

Green synthesis of zinc oxide nanoparticles by using *Mentha spicata* and *Ocimum basilicum* leaves: Physical properties, foliar treatment, plant uptake and antibacterial potential

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Abstract

In this study, plant extracts were used as stabilizing agents to synthesize ZnO-NPs with a green chemistry approach. The plant extracts were obtained from the aromatic plants of mint and basil leaves. The physical properties of the produced nanoparticles were investigated using XRD, SEM and FTIR. The shape of ZnO-NPs was triangular, as revealed by SEM analysis. The effects of biologically synthesized ZnO-NPs on plants when they reach the soil were investigated on the growth of wheat. In addition, antibacterial potential of ZnO-NPs on gram-positive and gram-negative bacteria was investigated. Results showed that *S. aureus* was more sensitive to all three types of ZnO-NPs than *E. coli*. Synthesized ZnO-NPs were also given as foliar treatment in order to determine Zn^{+2} uptake by plants and potential toxic effects on the growth of wheat. M-ZnO-NPs and B-ZnO-NPs improved the germination percentage of wheat at 400 mg/L concentration (100%), while raw ZnO-NPs showed 90% germination at the same concentration. It was also revealed that plant growth varies depending on the species and concentrations of ZnO-NPs, therefore, if foliar treatments are applied to wheat, the threshold concentrations, sizes and species of ZnO-NPs should be carefully evaluated.

1. Introduction

Nano-sized semiconductors have recently received an increasing amount of interest due to their unique features, which make them suitable materials for a variety of applications, particularly in optoelectronic devices (Aldeen et al. 2022). Zinc oxide (ZnO) is thought to be one of the most promising semiconductor materials available for various applications. ZnO is cheap since it is the second most common metal oxide in the earth's crust (Lakshmipriya and Gopinath 2021). Zinc oxide nanoparticles (ZnO-NPs) with a large band gap (3.37 eV) and high binding energy (60 meV) are currently receiving a lot of interest due to the growth in nanotechnological advancements and research. Due to its low cost, non-toxicity at low concentrations, chemical stability, and capacity to modify its optical and electrical properties, it has a wide range of uses in fields such as environmental applications, chemistry, physics, biology, medicine, and electronics (Jayappa et al. 2020). ZnO-NPs distinguish out among metallic nanoparticles due to their biocompatible structure, possible antimicrobial effects, and optical, electrical, and photocatalytic qualities (Arumugam et al. 2021; Lal et al. 2022). This highlights the application of ZnO-NPs in various domains, including in solar cells, photocatalysis, and chemical sensors (Lakshmipriya and Gopinath 2021). Seeds, leaves, bark, plant biomass, and microorganisms have recently emerged as the most practical and environmentally safe method for creating nanoparticles (Kalishwaralal et al. 2010).

There are numerous straightforward methods for synthesizing ZnO-NPs. The most often utilized procedures include wet chemical, hydrolysis/condensation, and sol-gel processing (Erat and Yildirimcan 2019). The physical and chemical behavior of ZnO-NPs can be easily modified depending on the used methods and method conditions when producing nanoparticles. Most of these synthesis techniques are expensive and necessitate stringent experimental conditions (such as pressure, temperature, energy, and time). More importantly, these techniques call for hazardous chemicals that worsen environmental pollution, which is currently the most pressing issue, and threaten the viability of ecosystems. Due to

several of their characteristics, such as being quicker, less expensive, and more environmentally friendly than conventional methods, green synthesis of ZnO-NPs is receiving a lot of attention these days. In recent years, it is evident that there has been an increase in interest in metal oxide nanoparticles produced by the green synthesis method in order to create a strong antimicrobial effect. The most promising synthesis technique is the use of plant extracts in "green synthesis," which can be accomplished by using plant extracts or specific microorganisms (algae, fungi, bacteria, etc) (Aldeen et al. 2022; Dabhane et al. 2021). According to Alamdari et al. (2020), functional groups in the extract donate electrons, converting Zn^{2+} to Zn^+ . By converting Zn^{2+} to Zn^+ ions, the hydroxyl and phenolic chemicals in the leaf extracts are to blame for the production of NPs (Khan et al. 2019). Similar findings were proposed by Ezealisiji et al. (2019), who claimed that zinc nitrate was ionized in an aqueous formed Zn^{2+} , which was then reduced to Zn^+ by a phytochemical found in the extract. By hydrolysis process, the hydroxyl group present in the polyphenols might turn into zinc hydroxide (Basnet et al. 2018). Numerous plant and fruit extracts have been used to date in the synthesis of numerous metallic nanoparticles such as *Rubus fairholmianus* (Rajendran et al. 2021), *Thymus syriacus* (Şahin et al. 2022), *Mussaenda frondosa* (Jayappa et al. 2020), *Myrica esculenta* (Lal et al. 2022), and *Syzygium cumini* (Singh et al. 2022). Studies have also shown that ZnO-NPs made using green synthesis have a potent bactericidal effect on a variety of microorganisms (Aldeen et al. 2022; Lal et al. 2022). The primary mechanism at play in this case is that the antibacterial effect is caused by a contact between the receptors on the microorganism's cell wall and the surface of the nanoparticles created through green synthesis. However, there are no prior reports on the comparison of ZnO-NPs synthesized as green with plant extracts such as *Mentha spicata* (mint) and *Ocimum basilicum* (basil) to the best of our knowledge.

Therefore, in the present investigation, we aimed to establish a simple and sustainable procedure for the production of ZnO-NPs using the plants' extracts of mint and basil as a capping, stabilizing and reducing agents. The structure and morphology of the synthesized ZnO-NPs were studied by the standard characterization techniques. The antimicrobial activities of the green synthesized ZnO-NPs were evaluated by the agar well diffusion method. In addition, it was also aimed to determine the effect of ZnO-NPs synthesized from two different plant extracts on the growth factors and chlorophyll content of wheat (*Triticum aestivum* -İkizce 96). These nanoparticles were applied to the leaves of the plant by spray method and the effects of ZnO-NPs on plant growth were determined. In order to test whether the nanoparticles given by spraying are taken up by the plant, the plants were dissolved in the acid medium and their Zn contents were determined.

2. Materials And Methods

2.1. Materials

The following ingredients were obtained from Sigma-Aldrich: Ethanol (C_2H_6O), sodium hydroxide (NaOH), and zinc acetate dihydrate ($Zn(CH_3COO)_2 \cdot 2H_2O$, >98%). All other chemicals used in this study were of analytical grade. The two-stage Millipore Direct-Q₃ was used to create distilled water (DI).

2.1. Preparation of the plants' extract

Plant leaves used (mint and basil) were obtained from the market and washed twice with running tap water and then distilled water to remove any dust or other impurities before being utilized to prepare plant's extract. In a conical flask, 450 mL of double-distilled water and 30 g of leaves were mixed. The flask was then set on a hot plate that had been preheated to 80°C for 30 minutes. After cooling to room temperature, the light brown extracts were filtered twice using Whatman No.1 filter papers to get remove of any residues before being stored in a refrigerator for later use.

2.2. Green synthesis of ZnO-NPs

A typical biosynthesis of the ZnO-NPs was as follows (Abdullah et al. 2020): 33.33 g of the zinc precursor salt ($\text{Zn}(\text{CH}_3\text{COO})_2 \cdot 2\text{H}_2\text{O}$); Sigma Aldrich, 99.99%) was dissolved in 1000 mL distilled water that prepared as stock solution. Then 150 mL of this stock solution was added to 90 mL of the plants' extract and the mixture was vigorously stirred for 10 min. Subsequently, NaOH was added accordingly up to pH 12.0. The solution was continuously stirred at room temperature for 2 h to produce ZnO-NPs (Fig. 1). The obtained precipitate that revealed the presence of ZnO was filtered and washed with deionized water repeatedly. Finally, the precipitate was dried in an oven overnight at 60°C. The light-yellow powder of ZnO-NPs was ground into a fine powder, weighed for calculation of production efficiency per unit plant biomass and then stored for the characterization.

2.3. Characterization techniques

The morphology of ZnO-NPs was studied using scanning electron microscopy (SEM, FEI, Quanta FEG 250) with Cu-K radiation. To ascertain the chemical composition of the ZnO-NPs and the purity of the phase, X-ray Diffraction Spectroscopy (XRD, Bruker, D8 Advance) with a 2 scan was utilized. Using a Hach Lange DR 3900 UV/VIS spectrometer, the optical characteristics of the ZnO-NPs were investigated between 200 and 700 nm. A Bruker Alpha spectrometer was used to perform Fourier transform infrared (FT-IR) spectroscopy spanning the wavelength range of $400\text{--}4000\text{ cm}^{-1}$ to examine functional groups of ZnO-NPs. The powder samples were analyzed with the KBr pellet method.

2.4. Preparation of ZnO-NPs suspensions and seed germination test

Green synthesized ZnO-NPs suspensions at different concentrations (25, 50, 100, 200, and 400 mg/L) were prepared by using deionized water. The ultrasonication process were employed to complete homogenization for 30 min at 40 °C. By the way, the wheat (*Triticum aestivum*- İkizce-96) seeds were pre-sterilized with 70% ethanol for 30 s and then exposed to 3% NaOCl for 10 min. The sterilized seeds were washed with deionized water 5 times for 5 min. The 10 wheat seeds were placed in petri dishes which include double layer filter paper as an inert material. After the ultrasonication 5 mL suspension at different concentration of ZnO-NPs synthesized with mint (M-ZnO-NPs), basil (B-ZnO-NPs) extract or pure ZnO-NPs (ZnO-NPs) were added to petri dishes. The petri dishes were left for incubation for 7 days at

25°C in dark. For the control groups, the seeds were exposed to 5 mL deionized water. All the treatments were conducted in three replicates and completely randomized designed. After the seed germination process, the number of germinated seeds were counted, and the radicle and plumule length was measured using millimetric paper (Doğaroğlu 2019). The germination percentage and the seedling vigor index was calculated using Eq. 1.

$$\text{SeedlingVigorIndex (SVI)} = GP (\%) \times (RL + PL) \text{ Eq. 1}$$

Where GP means germination percentage (%), RL means radicle length (cm), and PL means plumule length (cm).

2.5. Pots experiments and foliar exposure

The germinated three healthy seedlings were transferred to the pots included 80 g turf (pH 6.0–7.0, organic matter: 76.4%, moisture equivalent: 107%) and watered with tap water (pH 7.3 and conductivity 108.9 mS/cm). The first foliar treatment was applied with the hand sprayer after a week of the seedlings were sown in pots. The three foliar treatments in total experiments were conducted in whole experiments with one-week interval. To prevent contamination of the turf, the pots (turf surface) were covered with aluminum foil before the foliar application (Adrees et al. 2021). Total quantity of green synthesized ZnO-NPs used per treatment in three foliar applications were 15 mL. Besides that, the chlorophyll content was measured by using (SPAD-502 chlorophyll meter (Konica–Minolta, Japan, 0.06 cm² measurement area) every week before foliar application and the chlorophyll values were given as SPAD value. Before the plant harvest, plant heights, fresh and dry weights of wheat (aerial parts) were measured.

2.6. Zn concentration in plants

To determine the effects of ZnO-NPs on plants after foliar applications, the harvested plants were weighed (as shoot fresh weight) and left in an oven to reach constant weight for 2 h at 65 °C. After the time went out, the dry plants were weighed, and they were digested in 5 mL 12 M HNO₃ on a hot plate at 220 °C (Doğaroğlu and Köleli 2017). In order to determine the Zn concentration of digested plant materials, they were analyzed by inductively coupled plasma (ICP-MS) (Agilent 7500ce Model).

