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Research Article

Biosynthesis of Zinc Oxide Nanoparticles via Leaf Extracts of *Catharanthus roseus* (L.) G. Don and Their Application in Improving Seed Germination Potential and Seedling Vigor of *Eleusine coracana* (L.) Gaertn

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The ecofriendly nature of materials used in synthesis and their low cost make biosynthesized nanoparticles excellent stuff for a broad range of applications in bioscience. Green nanomaterials are progressively used in agriculture to deliver plant nutrients efficiently and effectively. The present work aimed to biosynthesize zinc oxide nanoparticles (ZnO NPs) utilizing *Catharanthus roseus* (L.) G. Don leaf extracts to use them as a nanopriming agent for improving seed germination and seedling growth in *Eleusine coracana* (L.) Gaertn (finger millet). UV-Vis spectroscopy, FTIR, FE-SEM, EDX, and TEM were used to characterize biosynthesized nanoparticles (NPs). The peaks at 362 nm characterized UV-Vis spectra of ZnO NPs. The FTIR absorption spectrum of ZnO NPs showed Zn-O bending at 547 cm^{-1}. The size (44.5 nm) and shape (nonspherical) of ZnO NPs were revealed by TEM image analysis. XRD confirmed the hexagonal wurtzite phase of ZnO with an average particle size of 35.19. The seed germination results revealed that ZnO-nanoprimed seeds at 500 mg/L substantially improved all the seed germination parameters, viz., plumule length (23.4%), radicle length (55%), vigor index (41.94%), and dry matter production (54.6%) compared to hydropriming (control).

1. Introduction

Nanotechnology has revolutionized various sectors, including agriculture, pharmaceuticals, and biotechnology, by enabling the manipulation of materials at the nanoscale [1, 2]. Nanoparticles (NPs) are the key building elements in the burgeoning field of nanotechnology. NPs have high light absorption and excellent catalytic properties due to their large surface area to the volume ratio and the broad gap between their conduction and valence bands [3]. Among various NPs, zinc oxide NPs have performed a notable role in inhibiting harmful microbial activities concerning plant disease management, improving feed digestibility of animals and cytotoxicity towards human cancer cells, etc. [4–7]. ZnO-coated commodity used in the textile industries absorbs harmful UV radiation and transforms it into harmless infrared light [8]. ZnO also possesses a strong photochemical activity, a large binding energy, a broad band gap, and good piezoelectric properties, etc [9].

NPs could be synthesized by biological, chemical, and physical means, but biological means of NP generation using microbes, plants, and macroalgae have been considered an alternative and an ecofriendly strategy. Nonbiological synthesis of NPs is toxic, exorbitantly expensive, and harms the environment [10–12]. Fouda and Sofy [13], Hajian et al. [14], Begum et al. [15], and Girilal et al. [16] observed that biologically synthesized NPs boosted plant growth and development compared to chemically manufactured (synthetic) NPs. Using phytoconstituents as reduction and capping agents in the synthesis of NPs provides a cost-effective alternative to conventional NP manufacturing. Plant leaf extract contains a manifold of metabolites that act as reducing agents during nanoparticle synthesis [17].

ZnO NPs can also be synthesized by aqueous leaf extracts of *Azadirachta indica* A. Juss., Arshi [18], which depicted a 50 nm diameter in TEM imaging analysis. Leaf extracts from *Carissa carandas* L. are also used as a reducing and capping agent in NP synthesis [19], and the average crystallite size of ZnO NPs has been observed at 35.2 nm at 25°C and at 30.3 nm at 60°C.

Particle development and regulation of stabilization and aggregation, morphology, size, and dispersion in generating metal nanoparticles are a great challenge [20]. Plant extracts provide an ecologically innocuous approach for the biological synthesis of various metallic NPs, allowing for graded synthesis with well-defined size and form. *Catharanthus roseus* (L.) G. Don, an important medicinal plant of the Apocynaceae family, was used in this study to produce ZnO NPs. *C. roseus* is a multipurpose medicinal herb that is widely used because it contains vital anticancer drugs, viz., vincristine and vinblastine, as well as secretes 100 types of alkaloid compounds [21].

Zinc (Zn) has played a pivotal role in upregulating vital physiological and metabolic processes in enhancing seed germination, pollen grain formation, and fertilization and preserving the plant pigment system [22] and crop biomass production [23]. In addition, it functions as a cofactor in regulating enzymes such as oxidoreductases, transferases, hydrolases, isomerases, lyases, and ligases [24]. A direct yield loss of US $ 1.5 billion/year is estimated due to low crop yields and massive loss due to disease concerns arising from Zn malnutrition. Zn malnutrition has become a significant health concern among resource-poor people [25]. In the Indian situation, Zn-deficient soils are expected to increase from 42 percent in 1970 to 63 percent by 2025 due to the continuous depletion of soil fertility [26].

