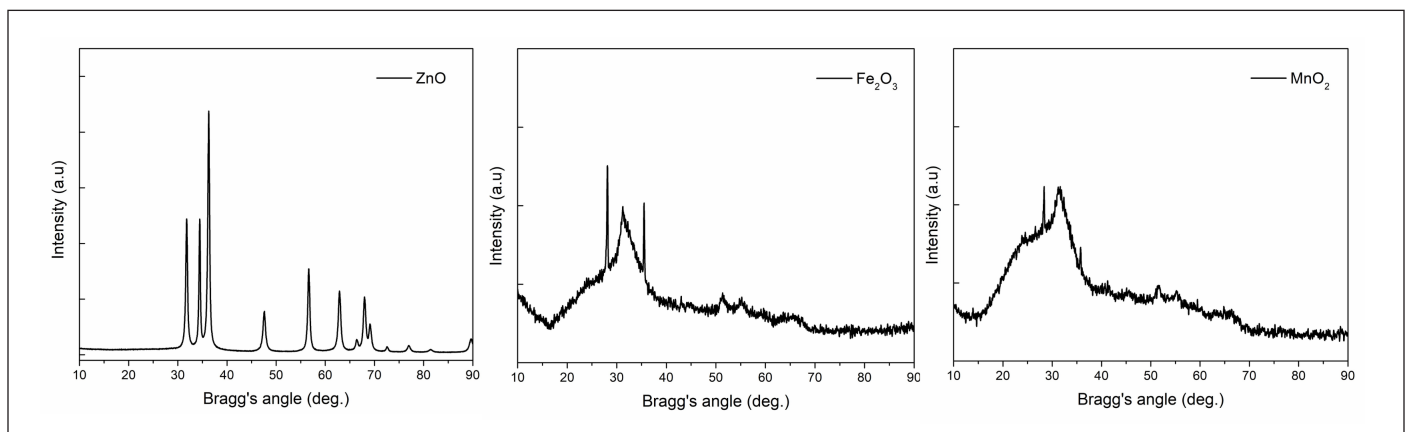


Figure 3. XRD pattern of green synthesized a-ZnO, b- Fe_2O_3 , and c- MnO_2 -NPs.

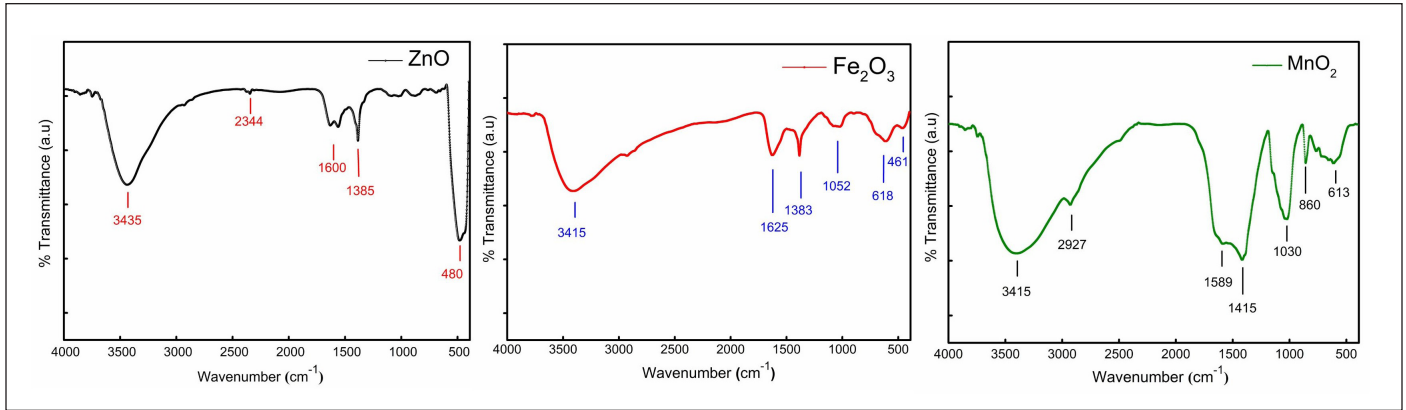


Figure 4. FTIR analysis of green synthesized a- ZnO, b- Fe₂O₃, c- MnO₂-NPs.