2.7. Antimicrobial activity

The agar well diffusion method was used to determine the antibacterial effects of synthesized three types of NPs (raw ZnO-NPs, M-ZnO-NPs, and B-ZnO-NPs) on a gram-positive (*Staphylococcus aureus*) and a gram-negative (*Escherichia coli*) bacterial colony. In the first step, the bacterial colonies were inoculated to a Tryptic Soy Broth (TSB) and incubated at 37 °C for 24 h. After the incubation, *S. aureus* and *E. coli* active culture was transferred to new tubes include TSB and incubated again at 37 °C for 5 h. Then the bacterial cultures were transferred to TSB again and the absorbance was determined at 625 nm (~ 10⁶–10⁷ cfu/mL). For the spread plate technique, Tryptic Soy Agar (TSA) was poured into petri dishes, and after the solidification, 0.1 mL of activated bacterial cultures were spread on the surface of TSA homogenously. The 10 mm well diameters were formed, and wells were filled with 100 µL of three

concentrations of synthesized ZnO-NPs, M-ZnO-NPs, or B-ZnO-NPs (0.01, 0.02, 0.04 g/mL) solved in DMSO (dimethylsulfoxide) (10%) (Lal et al. 2022). The DMSO and antibiotic (Amoxicillin, 50 µg) were employed as a negative control (NC) and a positive control (PC), respectively. The petri dishes were left for the incubation at 37 °C for a night and the zone diameters (the diameters around the wells) were determined via Vernier caliper (Aldeen et al. 2022). All the antibacterial assays were performed in triplicate.

2.8. Statistical analyses

All the treatments were realized in three replicates. Statistical analysis was performed by one-way analysis of variance (ANOVA) using the statistical software SPSS version 20 (IBM Inc., New York, USA). The means were compared using Post Hoc least significant difference (LSD) test and the significance level is 5%.

3. Results And Discussion

3.1. Characterization of synthesized ZnO-NPs

The surface morphology of ZnO-NPs was determined and shown in Fig. 2. The clear crystals of triangle-like shape ZnO-NPs produced by the extracts of the mint and basil were visible in the SEM images. In SEM images, the particles are depicted in a uniform state. As is typical of nanoparticles made through green synthesis, the produced ZnO-NPs occasionally showed considerable aggregation formation. This is due to the fact that biosynthetic NPs have a higher surface area and that their long-lasting affinities cause them to aggregate or clump together (Vidya et al. 2013). Particle agglomeration is caused by the polarity of the nanoparticles and their electrostatic attraction (Aminuzzaman et al. 2018). As the pH value rises, the amount of H^+ and OH^- ions varies, which has an impact on the structure, morphology, and formation of ZnO, and smaller nanoparticles form at high pHs. Because there are more OH ions present, they are more strongly attracted to the positively charged Zn^{2+} , which encourages the development of strong Zn-O bonds in the structure (Wahab et al. 2009). According to Wang et al. (2008), altering the basicity of the solution allows for the customization of various ZnO morphologies, including rods and flowers. In SEM images, the average particle size of pure ZnO-NPs was 94.11 nm, while the average particle size of M-ZnO-NPs was 73.76 nm and the average particle size of B-ZnO-NPs was 86.82 nm. This result revealed that pure ZnO-NPs had larger particle size, the grain size of ZnO-NPs produced by green synthesis decreased, and the smallest grain size was in M-ZnO-NPs.

3.1.1. XRD analysis

The hexagonal wurtzite structure of ZnO nanoparticles is characterized by these peaks (JCPDS card number: 36-1451) (Kaliraj et al. 2019). The XRD diffractograms of the prepared green ZnO photocatalysts of B-ZnO-NPs and M-ZnO-NPs and raw ZnO-NPs were shown in in Fig. 3. Figure 3a and b showed distinct peaks in the XRD patterns at $2\theta = 31.76^\circ, 34.40^\circ, 36.20^\circ, 47.52^\circ, 56.58^\circ, 62.83^\circ, 66.36^\circ, 67.93^\circ, 69.07^\circ,$

72.52°, and 76.94° that match well with planes Miller indices (100), (002), (101), (102), (110), (103), (200), (112), (201), (004), and (202) for B-ZnO-NPs and M-ZnO-NPs (Kaliraj et al. 2019). The XRD peaks' distinct line widening validates the material's nanoscale particle size. The strong diffraction peaks and lack of impure reflections respectively validated the high crystallinity and purity of the produced ZnO-NPs. No peaks connected to impurities were discovered, and ZnO made by green synthesis was completely pure and incredibly crystalline in its unaltered native condition. These results support earlier findings (Abdullah et al. 2020; Rana et al. 2016). We conducted the experiment at ambient temperature rather than using high temperatures, in contrast to earlier investigations (Anbuvaran et al. 2015; Karnan et al. 2016). This implies that a quick, easy, and environmentally friendly method of synthesis might be able to create highly crystalline nanomaterial. Therefore, in terms of energy efficiency, this study has proven to be a superior and more viable choice for synthesizing ZnO-NPs.

3.1.2. FTIR analysis

The biomolecules in charge of the synthesis of ZnO-NPs were described and identified using FTIR analyzes in the range of 4000 and 400 cm^{-1} (Fig. 4). Because Zn^{2+} ions from zinc acetate attached with polyphenols present in the plant extract and reduced to Zn^+ during the synthesis of ZnO-NPs, it is known as a complex production of Zn^+ - polyphenols in the reaction solution. The FT-IR spectra of raw ZnO-NPs and green synthesized ZnO-NPs from plant extracts revealed similar peaks, which suggested this complex phenomenon. The FTIR spectrum of the M-ZnO-NPs (Fig. 4a) showed absorption bands at 3837, 3383, 2979, 2903, 1563, 1405, 1252, 1055, 889, 688, 543 and 484 cm^{-1} . The FTIR spectrum of the B-ZnO-NPs (Fig. 4b) showed absorption bands at 3385, 1563, 1414, 877, 688, 582, 552 and 481 cm^{-1} . The broad and very intense bands at 3383 and 3385 cm^{-1} could be attributed to the presence of the hydroxyl functional groups of alcohols and phenolic compounds that may exist in plant extracts. The peaks that appeared at around 2979 – 2903 cm^{-1} are assigned to C–H stretching in alkanes respectively (Abdullah et al. 2020). The peaks located at 1563 cm^{-1} are related to the C = C stretching vibrations of aromatic rings in polyphenolic compounds (Matinise et al. 2017). The peaks at 1405 and 1414 cm^{-1} arise from the COO- stretching mode of symmetrical acids. The peak of 1252 cm^{-1} may be related to the optical character identification of C-O stretching (Bharathi and Bhuvaneshwari 2019). The marked peak at FTIR spectrum of mint 1055 cm^{-1} can also be related to the stretching of the C–O, C-C and C-O-C bonds from saturated esters, alcohols, phenols, cycloalkanes and acid anhydrides in the plant extract (Aramugam et al. 2021; Moghaddam et al. 2017). ZnO's primary absorption band is located in the 400–600 cm^{-1} range (Ishwarya et al. 2018; Kumar et al. 2019). The Zn-O vibration is associated to the wide, strong band at 500 cm^{-1} (Tantiwatcharothai and Prachayawarakorn 2019). The bending vibrations of alkanes and alkenes (C = C bending) are observed at 889, 877 cm^{-1} and 688 cm^{-1} (Abdullah et al. 2021; Bharathi and Bhuvaneshwari 2019; Larkin 2017) (Table 1)

Table 1
The FTIR spectrum of the biosynthesized ZnO-NFs

Wavenumber (cm ⁻¹)		Type of vibration	Functional groups	References
M-ZnO-NPs	B-ZnO-NPs			
484, 543	481, 552, 582	stretching	Zn-O	Tantiwatcharothai and Prachayawarakorn, 2019
688	688	deformation (γ) not in plain CAr-H deformation (γ) O-H not in plain deformation (γ) = C-A not in plain	benzene-H liquid alcohols or phenols cykloallenes polienes	Larkin, 2017
889	877	C = C	bending alkene vinylidene	Abdullah et al., 2021 Bharathi and Bhuvaneshwari, 2019
1055	-	stretching (ν) C-O skeletal (ν) C-C stretching (ν) C-O-C	saturated esters alcohols and phenols cycloalkanes acid anhydrides	Aramugam et al., 2021; Moghaddam et al., 2017
1252	-	stretching (ν) C-O stretching (γ) C-C deformation (γ) not in plain = C-H	saturated esters-acetates dimers alcohols and phenols alkanes	Bharathi and Bhuvaneshwari, 2019
1405	1414	stretching (ν) -COO-	salts of symmetrical acids	Matinise et al., 2017
1563	1563	stretching (ν) -COO- stretching (ν) C = O	aromatic acids salts β-diketones (phenolic forms)	
2903-2979	-	stretching (ν) C-H	-CH ₂ -	Bharathi and Bhuvaneshwari, 2019; Abdullah et al., 2020

Wavenumber (cm ⁻¹)		Type of vibration	Functional groups	References
M-ZnO-NPs	B-ZnO-NPs			
3383	3385	stretching (ν) O–H	free –OH group	Abdullah et al., 2020

3.2. Seed germination and seedling parameters

The seed germination tests were conducted to determine the toxic effects of green synthesized ZnO-NPs on wheat seeds. According to the seed germination test results, it was determined that the M-ZnO-NPs and B-ZnO-NPs had not any significantly toxic effects on wheat seed germination. Green synthesized ZnO-NPs using mint plant extract was significantly increased seed germination of wheat ($p < 0.05$). Maximum and minimum seed germination percentages were determined as 100% in 400 mg/L M-ZnO-NP concentration and 83.3% in control groups, respectively. In addition, the synthesized B-ZnO-NPs significantly increased seed germination at 400 mg/L concentration ($p < 0.05$). Maximum and minimum seed germination were determined as 100% at 400 mg/L B-ZnO-NPs concentration and 80% at 50 mg/L concentration. The raw ZnO-NPs enhanced the seed germination of wheat, compared to control. However, this enhancement did not reach to the M-ZnO and B-ZnO-NPs treatments (Fig. 5a). Maximum seed germination percentage was determined at the concentration of 25, 50, and 200 mg/L ZnO-NPs (as 96.7%). It was determined that the best yield of seed germination percentage was obtained at all concentrations of M-ZnO-NPs, compared to control groups, raw ZnO-NPs, and B-ZnO-NPs. In addition, it was determined that the seed germination percentage of wheat increased with increasing M-ZnO-NPs concentrations. Results also showed that the presence of ZnO-NPs in three types increased the seed germination percentage of wheat at the almost all concentrations (Fig. 5a). These results can be explained by the nanoparticles can penetrate through the seed pores depending on their sizes. Also, since Zn is an essential micronutrient for living organisms, seeds are willing to take up these materials. The seed germination test is an important parameter to determine the positive, negative or no effects on plants of external materials that release to the environment. In addition, germination percentage is a parameter used to evaluate seed viability (Meher et al. 2020). Literature studies showed that the different Zn sources (bulk ZnO, ZnO-NPs, ZnSO₄, etc.) effects the seed germination process of different plants (Du et al. 2019; Adrees et al. 2021). However, there are little studies that conducted about green synthesized ZnO-NPs on wheat seed germination process (Meher et al. 2020; Sharma et al. 2022). Ittroutwar et al. (2020) showed that seed germination percentage of maize was promoted by the green synthesized ZnO-NPs using seaweed, compared to bulk ZnO and control groups. Singh et al. (2019) reported the similar results, the authors showed that green synthesized ZnO-NPs using aloe vera extract caused better common bread wheat seed germination than control groups. In the current study, seed germination results showed the same trend as the others. Meher et al. (2020) reported that green synthesized ZnO-NPs using periwinkle leaf extract had no significant effect on seed germination of wheat (*Triticum*

aestivum-WH1105 variety) at different concentrations (20–100 mg/L). These differences are estimated to depend on the biological source of the synthesis materials, the size and shape of the ZnO-NPs, as well as the wheat species.