Eleusine coracana is an important millet crop in India, which has a rich source of calcium and polyphenols as well as a good nutritive value than major cereals [27]. Moreover, the crop is climate-resilient and can be cultivated on marginal lands. Long-term storage of seeds reduces seed germination percentage. Successful establishment in the field, however, requires good germination potential. Rapid seed germination and seedling establishment are the essential elements determining reproductive capacity [28] and productivity of crops [29].

For this reason, the seed priming approach has been established to promote seed germination and vigor [30] in agricultural systems. In the present study, we developed low-cost biosynthesized ZnO NPs, yet the application of ZnO nanoparticles at a broader level has been unexplored. In our research, we aimed to explore the positive interaction of the ZnO NPs with plants. During the research study, green synthesis of ZnO NPs has been achieved for their use as a nanopriming agent to promote seed germination and seedling growth parameters of finger millet (aged seeds).

2. Materials and Methods

2.1. Reagents and Chemicals. Zinc sulfate heptahydrate (99.5%) and sodium hydroxide (99%) were used as the introductory material (Sigma-Aldrich chemicals).

2.2. Preparation of Leaf Extracts. Fresh green leaves of *C. roseus* were collected from the G. B. Pant University of Agriculture and Technology, Pantnagar campus. The plucked leaves were gently washed under running tap water. The leaves were dried in a hot air oven at 40°C, pulverized through a home mixer blender, and kept at room temperature until needed. After drying, the 5 g leaf powder was transferred to a 250 ml beaker containing 100 ml distilled water and heated for 15 minutes at 80°C. The mixture was kept at room temperature for cooling and then filtered through Whatman filter paper no. 1. The filtrate was collected in vials and stored at 4°C for further experimentation. The presence or absence of phytochemicals in the extract had been studied earlier using standard procedures [31].

2.3. Preparation and Characterization of ZnO Nanoparticles. A 10 ml leaf extract of *C. roseus* was added dropwise to 90 ml of 1 mM zinc sulfate heptahydrate ($ZnSO_4.7H_2O$). The mixer was kept on a magnetic stirrer for 1 h. After 1 h, 2 M NaOH was appended, and the solution was placed at 65°C with stirring for 2 h. The yellow mixture (Figure 1) was washed with alcohol and distilled water. A high concentration of phytochemicals (alkaloids, phenols, and flavonoids) in the leaf extract functioned as effective stabilizing and capping agents. Alkaloids, flavonoids, and phenols are biological antioxidants that contain anionic radicals that cause zinc salts to be reduced to ZnO NPs. If the mixture's alkalinity is high, the amount of OH^- is generally large, generating significant attraction between positively charged Zn^{2+} and OH^-, resulting in increased crystallization and the production of tiny ZnO NPs (Figure 2) [32].

The generated nanopowder was dispersed in sterile distilled water, and spectrum scans were taken in the wavelength range of 300–500 nm using a GENESYS 10S UV-Vis spectrophotometer. Plotting tool Origin Pro 8 was used to replot the absorption results. UV-Vis spectrophotometric measurement confirmed NP production. The Thermo Fisher Nicolet i6700 FTIR spectrometer was used to perform FTIR. The data were recorded in the 400–4000 cm^{-1} range. The Malvern Zetasizer instrument was used to perform zeta potential studies (AIIMS, Delhi). The stability of the produced ZnO NPs was validated using zeta potential measurements. A field-emission scanning electron microscope