Table 1. Effect of different concentrations of ZnO, Fe₂O₃, and MnO₂ NPs on shoot and root length, shoot and root fresh wt., shoot and root dry wt. of germinated seeds of Brinjal.

Conc.	Cl.	Zn ppm			Fe ppm			Mn ppm			Zn+Fe ppm			Fe+Mn ppm			Mn+Zn ppm			Zn+Fe+Mn ppm		
		10	20	50	10	20	50	10	20	50	10	20	50	10	20	50	10	20	50	10	20	50
Shoot length cm.	2.96 ± 0.98	4.1 ± 1.36	4.89 ± 1.63	3.11 ± 1.03	2.9 ± 0.96	3.76 ± 1.25	2.41 ± 0.803	4.97 ± 1.65	7.91 ± 2.63	3.98 ± 1.326	3.84 ± 1.28	4.97 ± 1.65	2.86 ± 0.95	3.16 ± 1.05	3.92 ± 1.306	3.23 ± 1.07	4.16 ± 1.38	5.11 ± 1.703	3.9 ± 1.3	3.83 ± 1.27	5.9 ± 1.96	3.36 ± 1.12
Root length cm.	2.6 ± 0.86	3.43 ± 1.14	4.52 ± 1.50	2.94 ± 0.98	2.41 ± 0.803	3.24 ± 1.08	0.06 ± 0.02	4.21 ± 1.403	6.34 ± 2.11	3.48 ± 1.16	3.21 ± 1.07	4.38 ± 1.46	1.53 ± 0.51	2.54 ± 0.84	3.23 ± 1.07	2.41 ± 0.803	3.51 ± 1.17	4.29 ± 1.43	2.64 ± 0.88	3.16 ± 1.05	4.84 ± 1.61	2.34 ± 0.78
Shoot fresh wt. gm	0.08 ± 0.02	0.32 ± 0.10	0.38 ± 0.12	0.09 ± 0.03	0.09 ± 0.03	0.15 ± 0.05	0.06 ± 0.02	0.42 ± 0.14	0.75 ± 0.25	0.16 ± 0.053	0.14 ± 0.04	0.3 ± 0.1	0.07 ± 0.02	0.11 ± 0.036	0.18 ± 0.06	0.11 ± 0.036	0.37 ± 0.123	0.48 ± 0.16	0.14 ± 0.046	0.21 ± 0.07	0.42 ± 0.14	0.12 ± 0.04
Shoot dry wt. gm	0.04 ± 0.013	0.15 ± 0.05	0.17 ± 0.056	0.04 ± 0.013	0.05 ± 0.016	0.07 ± 0.023	0.03 ± 0.01	0.19 ± 0.063	0.36 ± 0.12	0.07 ± 0.023	0.06 ± 0.02	0.16 ± 0.053	0.04 ± 0.013	0.05 ± 0.016	0.08 ± 0.026	0.06 ± 0.02	0.17 ± 0.056	0.21 ± 0.07	0.09 ± 0.03	0.11 ± 0.036	0.22 ± 0.07	0.05 ± 0.016
Root fresh wt. gm	0.05 ± 0.016	0.27 ± 0.09	0.31 ± 0.103	0.07 ± 0.023	0.08 ± 0.026	0.12 ± 0.04	0.05 ± 0.016	0.38 ± 0.126	0.71 ± 0.23	0.11 ± 0.036	0.11 ± 0.036	0.17 ± 0.056	0.06 ± 0.02	0.09 ± 0.03	0.16 ± 0.053	0.11 ± 0.036	0.32 ± 0.106	0.41 ± 0.136	0.09 ± 0.03	0.19 ± 0.063	0.38 ± 0.123	0.09 ± 0.03
Root dry wt. gm	0.03 ± 0.01	0.13 ± 0.04	0.13 ± 0.04	0.06 ± 0.02	0.04 ± 0.013	0.07 ± 0.023	0.02 ± 0.006	0.16 ± 0.053	0.31 ± 0.103	0.07 ± 0.023	0.06 ± 0.02	0.09 ± 0.03	0.03 ± 0.01	0.04 ± 0.013	0.07 ± 0.023	0.07 ± 0.023	0.14 ± 0.046	0.19 ± 0.063	0.04 ± 0.013	0.09 ± 0.03	0.14 ± 0.046	0.04 ± 0.013

Table 2. Effect of different concentrations of ZnO, Fe₂O₃, and MnO₂ NPs on shoot and root length, shoot and root fresh wt., shoot and root dry wt. of germinated seeds of tomato.