In this study, seedling growth parameters such as root-stem length and seedling viability index were evaluated after seed germination. Measurements showed that applications of both M-ZnO-NPs and B-ZnO-NPs and ZnO-NPs increased root elongation at concentrations of 50 mg/L and 100 mg/L. Maximum root elongation was determined as 34.7% at 50 mg/L M-ZnO-NPs concentration, 30.43% at 100 mg/L B-ZnO-NPs concentration and 27.37% at ZnO-NPs concentration. Root elongation is very important for healthy plants, as roots are the first organs to respond to environmental factors and affect the entire life cycle of plants (Takatsuka and Umeda 2019; Taylor et al. 2021). These results demonstrated that there is a threshold to increase root-shoot elongation and increase metabolic/enzymatic activity. In this study, the root threshold was determined as 50 mg/L for the M-ZnO-NPs, while 25 mg/L was determined for the other ZnO-NPs types (Fig. 5b). In addition, the shoot threshold values were determined as 100 mg/L for M-ZnO-NPs and 50 mg/L for B-ZnO-NPs (Fig. 5c). It was determined that the application of B-ZnO-NPs ($p > 0.05$) and ZnO-NPs ($p < 0.05$) inhibited the shoot elongation of wheat at almost all concentrations compared to the control. Maximum shoot inhibition was observed as 14.45% at 400 mg/L B-ZnO-NPs concentration and 16.3% at 200 mg/L ZnO-NPs concentration. Asmat-Campos et al. (2022) reported this threshold of tomato seeds at the concentration of 100 mg/L green synthesized ZnO-NPs using coriander plant extract. Other authors reported that the maximum root and shoot elongation was determined at the concentration of 100 mg/L green synthesized ZnO-NPs as 12.1 cm and 20 cm, respectively (Ittroutwar et al. 2020). Depending on root and shoot length and germination percentage, the maximum seedling viability index (SVI) was measured at concentrations of 50 mg/L for M-ZnO-NPs and 100 mg/L for B-ZnO-NPs and ZnO-NPs (Fig. 5d).

3.3. Plant growth parameters

In order to determine the effects of foliar treatment of M-ZnO-NPs, B-ZnO-NPs and ZnO-NPs on wheat seedlings, some plant growth parameters such as chlorophyll content, plant height, plant fresh and dry weight were investigated. The chlorophyll content was measured using a chlorophyll meter and the results were given as the SPAD value (Fig. 6). The plants were grown for four weeks after the germination period. The foliar treatments were employed to seedlings sown in pots after first week, and the chlorophyll content was measured before every foliar treatment. Results showed that the chlorophyll contents of wheat treated with M-ZnO-NPs at different concentration decreased with time (from first week to fourth week) (Fig. 6a), while the chlorophyll content of control groups increased. The maximum chlorophyll content was determined at the concentration of 25 mg/L M-ZnO-NPs as 37.46 SPAD value at 1st week (before foliar treatment) (Fig. 6a) ($p < 0.05$). After the foliar treatment (2nd week), results showed that the foliar treatment caused to decrease on the chlorophyll value. It was assumed that the ZnO-NPs prevented the sunlight to enter the plant cells by covering the leaf surface (just like sunscreen). Thus, the chlorophyll synthesis decreased in plant cells. It was also determined that the chlorophyll content did not significantly change at the foliar treatment of 2nd and 3rd weeks, compared to control

group ($p > 0.05$). However, it decreased at the 4th week, compared to control significantly with the treatment of M-ZnO-NPs. The minimum chlorophyll content was determined at the concentration of 100 mg/L M-ZnO-NPs ($p < 0.05$). In addition, while the chlorophyll content values of wheat were similar with M-ZnO-NPs in the 1st week compared to the control, it increased with increasing B-ZnO-NPs in the 2nd week as in the 4th week (Fig. 6b) ($p < 0.05$). It is clearly seen that, the increase in chlorophyll content were more evident with increasing M-ZnO-NPs concentration at the 4th week. The minimum chlorophyll contents were determined at the ZnO-NPs foliar treatments between three types of nanomaterials (Fig. 6). It was thought that these differences depend on their sizes and the ability to stay on the leaf surface of the materials. In this study, it was determined that the size of green synthesized ZnO-NPs smaller than the raw ZnO-NPs (Fig. 2), as like Sahoo et al. (2021). The authors reported that the foliar treatments of green synthesized ZnO-NPs to mung bean plants showed more chlorophyll content than raw ZnO-NPs, because of their sizes. The increasing time and increasing ZnO-NPs concentration caused decreases of chlorophyll content of wheat, especially after 4 weeks (Fig. 6c).

Zinc is an essential micronutrient for plant that have key role on photosynthetic activities (Ittroutwar et al. 2020). However, toxic effects can be observed on different plants, especially at nanoscale and high concentrations. Wang et al. (2016) reported that ZnO-NPs showed inhibitory effects on chlorophyll synthesize of *Arabidopsis* plants at the concentration of 300 mg/L. In addition, Arumugam et al. (2021) showed that green synthesized ZnO-NPs using *Syzygium cumini* inhibited the chlorophyll content of *Sesamum indicum* while increasing plant growth. The decreases in chlorophyll content of plants may related with DNA damage (Arumugam et al. 2021) and the electron transport chain in plant cells (Azarin et al. 2023).

In order to determine the effects of raw ZnO-NPs and green synthesized ZnO-NPs on wheat growth, the plant heights and fresh and dry weights of plants were also evaluated. Figure 7a and Fig. 8 clearly showed the differences of wheat plant height after three foliar treatments of M-ZnO-NPs, B-ZnO-NPs, and ZnO-NPs. It was determined that the foliar treatment of M-ZnO-NPs did not significantly affect the plant height compared to control ($p > 0.05$), but there were significantly differences between groups depending on M-ZnO-NPs concentrations ($p < 0.05$). Maximum plant height was measured at a concentration of 200 mg/L M-ZnO-NP (Fig. 7a). Application of B-ZnO-NPs showed an increase in plant height with increasing B-ZnO-NP concentration (Fig. 7a), but this increase did not reach the control plants. Foliar application of ZnO-NPs also did not significantly affect wheat plant height ($p > 0.05$). There are conflicting results in the literature about the effects of foliar applications of ZnO-NPs on different plants (Adil et al. 2022; Keerthana et al. 2021; Azarin et al. 2023). It is assumed that the amount of Zn required for auxin production, which plays a role in plant elongation, may not be adequately supplied by the tested chemicals (Meher et al. 2020).

Shoo et al. (2021) reported that the economic yield of cereal crops is related to the dry matter of the plant. The authors noted that the dry matter of mung bean plants treated by foliar exposure of green synthesized ZnO-NPs was higher than that of raw ZnO-NPs. In the current study, significant decreases were determined in dry weights of 23.68% (50 mg/L) and 19.74% (100 mg/L) in M-ZnO-NPs application

($p < 0.05$), while dry weight values increased by 6.6% (25 mg/L), 17.1% (200 mg/L) and 7.9% (400 mg/L) ($p < 0.05$) compared to control plant (Fig. 7c). In addition, fresh weights decreased 8.1% (25 mg/L), 33.2% (50 mg/L) ($p < 0.05$), 24.4% (100 mg/L) ($p < 0.05$), and 11.1% (400 mg/L) compared to control except 200 mg/L concentration of M-ZnO-NPs (fresh weights increased as 14.5%) (Fig. 7b). Foliar treatment of B-ZnO-NPs at almost all concentrations decreased the fresh ($p < 0.05$) and dry weights of the wheat plant compared to the control, as did the raw ZnO-NPs (Fig. 7b and c). Although there are studies in the literature showing the effects of ZnO-NPs on wheat seedling growth parameters, no studies were found in which green synthesized ZnO-NPs were exposed to leaves on wheat and pot trials were conducted. Studies conducted in pots have shown differential effects of green synthesized or raw ZnO-NPs on wheat. Meher et al. (2020) reported that green synthesized ZnO-NPs increased the fresh and dry weight of wheat seedlings. The most important factor affecting the effect of these parameters is the concentration of ZnO-NPs. Doğaroğlu and Köleli (2017) examined the ZnO-NP concentrations affecting the shoot dry weight of wheat, and they reported that the maximum and minimum dry weights were obtained at 40 mg/L and 10 mg/L ZnO-NP concentrations, respectively. Du et al. (2019) reported that 20 mg/L ZnO-NP and 200 mg/L ZnSO₄ concentration increased the growth parameters of wheat. However, the highest concentration of both Zn sources (1000 mg/L) caused an inhibition on the dry weight of wheat. Plant dry weight can be used as an indicator of accumulation of ZnO-NPs by plant tissue. Knowledge of the mechanism of penetration of ZnO-NPs into plant tissue is still unclear (Du et al. 2019; Zhu et al. 2020). For this reason, Zhu et al. (2020) provided information on the mechanism of entry of ZnO-NPs into the wheat leaf. According to the authors, ZnO-NPs can penetrate in the wheat cells via stomal aperture. After penetrating into cells, they accumulate in the apoplast and are released as Zn ions and can be absorbed by mesophyll cells (Zhu et al. 2020). In the present study, the ZnO-NP concentration in the leaves of the wheat plant was also determined. The results showed that all sources of ZnO-NPs entered wheat leaves at almost all concentrations (Fig. 9). It was determined that the M-ZnO-NPs significantly penetrated in wheat leaves low concentrations (25mg/L and 50 mg/L) compared to control, however at the higher concentrations, the Zn uptake was decreased. However, these uptakes were lower than the other types of ZnO-NPs. The order of Zn concentration in plants was found as ZnO-NPs > B-ZnO-NPs > M-ZnO-NPs. In B-ZnO-NPs treatment, Zn uptake decreased with increasing concentration, but was still higher than in control. The maximum Zn uptake by wheat was determined at the concentration of 50 mg/L ZnO-NPs, and it increased with increasing ZnO-NPs concentrations. The results showed that phytotoxic effects were related to Zn uptake by plants, depending on other parameters. It is known that nanoparticle sizes are effective in penetration into the stomatal opening and accumulation in plant cells. However, in this study, it was determined that three types of ZnO-NPs were not bioavailable for plants.

3.4. Antibacterial Activity

Antimicrobial activity tests were performed to determine the effects of ZnO-NPs and green synthesized M-ZnO-NPs and B-ZnO-NPs on two different bacteria. For this, *Staphylococcus aureus* as gram positive bacteria and *Escherichia coli* as gram negative bacteria were chosen because they are the most used