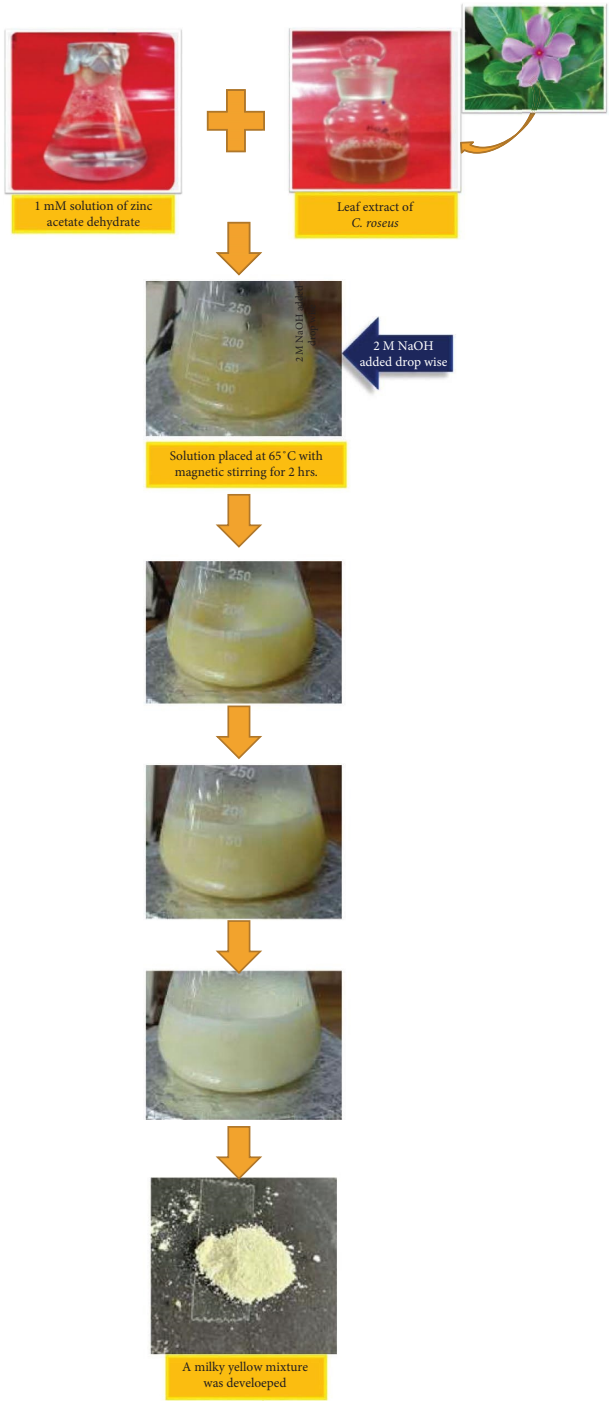

Figure 1: Schematic representation for the formation of ZnO NPs.

(FE-SEM) was used to characterize the external morphology of nanoparticles (JEOL FE-SEM). Elemental analysis was accomplished using energy dispersive X-ray (EDX) diffraction in conjunction with SEM. The shape and size of the NPs were determined using transmission electron microscopy (TALOS HR-TEM) (AIIMS, Delhi). Furthermore, the size and shape of NPs were determined using an X-ray diffractometer (Bruker). A Sonic VCX 750 ultrasonicator (750 watt power and 20 kHz frequency) was used to prepare a homogenous solution.

2.4. Seed Priming Experiment. Finger millet aged seeds were tested with different concentrations of ZnO NPs before being sown for the Petri dish germination experiment. First, a small amount of deionized water (DW) was added to ZnO NPs and ultrasonication (30 min, 3 cycles each 10 min) was used for good dispersion of NPs before normalizing the final volume with DW to achieve the necessary concentrations of 100, 500, and 1000 mg/L for preparing nanopriming solution. Next, the seeds were soaked in various ZnO NP (100 mL) concentrations for 4 hours in the dark at 25°C with continuous shaking on an incubator shaker, while the seeds primed with deionized water served as the control. Finally, the treated seeds were rinsed 3–4 times with distilled water and dried using tissue paper.

The most effective treatment levels were selected based on germination percentage, radicle and plumule length, fresh and dry weight, and seedling vigor of treated seeds. The emergence of seedlings was monitored daily until a consistent count was attained [33]. Seedling emergence and seed vigor were recorded using the following formula:

$$\begin{aligned}\text{Seed vigor} = {} & \text{plumule length (cm)} \\ & + \text{radicle length (cm)} \\ & \times \text{germination percentage.}\end{aligned} \quad (1)$$

Seedlings were retrieved 9 days after seeding, followed by radicle and plumule length measurements. Furthermore, the seedling sample was dried at 75°C for 48 hours until a constant weight was observed.

3. Results

Flavonoids, alkaloids, phenols, and proteins were found in the *C. roseus* extract [31]. These phytochemical constituents may act as reducing and stabilizing agents during ZnO NP synthesis.

3.1. Visual Identification of ZnO NPs. The color of zinc salt altered from white to yellow when adding the leaf extract. The production of ZnO NPs is mostly responsible for color change (Figure 1).

3.2. Spectroscopy Analysis of ZnO NPs. A UV-Vis spectrum verified the reduction of zinc ions to ZnO NPs with a strong peak at 362 nm (Figure 3(a)), but in the plant extract, there was no peak observed (Figure 3(c)). The FTIR technique is useful for determining the composition of materials. Figure 3(b) shows the typical FTIR spectra of ZnO NPs. The wide absorption peak at 3328 cm^{-1} may be related to the stretching of –OH or phenolic groups, whereas the peak at 547 cm^{-1} represents the characteristic absorption of the Zn–O bond; however, in the plant extract, no absorption spectrum was observed (Figure 3(d)).

3.3. Stability of ZnO NPs. Zeta potential (ZP) is an important measurement for studying the NP surface charge and colloidal stability. ZnO NPs have a ZP of −18.8 mV (Figure 4), indicating that it forms stable ZnO NPs at basic pH (8.1).