Conc.	Cl.	Zn ppm			Fe ppm			Mn ppm			Zn+Fe ppm			Fe+Mn ppm			Mn+Zn ppm			Zn+Fe+Mn ppm		
		10	20	50	10	20	50	10	20	50	10	20	50	10	20	50	10	20	50	10	20	50
Shoot length cm.	3.9 ± 1.3	5.11 ± 1.70	6.21 ± 2.07	4.2 ± 1.4	4.89 ± 1.63	5.11 ± 1.703	3.4 ± 1.13	6.2 ± 2.06	7.55 ± 2.51	3.36 ± 1.12	5.9 ± 1.96	6.78 ± 2.26	4.27 ± 1.423	4.1 ± 1.36	5.8 ± 1.93	3.23 ± 1.076	5.62 ± 1.87	6.13 ± 2.04	4.4 ± 1.46	5.11 ± 1.703	6.56 ± 2.18	4.63 ± 1.54
Root length cm.	4.1 ± 1.36	4.88 ± 1.62	5.23 ± 1.74	3.21 ± 1.07	2.4 ± 0.8	3.7 ± 1.23	3.53 ± 1.17	5.64 ± 1.88	5.89 ± 1.96	2.76 ± 0.92	5 ± 1.66	6.2 ± 2.06	3.46 ± 1.15	5.63 ± 1.87	4.56 ± 1.52	3.96 ± 1.32	3.43 ± 1.14	6.03 ± 2.01	7.4 ± 2.46	4.34 ± 1.44	5 ± 1.66	2.33 ± 0.77
Shoot fresh wt. gm	0.16 ± 0.05	0.58 ± 0.19	0.64 ± 0.21	0.41 ± 0.136	0.41 ± 0.136	0.45 ± 0.15	0.11 ± 0.036	0.61 ± 0.203	0.73 ± 0.243	0.32 ± 0.106	0.57 ± 0.19	0.58 ± 0.193	0.32 ± 0.106	0.45 ± 0.15	0.39 ± 0.13	0.08 ± 0.026	0.49 ± 0.163	0.62 ± 0.206	0.41 ± 0.136	0.44 ± 0.146	0.63 ± 0.21	0.37 ± 0.123
Shoot dry wt. gm	0.07 ± 0.023	0.24 ± 0.08	0.29 ± 0.09	0.19 ± 0.06	0.21 ± 0.07	0.21 ± 0.07	0.05 ± 0.016	0.32 ± 0.106	0.34 ± 0.113	0.13 ± 0.043	0.28 ± 0.09	0.23 ± 0.076	0.23 ± 0.076	0.24 ± 0.08	0.17 ± 0.056	0.04 ± 0.013	0.24 ± 0.08	0.37 ± 0.123	0.17 ± 0.056	0.2 ± 0.06	0.24 ± 0.08	0.15 ± 0.05
Root fresh wt. gm	0.15 ± 0.05	0.46 ± 0.153	0.53 ± 0.176	0.38 ± 0.126	0.32 ± 0.106	0.37 ± 0.123	0.09 ± 0.03	0.52 ± 0.173	0.68 ± 0.226	0.27 ± 0.09	0.48 ± 0.16	0.46 ± 0.153	0.19 ± 0.063	0.37 ± 0.123	0.32 ± 0.106	0.07 ± 0.023	0.35 ± 0.116	0.52 ± 0.173	0.27 ± 0.09	0.36 ± 0.12	0.57 ± 0.19	0.31 ± 0.103
Root dry wt. gm	0.05 ± 0.016	0.19 ± 0.063	0.2 ± 0.066	0.15 ± 0.05	0.16 ± 0.053	0.16 ± 0.053	0.04 ± 0.013	0.23 ± 0.076	0.27 ± 0.09	0.11 ± 0.036	0.18 ± 0.06	0.19 ± 0.063	0.09 ± 0.03	0.17 ± 0.056	0.11 ± 0.036	0.03 ± 0.01	0.19 ± 0.063	0.23 ± 0.076	0.12 ± 0.04	0.11 ± 0.036	0.26 ± 0.086	0.14 ± 0.04