bacterial species in the literature (Siddiqi et al. 2018; Dabhane et al. 2021; Lal et al. 2022). There are various studies in the literature about the antibacterial potential of different NPs synthesized via green approach or chemically, such as Fe_2O_3 , Co_3O_4 (Batool et al. 2022), Cu-Ni hybrid nanomaterials (Abdullah et al. 2022), Co-ZnO-NPs (Ameur et al. 2019), Ag NPs (Bankar et al. 2010), etc. Synthesized ZnO-NPs via green approach or chemically route have the largest share among these studies due to the properties mentioned in the introduction (Joshi et al. 2018; Ansari et al. 2020; Dmochowska et al. 2020; Daphane et al. 2021; Lal et al. 2022). In these reports, it was stated that metallic NPs have a good antimicrobial potential compared to the positive control (antibiotics) (Batool et al. 2022). It is known that the magnitude of the effects (zone inhibition) here usually depends on the NPs concentrations. In the current study, the bacterial strains exposed to 0.01, 0.02, 0.04 g/mL synthesized ZnO-NPs. Results showed that the 0.01 g/mL and 0.02 g/mL concentration of M-ZnO-NPs was not sufficient amount to determine the effectiveness on *E. coli*. It was determined that the M-ZnO-NPs negatively affected on gram-negative bacterial (*E. coli*) strains at the concentration of 0.04 g/mL, while the zone inhibition of gram positive bacterial (*S. aureus*) strains slightly increased with increasing M-ZnO-NPs (Fig. 10). The inhibition zone diameters were determined at the concentration of 0.01, 0.02, and 0.04 g/mL M-ZnO-NPs on *S. aureus* as 14.73 mm (45.9%), 15.37 mm (47.9%), and 17.83 mm (55.6%), respectively. In addition, the synthesized B-ZnO-NPs negatively affected the activation of *E. coli* only at the highest concentration (0.04 g/mL, 71.42%), while M-ZnO-NPs were more effective on *S. aureus* at all concentrations. The inhibition zone diameters were measured as 14.51 mm, 15.24 mm, and 16.14 mm for *S. aureus* and the percentage was calculated as 44.08%, 46.26%, and 48.99%, depending on the increasing B-ZnO-NPs concentration, respectively. The maximum inhibition zone diameters were determined in the treatment of raw ZnO-NPs compared to M-ZnO and B-ZnO-NPs. It was measured as 81.67% for *E. coli* and 72.81% for *S. aureus* at the concentration of 0.04 g/mL ZnO-NPs. It was determined that the synthesized three types of ZnO-NPs can be toxic on both *E. coli* and *S. aureus* bacteria when there were enough materials in the growth media. These results showed that there is a threshold concentration in the resistance of each bacterium to different materials. The results also indicated that gram-positive bacteria (*S. aureus*) were more susceptible to all three types of ZnO-NPs compared to gram-negatives (*E. coli*), as reported by Ansari et al. (2020). In this study, although the effect mechanism of ZnO-NPs on bacterial activation was not studied, the other researchers explained the effect mechanisms. The effect mechanisms of external materials on bacterial activities can be explained as cell membrane and cell wall destruction due to NPs deposited on the bacterial surface, electrostatic interaction between Zn^{+} and cell wall, cellular destruction caused by the production of reactive oxygen species in the bacterial cell and/or cell wall structures of the bacteria (Ansari et al. 2020; Aldeen et al. 2022). Table 2 shows the comparative study of the antibacterial activity of ZnO-NPs produced by green synthesis in previous studies.

Table 2

Comparative study of antibacterial activity of green synthesized of ZnO-NPs with previous works

Plant	Morphology	Crystal Size	Antibacterial assay (zone diameter) (mm)		Effective concentration of ZnO-NPs (mg/mL)	Ref.
			Gram-positive	Gram-negative		
<i>Thymus syriacus</i>	Spherical-like	17.19	35.00	19.33	500 mg/mL	Şahin et al., 2022
<i>Phoenix roebelenii</i> leaves	Spherical	15 nm	15.8	15.00	4 mg/mL	Aldeen et al., 2022
<i>Garcinia Cambogia</i> fruit pulp	Semi-spherical	32.8 nm	-	19.00	0.1 mg/mL	Sasi et al., 2022
<i>Cinnamomum verum</i>	Hexagonal Wurtzite	45 nm	16.75	13.25	2 mg disc ⁻¹	Ansari et al., 2020
<i>Mentha spicata</i> extract	Hexagonal Wurtzite		17.83	13.51	40 mg/mL (0.04 g/mL)	Current study
<i>Ocimum basilicum</i> extract	Hexagonal Wurtzite		16.14	14.21	40 mg/mL (0.04 g/mL)	Current study

4. Conclusion

In this study, the effects of foliar treatment of green synthesized ZnO nanoparticles using mint (*Mentha spicata*) and basil (*Ocimum basilicum*) plant extract, and raw ZnO nanoparticles on wheat (*Triticum aestivum* -ikizce 96) growth parameters were evaluated. It was also tested the antibacterial potential of three types of synthesized ZnO-NPs. The characterization studies showed that the synthesized ZnO-NPs was in a uniform size and morphology, also they had purity and high crystallinity. The XRD results proved the synthesized ZnO-NPs were in hexagonal wurtzite morphology. Besides that, the M-ZnO-NPs and B-ZnO-NPs improved the wheat seed germination percentage at the concentration of 400 mg/L (100%), while raw ZnO-NPs showed 90% germination at the same concentration. It also concludes that green synthesized ZnO nanoparticles can be used as an effective material for seed germination. The improvement threshold was determined at the concentration of 50 mg/L of M-ZnO NPs and 25 mg/L for B-ZnO NPs and raw ZnO-NPs for root elongation, while it was determined at the concentration of 100 mg/L M-ZnO-NPs and 50 mg/L B-ZnO-NPs for shoot elongation. However, raw ZnO-NPs inhibited the shoot elongation of wheat seedling. The foliar exposure of M-ZnO-NPs and raw ZnO-NPs caused

decreases on chlorophyll content, while it increased at the treatment of B-ZnO-NPs. When the plant heights (aerial parts) were evaluated, the maximum yield was determined as ZnO-NPs > M-ZnO-NPs > B-ZnO-NPs. In addition to, plant growth and production changed depending on the types of ZnO-NPs and concentrations, such as, it was determined at the concentration of 200 mg/L foliar treatments of M-ZnO-NPs. Thus, if the foliar treatments are applied to wheat, the threshold concentrations, size, and types of ZnO-NPs should be considered carefully. Since, to determine and understand the maximum benefits and the minimum phytotoxicological effects of ZnO-NPs in agricultural application, it should be detailed investigated the interactions between plants and ZnO NPs. In addition to, in this study, the antibacterial potential of the synthesized ZnO-NPs on *E. coli* and *S. aureus* bacterial culture was determined. These bacterial species can be found all over the world and in the soil. Therefore, antibacterial potentials were also discussed. Results showed that *S. aureus* was more sensitive to all three types of ZnO-NPs than *E. coli*. The activity level on *E. coli* was determined as ZnO NPs > B-ZnO NPs > M-ZnO NPs, while on *S. aureus* it was determined as ZnO NPs > M-ZnO NPs > B-ZnO NPs. This result indicated that the synthesized ZnO NPs can be used effectively on *S. aureus* (gram-positive bacteria) to keep healthy plants rhizosphere in soil or to prevent contamination of plants leaves via foliar spraying.

Declarations

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Author Contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Yağmur Uysal, Zeynep Gökem Doğaroğlu, Zehranur Çaylalı and Delil Sefkan Karakulak. The first draft of the manuscript was written by Yağmur Uysal and Zeynep Gökem Doğaroğlu. All authors read and approved the final manuscript.

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Data availability

All data collected and reviewed are included in this article and properly referenced.

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References

1. Abdullah FH, Abu-Bakar NHH, Abu Bakar M (2020) Low temperature biosynthesis of crystalline zinc oxide nanoparticles from *Musa acuminata* peel extract for visible-light degradation of methylene blue. *Optik - Int J Light and Elect Optics* 206:164279
2. Abdullah FH, Abu Bakar NHH, Abu Bakar M (2021) Comparative study of chemically synthesized and low temperature bio-inspired *Musa acuminata* peel extract mediated zinc oxide nanoparticles for enhanced visible-photocatalytic degradation of organic contaminants in wastewater treatment. *J Haz Mat* 406:124779
3. Abdullah H, Ginting RT, Shuwanto H, Kuo D-H (2022) Banana peel biowaste-derived carbon composited with Zn(O,S) for solar-light photocatalytic hydrogen generation. *Int J of Hydro Ener* 47(97):41021–41033
4. Adil M, Bashir S, Bashir S et al (2022) Zinc oxide nanoparticles improved chlorophyll contents, physical parameters, and wheat yield under salt stress. *Front Plant Sci* 13:932861. [10.3389/fpls.2022.932861](https://doi.org/10.3389/fpls.2022.932861)
5. Adrees M, Khan ZS, Hafeez M, Rizwan M, Hussain K, Asrar M, Alyemeni MN, Wijaya L, Ali S (2021) Foliar exposure of zinc oxide nanoparticles improved the growth of wheat (*Triticum aestivum* L.) and decreased cadmium concentration in grains under simultaneous Cd and water deficient stress. *Ecotox and Enviro Safety* 208:111627
6. Alamdari S, Ghamsari MS, Tafreshi MJ (2020) Novel scintillation properties by entrapping ZnO: Ga nanocrystals in epoxy polymer. *Progress in Nucl Ener* 130:103495. <https://doi.org/10.1016/j.pnucene.2020.103495>
7. Aldeen TS, Mohamed HEA, Maaza M (2022) ZnO nanoparticles prepared via a green synthesis approach: Physical properties, photocatalytic and antibacterial activity. *J Phys and Chem of Solids* 160:110313
8. Ameer SB, BelHadjltaief H, Duponchel B et al (2019) Enhanced photocatalytic activity against crystal violet dye of Co and In doped ZnO thin films grown on PEI flexible substrate under UV and sunlight irradiations. *Heliyon* 5:e01912
9. Aminuzzaman M, Ying LP, Goh W-S, Watanabe A (2018) Green synthesis of zinc oxide nanoparticles using aqueous extract of *Garcinia mangostana* fruit pericarp and their photocatalytic activity. *Bull Mater Sci* 41. [10.1007/s12034-018-1568-4](https://doi.org/10.1007/s12034-018-1568-4)
10. Anbuvaran M, Ramesh M, Viruthagiri G, Shanmugam N, Kannadasan N (2015) *Anisochilus carnosus* leaf extract mediated synthesis of zinc oxide nanoparticles for antibacterial and

photocatalytic activities. Mater Sci Semicond Proc 39:621–628

11. Ansari MA, Murali M, Prasad D et al (2020) Cinnamomum verum bark extract mediated green synthesis of ZnO nanoparticles and their antibacterial potentiality. Biomolecules 10:336. 10.3390/biom10020336
12. Asmat-Campos D, López-Medina E, Montes de Oca-Vásquez G et al (2022) ZnO Nanoparticles obtained by green synthesis as an alternative to improve the germination characteristics of *L. esculentum*. Molecules 27:2343. <https://doi.org/10.3390/molecules27072343>
13. Arumugam M, Manikandan DB, Dhandapani E et al (2021) Green synthesis of zinc oxide nanoparticles (ZnO NPs) using *Syzygium cumini*: Potential multifaceted applications on antioxidants, cytotoxic and as nanonutrient for the growth of *Sesamum indicum*.. Environ Tech Innova 23:101653. <https://doi.org/10.1016/j.eti.2021.101653>
14. Azarin K, Usatov A, Minkina T et al (2023) Effects of bulk and nano-ZnO particles on functioning of photosynthetic apparatus in barley (*Hordeum vulgare* L.). Environ Res 216:114748. <https://doi.org/10.1016/j.envres.2022.114748>
15. Bankar A, Joshi B, Kumar AR, Zinjarde S (2010) Banana peel extract mediated novel route for the synthesis of silver nanoparticles. Coll and Surf A: Physicochem Eng Asp 368:58–63. 10.1016/j.colsurfa.2010.07.024
16. Basnet P, Chanu TI, Samanta D, Chatterjee S (2018) A review on bio-synthesized zinc oxide nanoparticles using plant extracts as reductants and stabilizing agents. J Photochem and Photobiol B: Biology 183:201–221. <https://doi.org/10.1016/j.jphotobiol.2018.04.036>
17. Batool S, Hasan M, Dilshad M et al (2022) Green synthesis of *Cordia myxa* incubated ZnO, Fe₂O₃, and Co₃O₄ nanoparticle: Characterization, and their response as biological and photocatalytic agent. Adv Pow Tech 33:103780. <https://doi.org/10.1016/j.appt.2022.103780>
18. Bharathi D, Bhuvaneshwari V (2019) Synthesis of zinc oxide nanoparticles (ZnO NPs) using pure bioflavonoid rutin and their biomedical applications: antibacterial, antioxidant and cytotoxic activities. Res Chem Intermed 45(4):2065–2078. <http://dx.doi.org/10.1007/s11164-018-03717-9>
19. Dabhane H, Zate M, Bharsat R, Jadhav G, Medhane V (2021) A novel bio-fabrication of ZnO nanoparticles using cow urine and study of their photocatalytic, antibacterial and antioxidant activities. Inorg Chem Comm 134:108984. <https://doi.org/10.1016/j.inoche.2021.108984>
20. Dmochowska A, Czajkowska J, Jędrzejewski R, Stawiński W, Migdał P, Fiedot-Toboła M (2020) Pectin based banana peel extract as a stabilizing agent in zinc oxide nanoparticles synthesis. Int J Biol Macromol 165:1581–1592. <https://doi.org/10.1016/j.ijbiomac.2020.10.042>
21. Doğaroğlu ZG (2019) Role of EDDS and ZnO-nanoparticles in wheat exposed to TiO₂Ag-nanoparticles. Arch of Environ Protec 45(4):78–83. 10.24425/aep.2019.130244
22. Doğaroğlu ZG, Köleli N (2017) TiO₂ and ZnO Nanoparticles Toxicity in Barley (*Hordeum vulgare* L.). Clean – Soil Air Water 45:1700096. 10.1002/clen.201700096