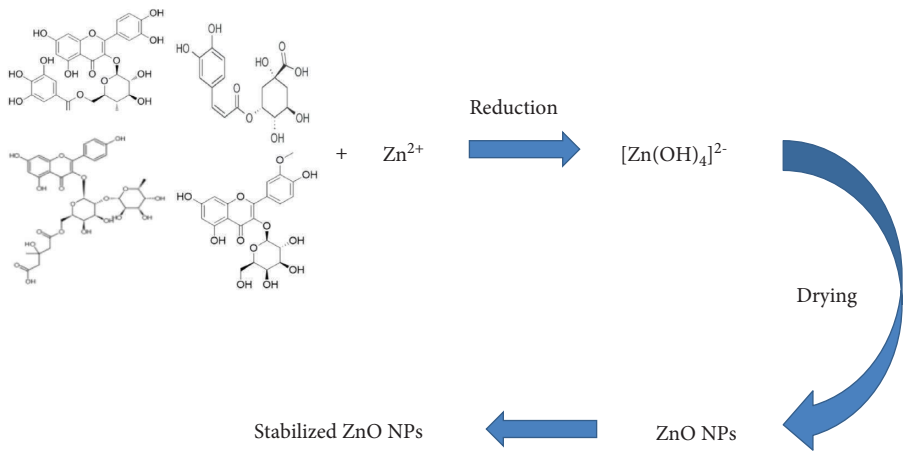

Figure 2: A proposed mechanism of synthesis of ZnO NPs utilizing the *C. roseus* leaf extract.

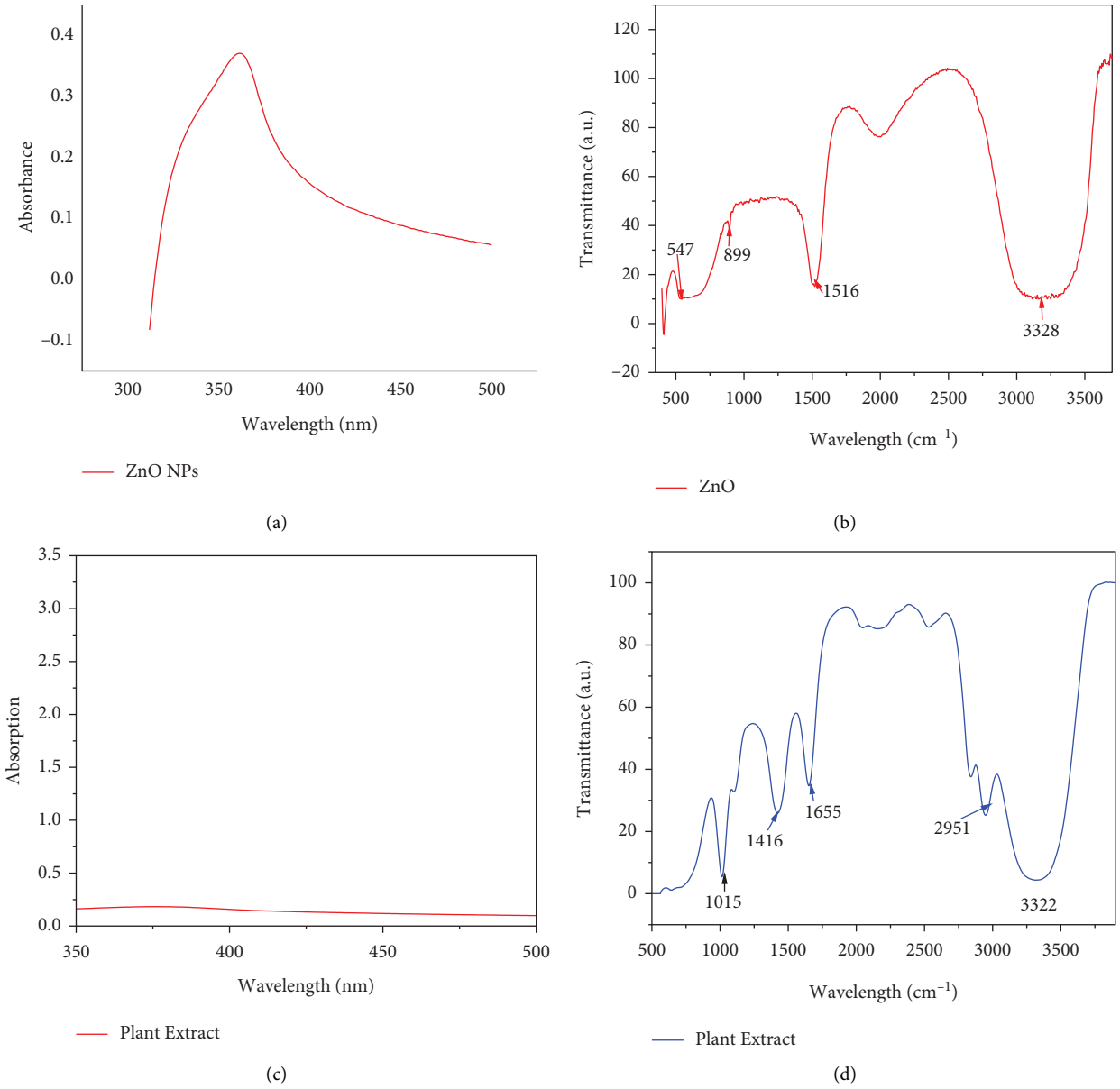

Figure 3: UV-Vis (a, c) and FTIR spectra (b, d) of synthesized ZnO NPs and plant extracts, respectively.