0.010). MnO₂ treatment led to the highest mean dry weight of shoots 0.1944 g, with MnO₂ZnO (0.1733 g) and ZnO (0.1522 g) also showing substantial increases compared to the control group's 0.04667 g (view plot 10d). These findings indicate that MnO₂ and its combinations

are effective in enhancing the dry biomass of shoots. For RFW, the analysis delineated significant variations (*f*-value = 3.36, *p*-value = 0.004). MnO₂ NPs resulted in the highest mean root fresh weight at 0.3767 g, followed by MnO₂ZnO at 0.2900 g and ZnO at 0.2889 g

Table 3. Effect of different concentrations of ZnO, Fe₂O₃, and MnO₂ NPs on shoot and root length, shoot and root fresh wt., shoot and root dry wt. of germinated seeds of chilli.

Conc.	Cl.	Zn ppm			Fe ppm			Mn ppm			Zn+Fe ppm			Fe+Mn ppm			Mn+Zn ppm			Zn+Fe+Mn ppm		
Hours		10	20	50	10	20	50	10	20	50	10	20	50	10	20	50	10	20	50	10	20	50
Shoot length cm.	1.98 ± 0.66	2.46 ± 0.88	4.91 ± 1.63	2.14 ± 0.71	2.3 ± 0.76	3.43 ± 1.14	1.97 ± 0.65	3.99 ± 1.33	4.7 ± 1.56	2.5 ± 0.83	3.1 ± 1.03	3.78 ± 1.26	2.7 ± 0.9	3.73 ± 1.24	4.23 ± 1.41	2.83 ± 0.94	3.86 ± 1.28	5.26 ± 1.75	2.4 ± 0.8	3.24 ± 1.08	3.98 ± 1.32	2.33 ± 0.77
Root length cm.	1.24 ± 0.41	2.6 ± 0.75	5.06 ± 1.68	2.1 ± 0.7	3.1 ± 1.03	3.73 ± 1.24	2.63 ± 0.87	3.78 ± 1.26	4.23 ± 1.41	3.6 ± 1.2	4.53 ± 1.51	4.76 ± 1.58	3.57 ± 1.19	4.3 ± 1.43	4.06 ± 1.35	3.4 ± 1.13	4.46 ± 1.48	4.87 ± 1.62	2.83 ± 0.94	3.88 ± 1.29	3.68 ± 1.22	2.67 ± 0.89
Shoot fresh wt. gm	0.08 ± 0.026	0.16 ± 0.053	0.48 ± 0.16	0.05 ± 0.016	0.05 ± 0.016	0.13 ± 0.043	0.04 ± 0.013	0.24 ± 0.08	0.52 ± 0.173	0.06 ± 0.02	0.08 ± 0.026	0.16 ± 0.053	0.07 ± 0.023	0.13 ± 0.043	0.32 ± 0.106	0.09 ± 0.03	0.19 ± 0.063	0.51 ± 0.17	0.05 ± 0.016	0.21 ± 0.07	0.38 ± 0.126	0.04 ± 0.013
Shoot dry wt. gm	0.04 ± 0.013	0.08 ± 0.026	0.19 ± 0.063	0.02 ± 0.006	0.02 ± 0.006	0.07 ± 0.023	0.01 ± 0.003	0.11 ± 0.036	0.19 ± 0.063	0.04 ± 0.013	0.04 ± 0.013	0.08 ± 0.026	0.03 ± 0.01	0.06 ± 0.02	0.14 ± 0.046	0.04 ± 0.013	0.08 ± 0.026	0.21 ± 0.07	0.02 ± 0.006	0.11 ± 0.036	0.14 ± 0.046	0.01 ± 0.003
Root fresh wt. gm	0.06 ± 0.02	0.13 ± 0.043	0.41 ± 0.136	0.04 ± 0.013	0.04 ± 0.013	0.11 ± 0.036	0.03 ± 0.01	0.19 ± 0.063	0.47 ± 0.156	0.06 ± 0.02	0.07 ± 0.023	0.13 ± 0.043	0.05 ± 0.016	0.12 ± 0.04	0.29 ± 0.096	0.07 ± 0.023	0.16 ± 0.053	0.45 ± 0.15	0.04 ± 0.013	0.16 ± 0.053	0.31 ± 0.103	0.03 ± 0.01
Root dry wt. gm	0.04 ± 0.013	0.06 ± 0.02	0.14 ± 0.046	0.01 ± 0.003	0.01 ± 0.003	0.05 ± 0.016	0.01 ± 0.003	0.09 ± 0.03	0.18 ± 0.06	0.03 ± 0.01	0.03 ± 0.01	0.06 ± 0.02	0.02 ± 0.006	0.05 ± 0.016	0.11 ± 0.036	0.03 ± 0.01	0.07 ± 0.023	0.18 ± 0.06	0.01 ± 0.003	0.06 ± 0.02	0.13 ± 0.043	0.01 ± 0.003