23. Du W, Yang J, Peng Q, Liang X, Mao H (2019) Comparison study of zinc nanoparticles and zinc sulphate on wheat growth: From toxicity and zinc biofortification. *Chemosphere* 227:109e116. <https://doi.org/10.1016/j.chemosphere.2019.03.168>
24. Erat S, Yildirimcan S (2019) Investigation of oxygen-related defects in ZnO: growing time and Mn concentration effects. *Turkish J of Eng* 3(2):68–75. 10.31127/tuje.453593
25. Ezealisiji KM, Siwe-Noundou X, Maduelosi B, Nwachukwu N, Krause RWM (2019) Green synthesis of zinc oxide nanoparticles using *Solanum torvum* (L) leaf extract and evaluation of the toxicological profile of the ZnO nanoparticles–hydrogel composite in Wistar albino rats. *Int Nano Lett* 9(2):99–107. <http://dx.doi.org/10.1007/s40089-018-0263-1>
26. Ishwarya R, Vaseeharan B, Subbaiah S et al (2018) Sargassum wightii-synthesized ZnO nanoparticles – from antibacterial and insecticidal activity to immunostimulatory effects on the green tiger shrimp *Penaeus semisulcatus*. *J Photochem and Photobiol B: Biol* 183:318–330. <https://doi.org/10.1016/j.jphotobiol.2018.04.049>
27. Ittroutwar PD, Kasivelu G, Raguraman V, Malaichamy K, Sevathapandian SK (2020) Effects of biogenic zinc oxide nanoparticles on seed germination and seedling vigor of maize (*Zea mays*). *Biocat and Agri Biotech* 29:101778. <https://doi.org/10.1016/j.bcab.2020.101778>
28. Jayappa MD, Ramaiah CK, Kumar MAP, Suresh D, Prabhu A, Devasya RP, Sheikh S (2020) Green synthesis of zinc oxide nanoparticles from the leaf, stem and in vitro grown callus of *Mussaenda frondosa* L.: characterization and their applications. *Appl Nanosci* 10:3057–3074. <https://doi.org/10.1007/s13204-020-01382-2>
29. Joshi S, Patel D, Chandra H, Bhatt SC (2018) Green synthesis of zinc oxide nanoparticle of *Thymus vulgaris* L. leaves and its antibacterial activity. *J Plant Develop Sci* 10(10):533–537
30. Kalishwaralal K, Deepak V, Pandian SRK, Kottaisamy M, BarathManiKanth S, Kartikeyan B, Gurunathan S (2010) Biosynthesis of silver and gold nanoparticles using *Brevibacterium casei*. *Colloids Surf B* 77(2):257–262. <http://dx.doi.org/10.1016/j.colsurfb.2010.02.007>
31. Kaliraj L, Ahn JC, Rupa EJ, Abid S, Lu J, Yang DC (2019) Synthesis of panos extract mediated ZnO nano-flowers as photocatalyst for industrial dye degradation by UV illumination. *J Photochem Photobiol B* 199:111588
32. Karnan T, Selvakumar SAS (2016) Biosynthesis of ZnO nanoparticles using rambutan (*Nephelium lappaceum* L.) peel extract and their photocatalytic activity on methyl orange dye. *J Mol Struct* 1125:358–365
33. Keerthana P, Vijayakumar S, Vidhya E, Punitha VN, Nilavukkarasi M, Praseetha PK (2021) Biogenesis of ZnO nanoparticles for revolutionizing agriculture: A step towards anti -infection and growth promotion in plants. *Indust Crops Prod* 170:113762
34. Khan I, Saeed K, Khan I (2019) Nanoparticles: Properties, applications and toxicities. *Arab J Chem* 12(7):908–931. <http://dx.doi.org/10.1016/j.arabjc.2017.05.011>
35. Kumar RS, Rajendran D, Balaraman HB, Viswanathan G (2019) Functional deep eutectic solvent-based chaotic extraction of phycobiliprotein using microwave-assisted liquid-liquid micro-extraction

- from *Spirulina (Arthrospira platensis)* and its biological activity determination. *Algal Res* 44:101709. <https://doi.org/10.1016/j.algal.2019.101709>
36. Lakshmipriya T, Gopinath SCB (2021) Introduction to nanoparticles and analytical devices. In: *Nanoparticles in Analytical and Medical Devices*, pp. 1–29, 2021
 37. Lal S, Verma R, Chauhan A et al (2022) Antioxidant, antimicrobial, and photocatalytic activity of green synthesized ZnO-NPs from *Myrica esculenta* fruits extract. *Inorg Chem Comm* 141:109518. <https://doi.org/10.1016/j.inoche.2022.109518>
 38. Larkin P (2017) *Infrared and Raman spectroscopy: principles and spectral interpretation*. Elsevier
 39. Matinise N, Fuku XG, Kaviyarasu K, Mayedwa N, Maaza M (2017) ZnO nanoparticles via *Moringa oleifera* green synthesis: Physical properties & mechanism of formation. *Appl Surf Sci* 406:339–347. <https://doi.org/10.1016/j.apsusc.2017.01.219>
 40. Meher BB, Sahu S, Singhal S, Joshi M, Maan P, Gautam S (2020) Influence of green synthesized zinc oxide nanoparticles on seed germination and seedling growth in wheat (*Triticum aestivum*). *Int J Curr Microbiol App Sci* 9(5):258–270. <https://doi.org/10.20546/ijcmas.2020.905.029>
 41. Moghaddam M, Mehdizadeh L (2017) Chemistry of essential oils and factors influencing their constituents. In: A. M. Grumezescu, A. M. Holban (Eds) *Handbook of Food Bioengineering, Soft Chemistry and Food Fermentation*, Academic Press pp. 379–419. <https://doi.org/10.1016/B978-0-12-811412-4.00013-8>
 42. Rajendran NK, George BP, Houreld NN, Abrahamse H (2021) Synthesis of zinc oxide nanoparticles using *Rubus fairholmianus* root extract and their activity against pathogenic bacteria. *Molecules* 26:3029. <https://doi.org/10.3390/molecules26103029>
 43. Rana N, Chand S, Gathania AK (2016) Green synthesis of zinc oxide nano-sized spherical particles using *Terminalia chebula* fruits extract for their photocatalytic applications. *Int Nano Lett* 6:91–98
 44. Sahoo SK, Dwivedi GK, Dey P, Praharaj S (2021) Green synthesized ZnO nanoparticles for sustainable production and nutritional biofortification of green gram. *Environ Tech Innov* 24:101957. <https://doi.org/10.1016/j.eti.2021.101957>
 45. Sasi S, Fasna PHF, Sharmila TKB et al (2022) Green synthesis of ZnO nanoparticles with enhanced photocatalytic and antibacterial activity. *J Alloys and Comp* 924:166431. <https://doi.org/10.1016/j.jallcom.2022.166431>
 46. Sharma R, Agrawal A, Sharma A et al (2022) Effect of biosynthesized ZnO nanoparticles on wheat seedlings. *Mat Today: Proce* 69:1587–1595. <https://doi.org/10.1016/j.matpr.2022.08.353>
 47. Siddiqi KS, Tajuddin RA, Husen A (2018) Properties of zinc oxide nanoparticles and their activity against microbes. *Nanoscale Res Lett* 13:141. <https://doi.org/10.1186/s11671-018-2532-3>
 48. Singh K, Nancy, Singh J, Singh G (2022) Efficient photocatalytic degradation of industrial dye using biogenic ZnO NPs. *Mat Today: Proce* 68(4):760–765. <https://doi.org/10.1016/j.matpr.2022.06.138>
 49. Singh J, Kumar S, Alok A, Upadhyay SK, Rawat M, Tsang DCW, Bolan N, Kim K-H (2019) The potential of green synthesized zinc oxide nanoparticles as nutrient source for plant growth. *J Clean Prod* 214:1061e1070. <https://doi.org/10.1016/j.jclepro.2019.01.018>

50. Şahin B, Aydın R, Soylu S, Türkmen M, Kara M, Akkaya A, Çetin H, Ayyıldız E (2022) The effect of *thymus syriacus* plant extract on the main physical and antibacterial activities of ZnO nanoparticles synthesized by SILAR method. Inorg Chem Comm 135:109088. <https://doi.org/10.1016/j.inoche.2021.109088>
51. Takatsuka H, Umeda M (2019) ABA inhibits root cell elongation through repressing the cytokinin signaling. Plant Sign & Behav 14(3):e1578632. <https://doi.org/10.1080/15592324.2019.1578632>
52. Tantiwatcharothai S, Prachayawarakorn J (2019) Characterization of an antibacterial wound dressing from basil seed (*Ocimum basilicum* L.) mucilage-ZnO nanocomposite. Int J Biol Macromol 135:133–140. <https://doi.org/10.1016/j.ijbiomac.2019.05.118>
53. Taylor I, Lehner K, McCaskey E et al (2021) Mechanism and function of root circumnutation. PNAS 118(8):e2018940118. <https://doi.org/10.1073/pnas.2018940118>
54. Vidya C, Hiremath S, Chandraprabha MN et al (2013) Green synthesis of ZnO nanoparticles by *Calotropis gigantea*. Int J Curr Eng Technol 1:118–120
55. Wahab R, Ansari S, Kim YS, Song M, Shin H-S (2009) The role of pH variation on the growth of zinc oxide nanostructures. Appl Surf Sci 255:4891–4896
56. Wang Y, Li X, Wang N, Quan X, Chen Y (2008) Controllable synthesis of ZnO nanoflowers and their morphology-dependent photocatalytic activities. Sep Purif Technol 62:727–732
57. Wang X, Yang X, Chen S et al (2016) Zinc oxide nanoparticles affect biomass accumulation and photosynthesis in *Arabidopsis*. Front Plant Sci 6:1243. [10.3389/fpls.2015.01243](https://doi.org/10.3389/fpls.2015.01243)
58. Zhu J, Li J, Shen Y et al (2020) Mechanism of zinc oxide nanoparticle entry into wheat seedling leaves. Environ Sci: Nano 7:3901–3913. <https://doi.org/10.1039/D0EN00658K>