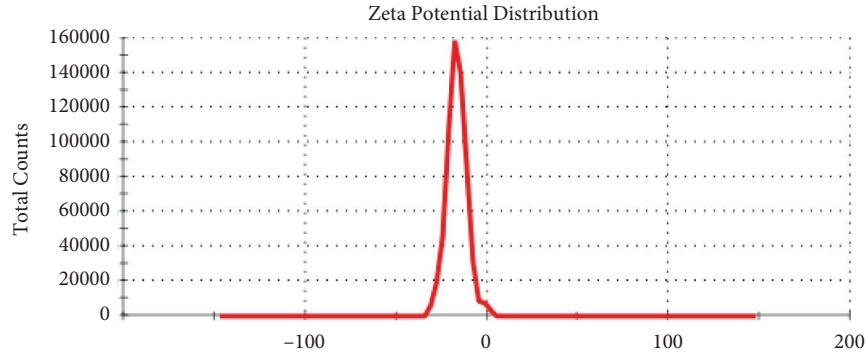

Figure 4: Zeta potential analysis of biosynthesized ZnO NPs.

3.4. Purity, Surface Morphology, and Size of ZnO NPs. Under the EDX spectrum analysis, EDX confirmed the presence of elemental zinc and oxygen (Figure 5(b)). In addition, Zn and O have weight % values of 79.8 and 20.1, respectively, compared with the other published research studies [34, 35]. SEM examination to ascertain the morphology of biologically produced ZnO NPs revealed nonspherical agglomerates (Figure 5(a)). The particle size and shape were determined by TEM. In Figure 5(c), the TEM image of ZnO NPs synthesized with the *C. roseus* extract shows nonspherical particles with a mean size of 43.6 nm. The XRD pattern of the ZnO powder is shown in Figure 5(e). The diffraction peaks located at 31.84°, 34.52°, 36.33°, 47.63°, 56.71°, and 62.96° have been indexed as a hexagonal wurtzite phase of ZnO (JCPDS no. 36–1451). Diffraction peaks corresponding to the impurity were not found in the XRD patterns, confirming the high purity of the synthesized ZnO NPs. The average crystallite sizes of the samples were calculated by the Debye–Scherrer equation (Equation (1)) using the full width at half maximum of 101 of the X-ray diffraction peaks.

$$d = \frac{0.89\lambda}{\beta \cos \theta}. \quad (2)$$

The average particle size of the sample was found to be 35.19 nm, which was derived from the FWHM of the more intense peak corresponding to the 101 plane located at 36.33° using Scherrer's formula.

3.5. Effect of ZnO NPs on Seed Germination and Seedling Vigor. Seed treatment with ZnO NPs at 100 and 500 mg/L showed a significant increase in germination percentage compared to the control, while at 1000 mg/L, seed germination was remarkably reduced (Figure 6(a)). In addition, seedling vigor also significantly increased at 100 and 500 mg/L, which was 7523.917 (29.17%) and 9180.083 (41.94%), respectively, compared to the control treatment (0 mg/L) (Figure 6(b)). These findings suggest that lower concentrations of ZnO NPs (100 and 500 mg/L) significantly increased seed germination and seedling vigor, while higher concentration (1000 mg/L) negatively affected these parameters.

Seed treatment with ZnO NPs significantly enhanced seedling growth (Figure 6). At 100 and 500 mg/L of ZnO NP treatment, plumule length increased by 27.4 and 23.4 percent (Figure 6(c)), while radicle length increased by 35.1 and 55 percent, respectively (Figure 6(d)). A similar trend was followed in the fresh and dry weight of the seedling. The fresh weight increased by 24.8 and 34.8 percent (Figure 6(e)), while the dry weight increased by 35.5 percent and 54.6 percent (Figure 6(f)). The root-shoot ratio of the seedlings also increased by 1.1 and 1.6, respectively (Figure 6(g)), compared to the control (0 mg/L). The results indicated that seed treatment with 500 mg/L ZnO NPs significantly increased seedling growth parameters followed by 100 mg/L ZnO NPs; however, higher concentration (1000 mg/L) reduced seedling growth (Figure 7).