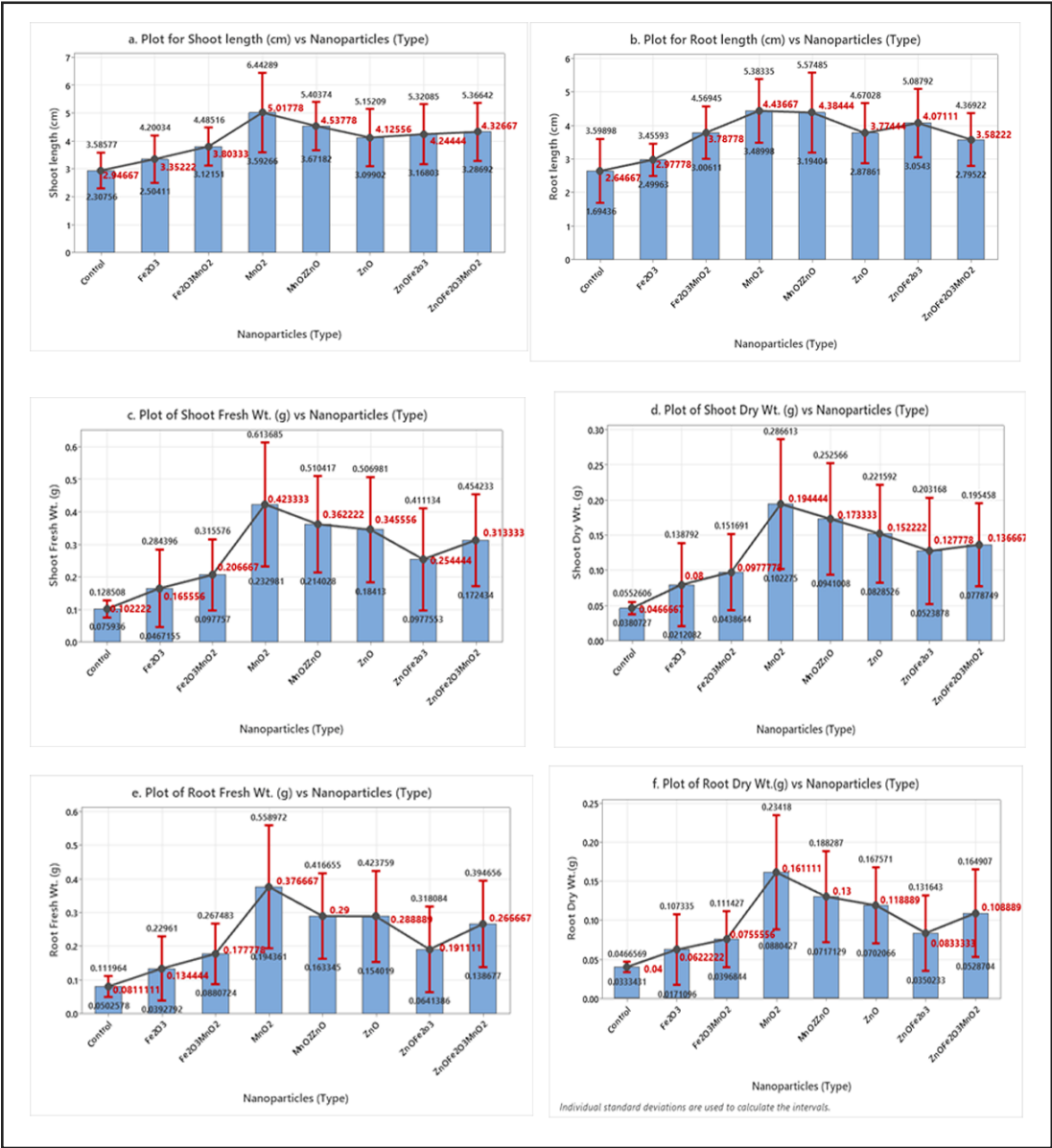


Figure 5. Error bar plots for various growth parameters.