Figures

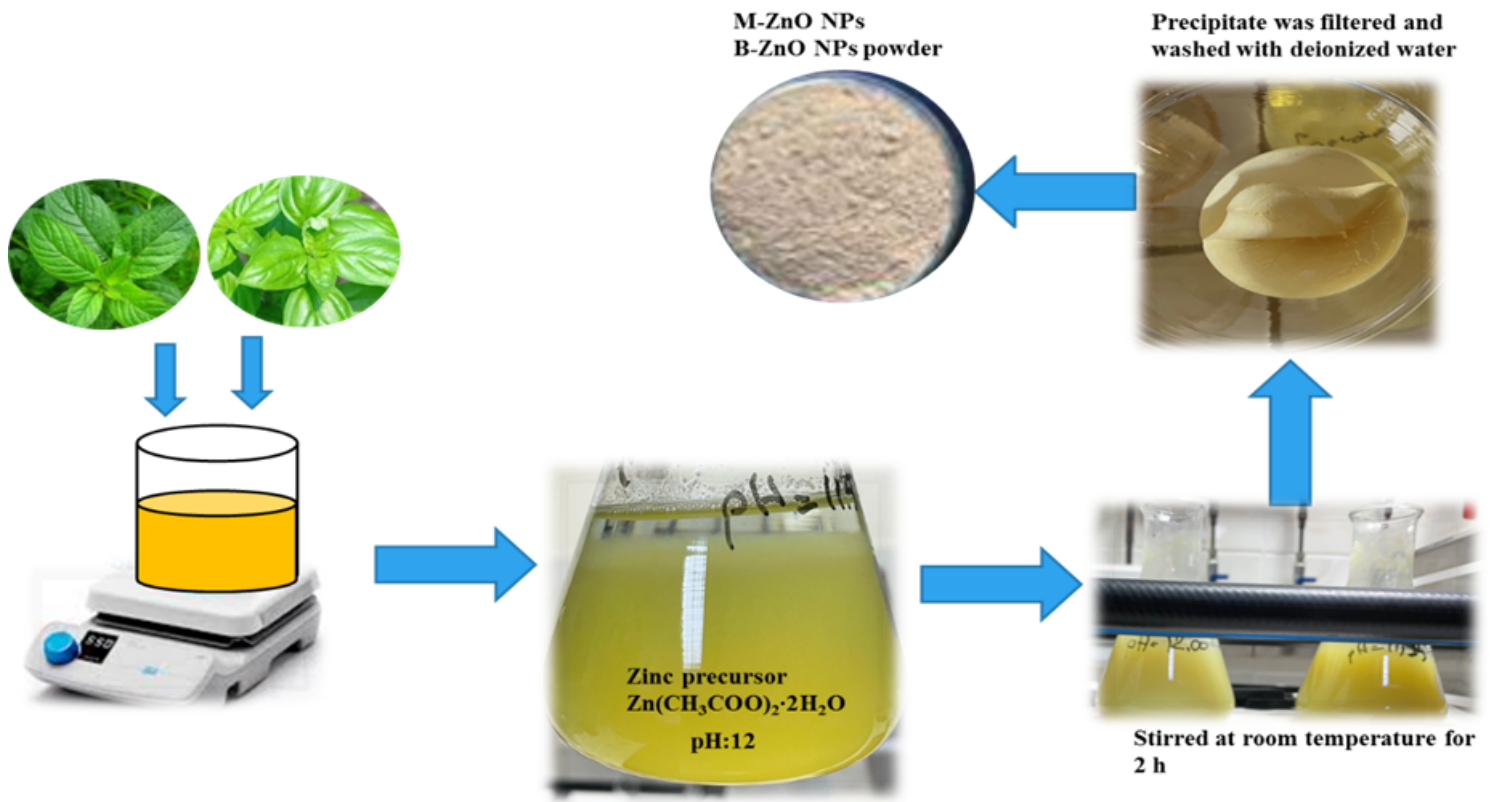


Figure 1

Schematic diagram of green synthesized ZnO-NPs using mint and basil

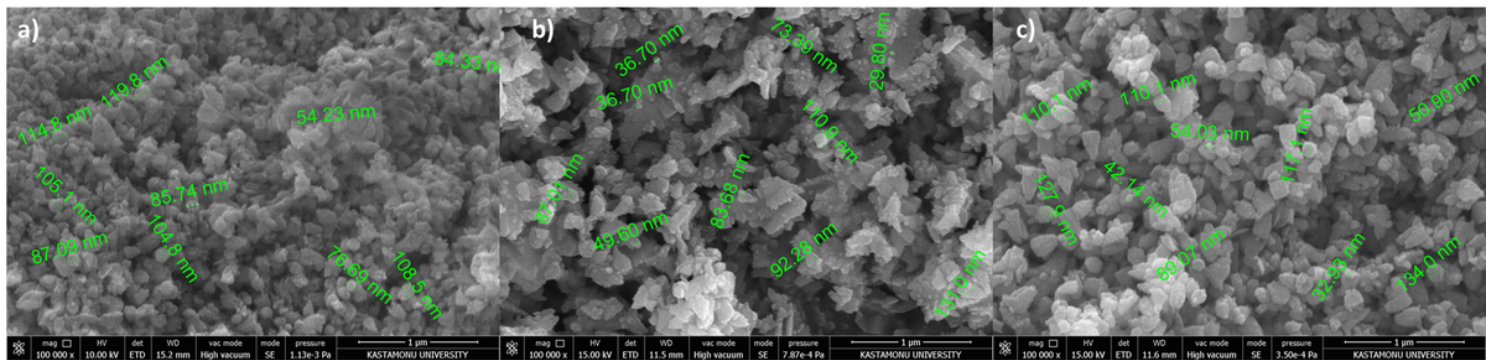


Figure 2

SEM images of a) Raw ZnO-NPs, b) M-ZnO-NPs and c) B-ZnO-NPs

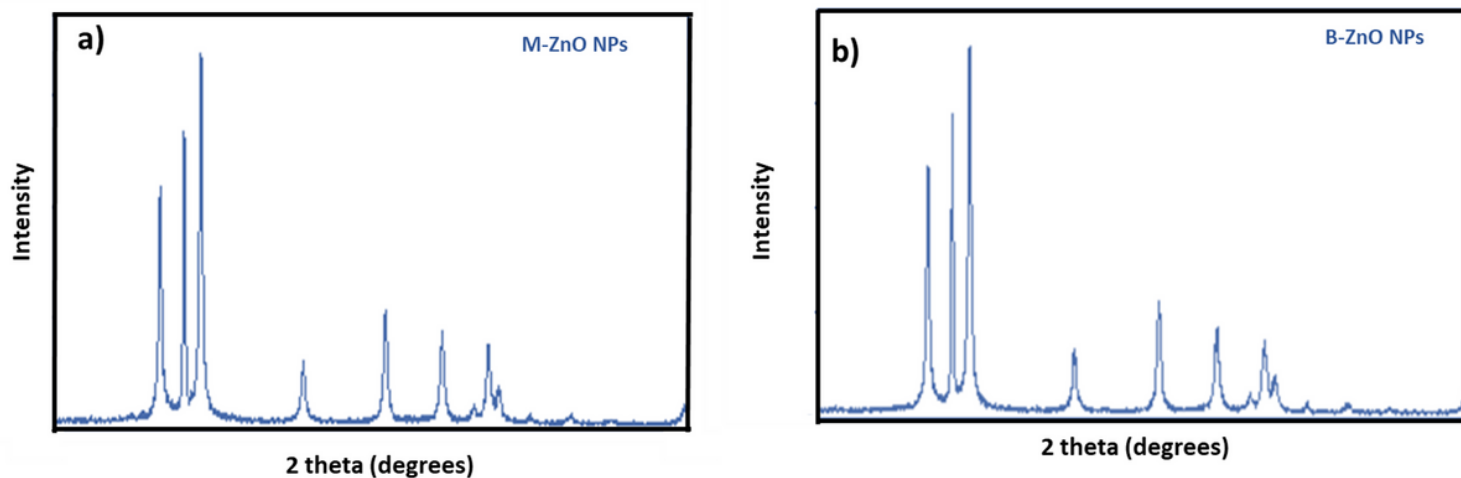


Figure 3

XRD patterns of a) M-ZnO NPs, and b) B-ZnO NPs

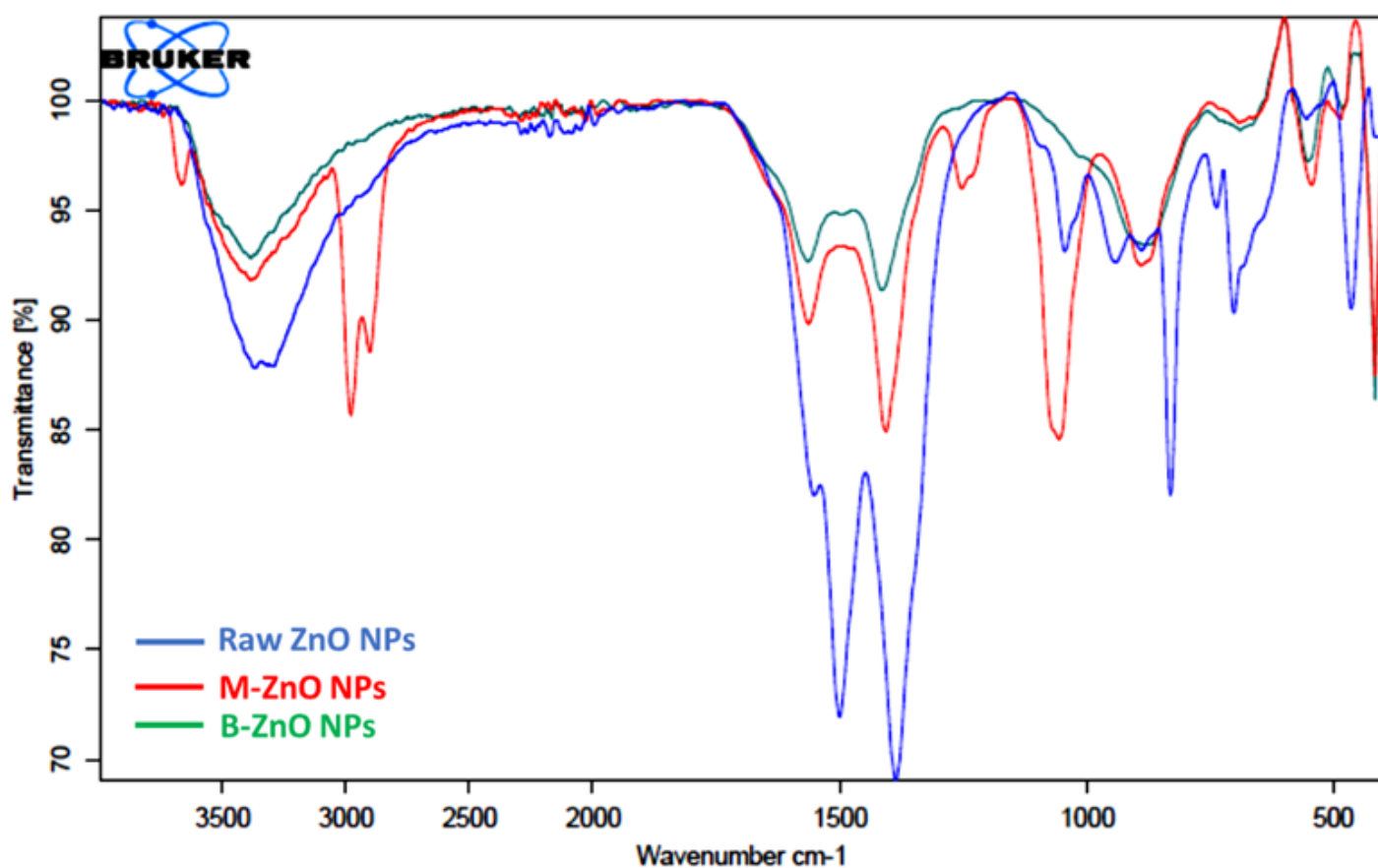


Figure 4

FT-IR spectrums of synthesized raw ZnO-NPs, M-ZnO-NPs (*Mentha spicata*) and B-ZnO-NPs (*Ocimum basilicum*)