3.6. Heatmap Clustering. Heatmap clustering was used in the finger millet seed germination experiment with various concentrations of ZnO NPs. The 4 concentrations could be separated into two clusters. Group A contains 2 concentrations of 0 mg/L and 1000 mg/L of ZnO NPs, and Group B comprises the other two concentrations (100 mg/L and 500 mg/L) (Figure 8). Group B is characterized by the highest vigor index, plumule length, radicle length, and fresh and dry weight of seedlings. It was observed that the vigor index is the most determinant factor of plant growth which is significantly improved at a concentration of 500 mg/L. According to Figure 6, maximum seedling growth promotion was observed at 500 mg/L of ZnO NPs, followed by 100 mg/L.

4. Discussion

Under changing climatic conditions, the monsoon regime shift causes negative consequences for crops. It reduces seed germination, vigor potential, and yield losses under severe stress conditions. On the contrary, applying the nanopriming agent to crops greatly enhances seed germination and plant growth parameters under adverse climatic conditions [36, 37].

The biosynthesis of NPs is a convenient, inexpensive, nontoxic, and ecologically sound process of NP synthesis. Using biological resources in synthesizing NPs is extremely important for the sustainability of NP production and usage. In the present research study, biosynthesis of yellow color ZnO NPs was achieved by using the leaf extract of *C. roseus*.

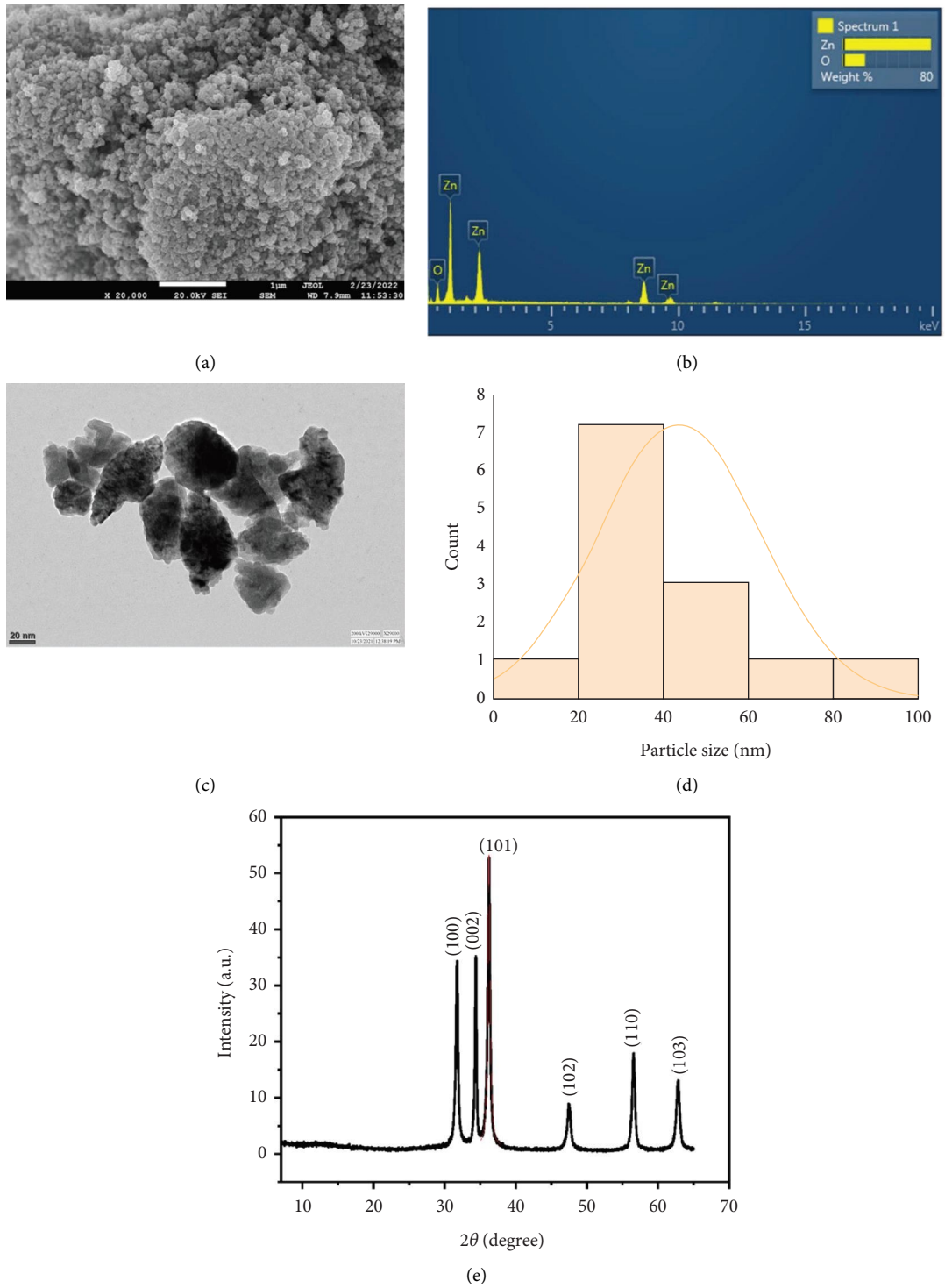

Figure 5: (a) SEM image and (b) EDX spectrum of ZnO NPs. (c) Zinc oxide nanoparticles under a TEM microscope. (d) Particle size distribution of ZnO NPs is represented by the histogram (TEM method) with an average size between 20 and 40 nm. (e) X-ray diffractometer (XRD) spectra of synthesized ZnO NPs.