(refer plot 10e). These results highlight MnO_2 's role in increasing root fresh biomass, making it a promising nanofertilizer. Finally, RDW also showed significant differences across treatments (f -value = 3.33, p -value = 0.004). The MnO_2 treatment achieved the highest root dry weight at 0.1611 g, with MnO_2ZnO (0.1300 g) and ZnO (0.1189 g) also showing significant improvements over the control (0.04,000 g). This underscores the effectiveness of MnO_2 NPss in enhancing root dry biomass (check plot 10f for elaborated explanation). Across all measured parameters, MnO_2 -based NPss consistently demonstrated the most significant impact on both shoot and root growth metrics. The MnO_2 , MnO_2ZnO , and $\text{ZnOFe}_2\text{O}_3\text{MnO}_2$ treatments significantly enhanced shoot length, root length, and both fresh and dry weights of shoots and roots. For instance, MnO_2 NPss increased shoot length to 5.018 cm and root length to 4.437 cm, far surpassing the control group's measurements. This suggests that MnO_2 NPss are particularly effective as nanofertilizers, offering a valuable tool for boosting the yield and biomass of vegetable crops, and potentially enhancing agricultural productivity.

4. DISCUSSION

Nanofertilizers supply the nutrients plants need at the right time and place, they offer a multitude of benefits to plants, including increased yield, quality, and quantity. This turns out to be the most extensively utilized application of NPss in farming [29]. The hardest problem is figuring out how much nanofertilizer to give the plant. Considering that boosting plant development and yield often relies on the applied nanofertilizer's concentration [30]. To manage the bioavailability of nutrients and ensure that they are exclusively taken by the plant and not lost to the surrounding environment, which includes soil, water, and related microbes, NPss can be employed as plant fertilizers [31]. However, using NPs-based nutrients topically offers a quicker and more effective means of capturing vital nutrients [32]. To protect the environment and attain sustainability, "green" NP has gained popularity to protect plants from biotic stress [33]. While improving seed germination and encouraging healthy seedlings, these nanofertilizers showed favorable and encouraging outcomes [34]. The data above show that all growth parameters rose significantly with NPss- MnO_2 , MnO_2ZnO , and $\text{ZnOFe}_2\text{O}_3\text{MnO}_2$ application over control with 20 ppm and 10 ppm, respectively. For instance, MnO_2 NPss resulted in an average shoot length of 5.018 cm and root length of 4.437 cm, substantially surpassing the control group's measurements. These findings highlight the potential of MnO_2 -based nanofertilizers to improve seedling biomass yield in vegetable crops, suggesting their promising application in sustainable agriculture.

The application of nanomaterials by foliar spraying offers a more secure method and keeps the soil safe from harm. As leaves are the fundamental components of photosynthesis, gas exchange, and transpiration, nanomaterials' foliar absorption can be accomplished with ease [35]. The study on Black gram revealed that treating seeds with 600 mg/l ZnO -NPs had a significant impact on the germination parameters; at this concentration, maximum root length, maximum germination length, maximum GP, and maximum vigor of seedlings were all noted [36]. Higher than 800 ppm ZnO -NP concentrations were found to possess detrimental and impeding effects on the growth of seedlings and germination in maize [37]. ZnO NPs decreased tomato growth at doses of 250, 500, 750, and 1,000 mg/l [38]. Even a low ZnO -NPs concentration has a noticeable impact on certain species. In a study on pearl millet, when ZnO -NPs (foliar application) were applied at concentrations less than 10 ppm, there was a noticeable increase in pigment, protein content, buds, and root growth [39]. The application

of modest concentrations of Zn has been found to stimulate the vigor of bean and wheat seedlings more than other ions [40]. García-López *et al.* [41] found no notable variations in the proportion of chilli seed germination (*Capsicum annuum* L.) interspersed between the various ZnO NP concentrations; yet, they discovered notable impacts on the vigor of seedlings that arose following ZnO NP treatment [41]. The ZnO -NPs increase the levels of phytohormones in the roots, such as indole acetic acid (IAA), which promotes root growth [42]. These findings would suggest that a high zinc content in seeds has a crucial physiological role in the early stages of seedling growth and seed germination [43].