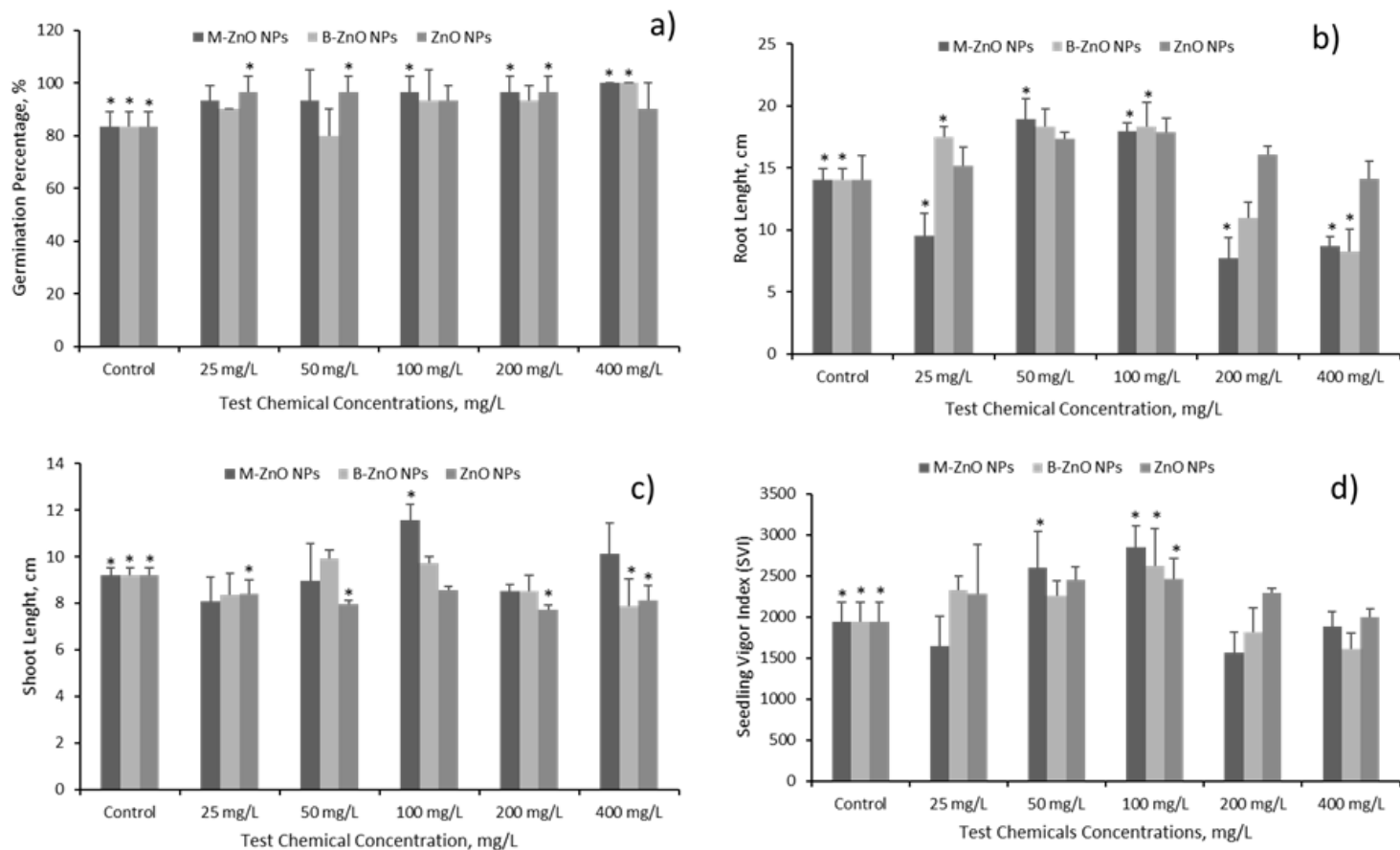


Figure 5

a) Germination percentage of wheat seed treated with M-ZnO-NPs, B-ZnO-NPs, and raw ZnO-NPs, b) average root lengths and c) shoot lengths of germinated wheat seedling, and d) seedling vigor index of wheat (* means there was a significant effect compared to control, $p < 0.05$)

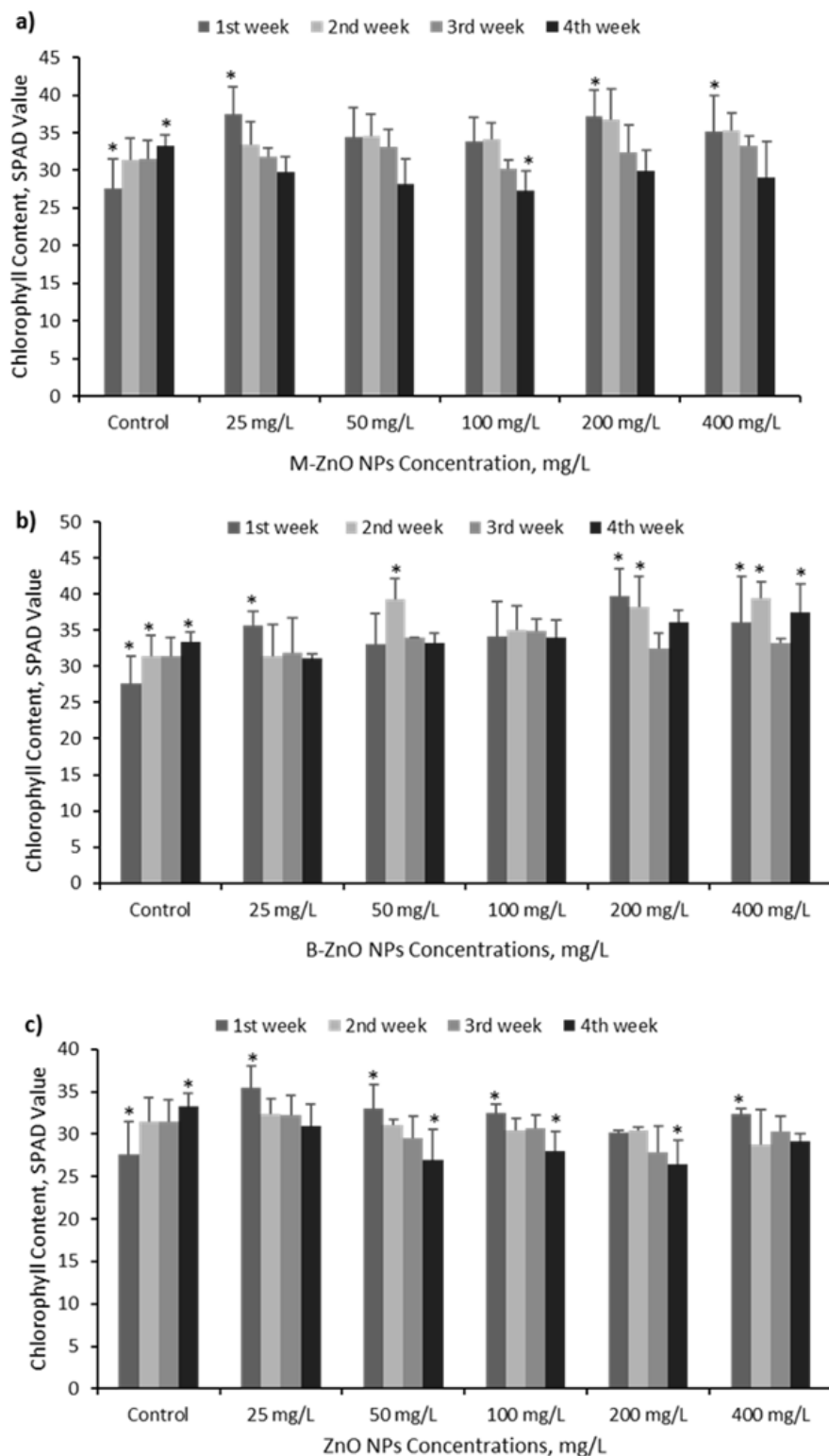


Figure 6

Chlorophyll content of wheat foliar treated with a) M-ZnO-NPs, b) B-ZnO-NPs, and c) ZnO-NPs (* means there was a significant effect compared to control, $p < 0.05$)

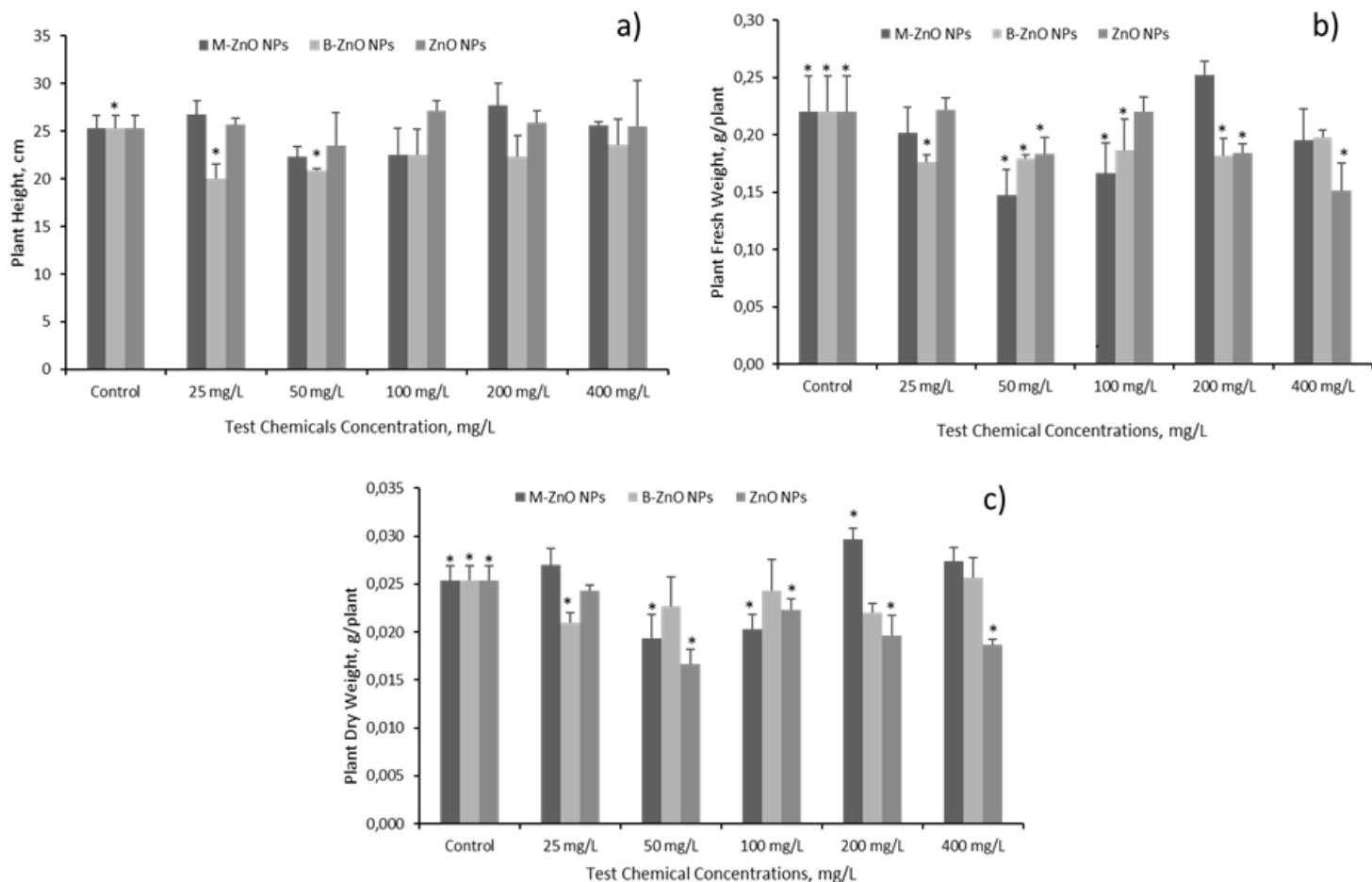


Figure 7

Growth parameters of wheat under treatment of M-ZnO-NPs, B-ZnO-NPs, and ZnO-NPs at different concentrations a) plant height, b) plant fresh weight, and c) plant dry weight (* means there was a significant effect compared to control, $p < 0.05$)