According to a previous study, the formation of ZnO NPs coincided with the transition of color to light yellow [38–40]. ZnO NPs can be synthesized from the plant leaf extract containing numerous phytochemicals like alkaloids, flavonoids, and phenols. These phytochemicals contain anionic radicals that are amenable to reducing zinc salts to ZnO NPs. If the mixture's alkalinity is high, the amount of OH^- is generally large, generating significant attraction between positively charged Zn^+ and OH^-, resulting in increased crystallization and the production of smaller ZnO NPs. The

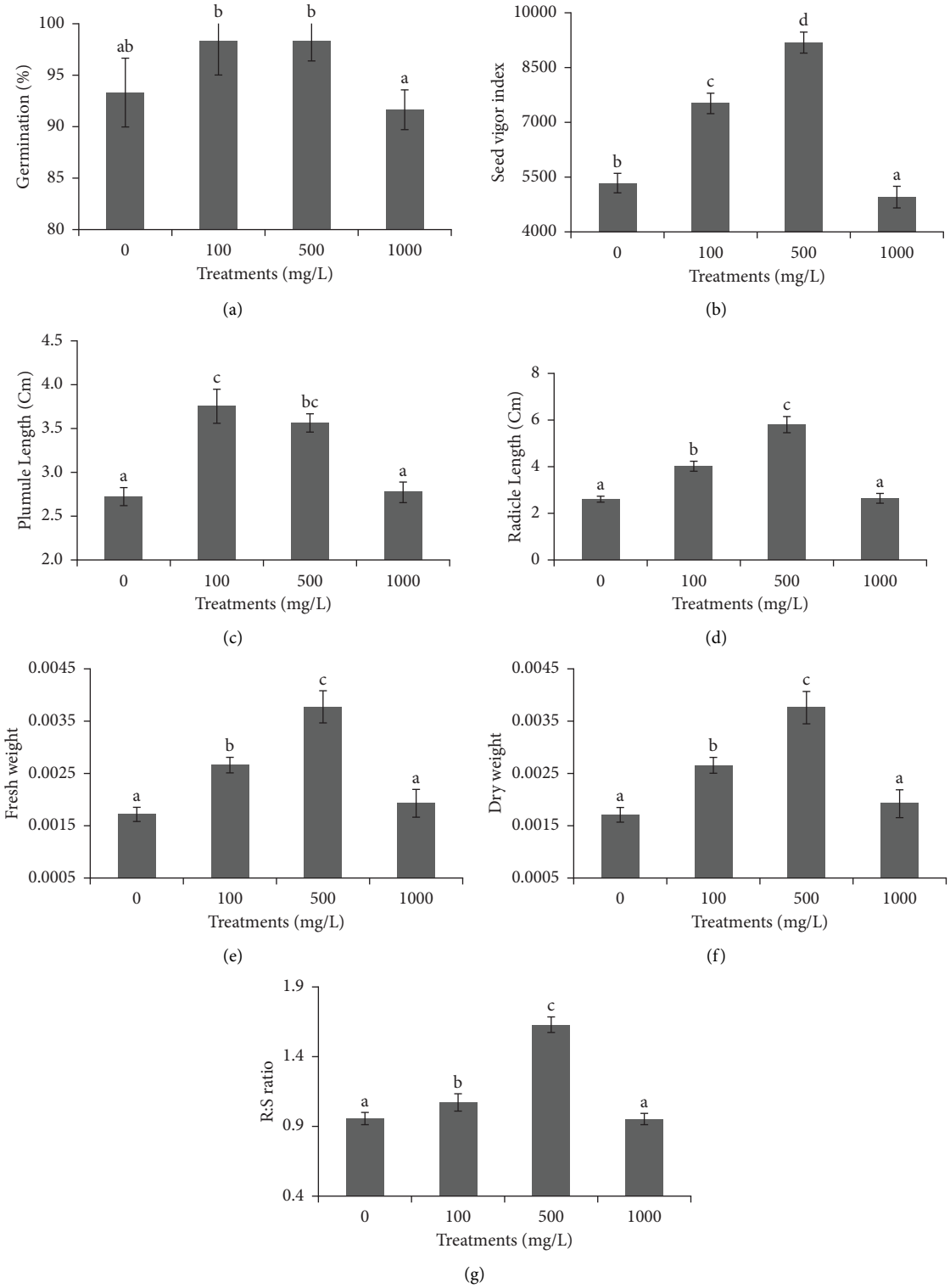

Figure 6: Effect of ZnO NPs at different concentrations (0, 100, 500, and 1000 mg/L) on seed germination (a), seedling vigor (b), plumule length (c), radicle length (d), fresh weight (e), dry weight (f), and R : S ratio (g) of *Eleusine coracana* (L.) *Gaertn* under *in vitro* conditions. Results are indicated as means of three replications, and vertical bars express the standard deviation (SD) of the means. Different letters denote significant differences among treatment outcomes taken at the same time interval according to Duncan's multiple range test at $P \leq 0.05$.