Zinc has a beneficial effect on seed germination by raising the level of IAA. This improvement was ascribed to zinc NPss' increased precursor activity in the synthesis of auxin. An enzyme that affects the release of IAA, a phytohormone (auxin) that greatly controls plant growth, contains zinc [42]. Valadkhan *et al.* [44] found that when chickpeas were treated foliarly with nano-Fe, nano-Zn, and nano-Ca, in comparison to the control, there was an increase in the quantity of pods per plant, the quantity of seeds per plant, and the weight of the seeds [44]. According to Abusaleem *et al.* [45], tomatoes' vigor index was dramatically increased by 40 nm green synthesized Fe_2O_3 NPs. According to Karunakaran *et al.* [46], applying 56 nm Fe_2O_3 NPss to the root length increased its length. Watermelon and maize seeds germinated more readily when exposed to 20 mg/l Fe_2O_3 NPs, and root elongation could be accelerated by exposure to 20 and 50 mg/l Fe_2O_3 NPs [47]. Reduced root development was observed at increasing iron oxide NPs concentrations.

The root and shoot growth in mung beans increased by about 52% and 38%, respectively, by using MnO_2 NPs treatment [48], in the case of eggplant yield improved by about 22% [49]. The small size MnO_2 NPs from 1 to 100 nm, higher surface area, and their interaction may improve the solubility, expansion, and availability of plants, hence the yield of plants as compared to other fertilizers [50–52].

The synthesis and characterization of NPss required to be confirmed since ZnO , Fe_2O_3 , and MnO_2 NPs absorb most light between 320 and 380 nm in wavelength, the test's outcome was regarded as a preliminary validation of ZnO , Fe_2O_3 , and MnO_2 -NPs synthesis [25]. The FESEM analysis was utilized to more precisely investigate the form and size of NPss. The functional groups of the chemicals in the extract may have contributed to the roughly spherical forms of these NPs [53]. Electrostatic attraction, collision, polarity, and the high surface energy of ZnO , Fe_2O_3 , and MnO_2 NPs are the forces that ultimately shape the NPss. The XRD technique has also been used to gauge the degree of crystallinity and estimate the size of crystalline particles [54]. Bragg's law guides XRD operations and aids in determining NPs' Bragg reflection [55].

According to present research, soaking brinjal, tomato, and chilli seeds in various NPs concentrations significantly increased their growth which was dose-dependent. They entered the plant cell quickly and aided in the biomass development of the plant. The application of these NPs showed an increase in both fresh and dry weights in the 20 ppm followed by 10 ppm concentration range which improved seedling biomass as compared to 50 ppm as well as to control. It could be a successful technique for increasing seedling development in brinjal, tomato, and chilli and the best is to treat seeds with lower concentrations of ZnO , Fe_2O_3 , and MnO_2 NPs (10 and 20 ppm).

5. CONCLUSION

The current study provides an effective and repeatable protocol for the environmentally friendly green synthesis of ZnO , Fe_2O_3 , and MnO_2

NPs and emphasizes its use in enhancing the parameters of brinjal, tomato, and chilli seedling growth, as well as the fresh and dried weight of the shoot *in vitro*. Applying green synthesized NPss, may enhance seed germination and promote plant growth. Additionally, these NPs could be utilized as an easily absorbed type of micronutrient, enhancing agricultural yield in farmers' fields and supporting the effective establishment of crops under stressful circumstances. The higher concentrations of these NPss exhibited detrimental effects, but modest NPs concentrations enhanced germination and seedling growth. In this study, 20 ppm concentration of NPs-singly, double and in triple combination significantly enhances the growth and biomass of vegetable seedlings.

6. AUTHOR'S CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be an author as per the International Committee of Medical Journal Editors (ICMJE) requirements/guidelines.

7. CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

8. FUNDING

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9. ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

10. DATA AVAILABILITY

The experimental data used to support the findings of the study are included in the paper.

11. PUBLISHER'S NOTE

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12. USE OF ARTIFICIAL INTELLIGENCE (AI)-ASSISTED TECHNOLOGY

The authors declares that they have not used artificial intelligence (AI)-tools for writing and editing of the manuscript, and no images were manipulated using AI.

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