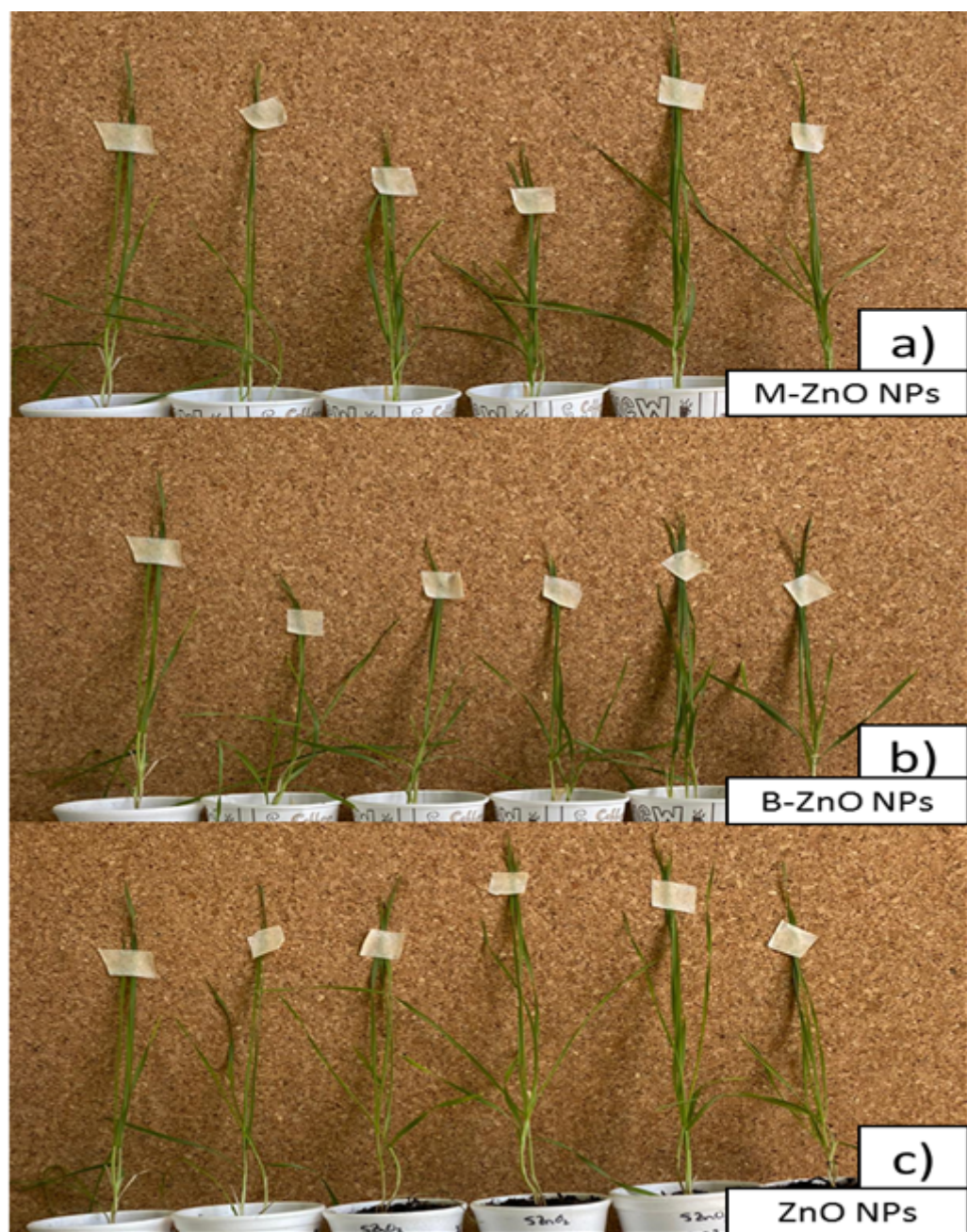


Figure 8

Effects of foliar treatment of a) M-ZnO-NPs, b) B-ZnO-NPs, and c) ZnO-NPs on wheat plant height

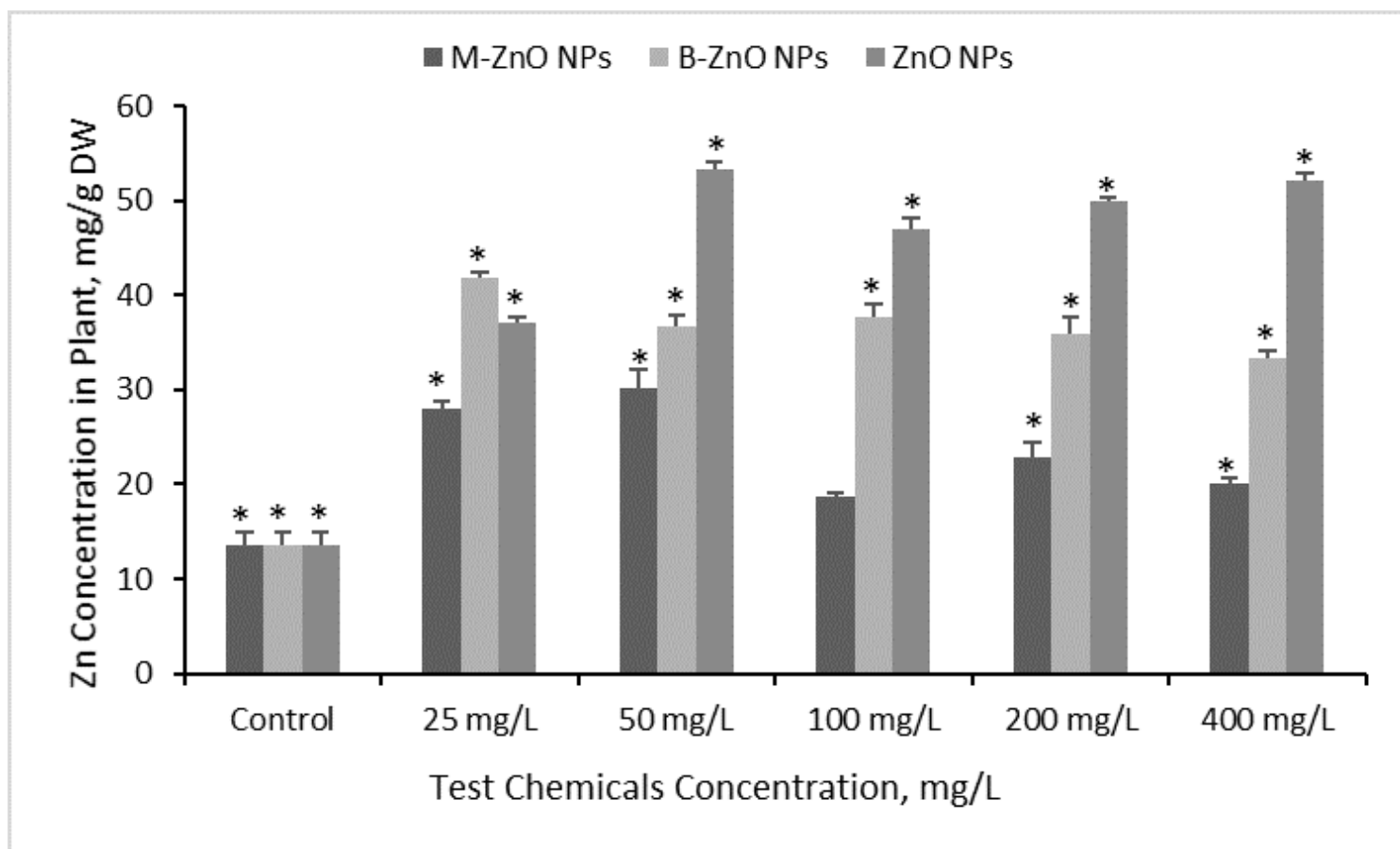


Figure 9

Zn concentration in plants foliar treated with at different concentration of M-ZnO-NPs, B-ZnO-NPs, and raw ZnO-NPs (* means there was a significant effect compared to control, $p < 0.05$)

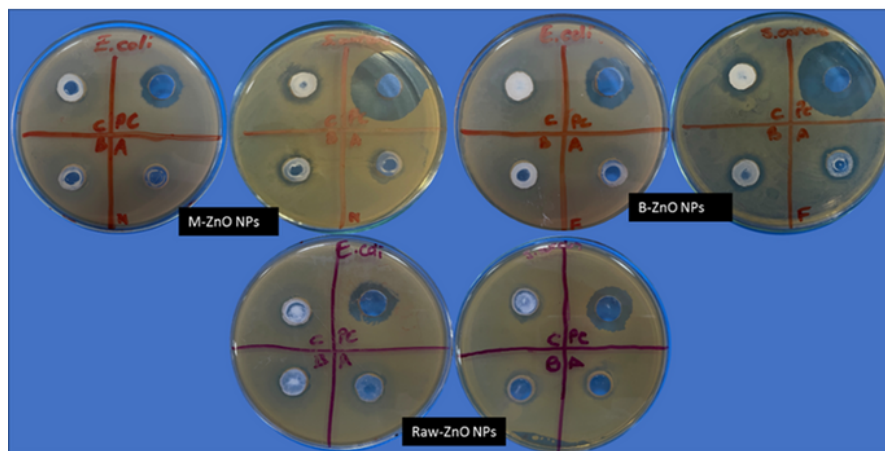
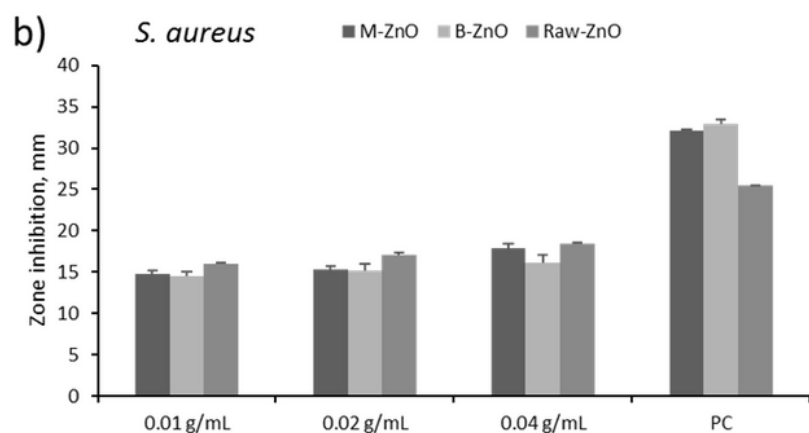
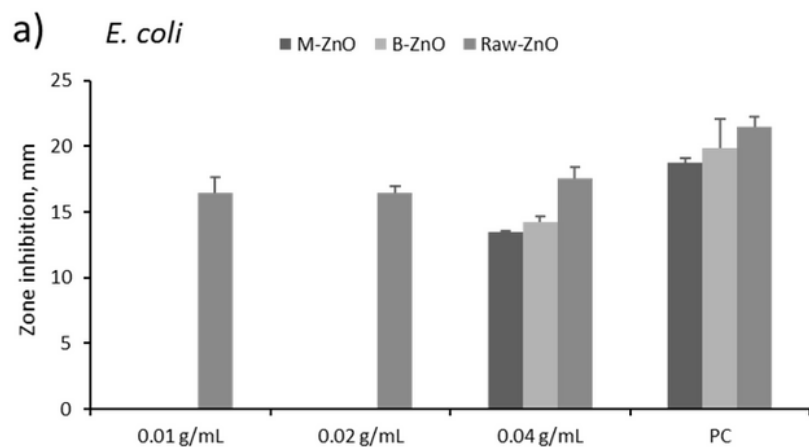


Figure 10

Antibacterial activity and zone inhibition of synthesized a-b) M-ZnO-NPs, c-d) B-ZnO-NPs, and e-f) ZnO-NPs; a-c-e) *E. coli*; b-d-f) *S. aureus*; in photograph A: 0.01g/mL, B: 0.02 g/mL, C: 0.04 g/mL, PC: 50 μ g amoxicillin.