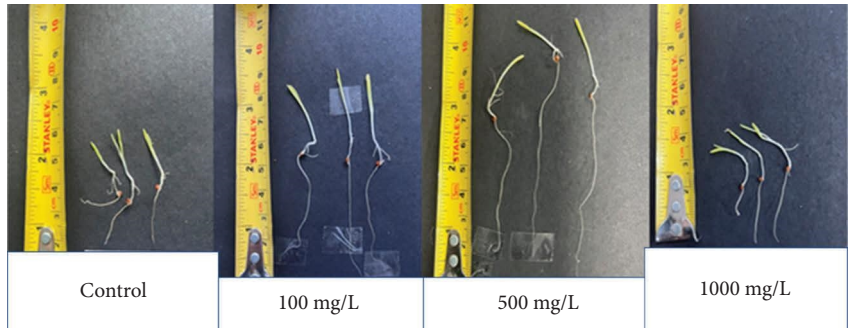

Figure 7: Effect of zinc oxide nanoparticles (ZnO NPs) on the growth of *Eleusine coracana* seedlings.

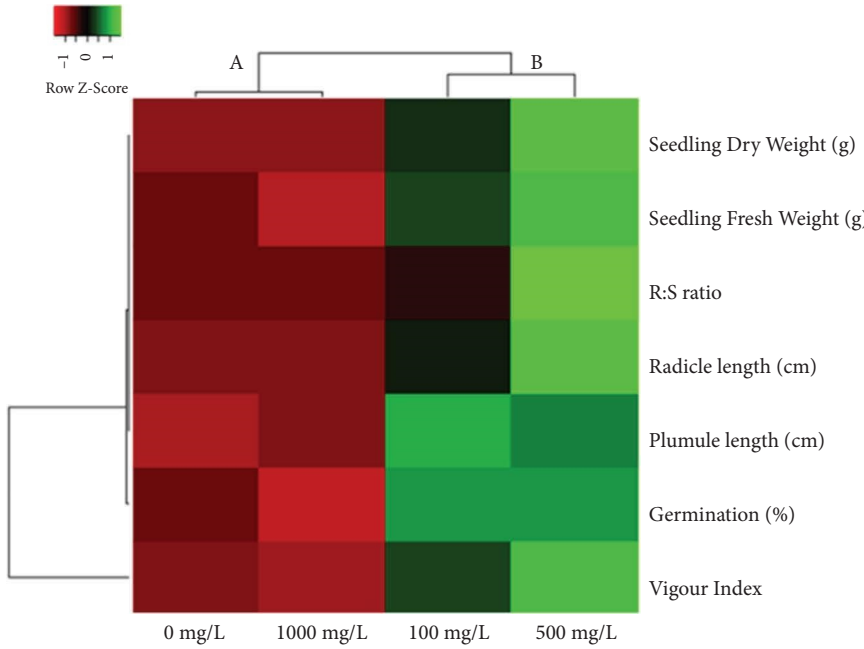

Figure 8: Heatmap clusters on the effect of different concentrations of ZnO NPs on seedling growth parameters. Mean values refer to colors from minimum displayed in red to maximum represented with green.

generation of ZnO NPs was checked by the UV-Vis spectrum, where a strong peak at 362 nm confirmed the reduction of zinc ions to ZnO NPs. Other studies, such as the studies by Khalafi et al. [41] and Muhammad et al. [42], support the present findings in the biosynthesis of ZnO NPs, exhibiting strong peaks at 362 nm and 360 nm, respectively. The production of ZnO NPs was confirmed by the presence of Zn and O elements in EDX spectrum analysis. FTIR spectra analysis was used to determine the composition of biosynthesized ZnO NPs. The findings of FTIR are consistent with those of the studies by Yedurkar et al. [43] and Ebadi et al. [44]. The stability of biosynthesized ZnO NPs was assured by zeta potential at −18.8 mV. SEM analysis of the powder sample identified the nonspherical agglomerates of ZnO NPs. The size and morphology of the biosynthesized ZnO NPs determined by TEM analysis revealed that nonspherical NPs have an average size of 43.6 nm. The particle size and shape of the biosynthesized ZnO NPs agree with several other studies [45–47]. The average particle size of the sample was found to be 35.19 nm under XRD analysis.

Nanoparticles have improved uptake and high nutrient use efficiency compared to bulk materials. Nanoscale materials could lead to more effective delivery of nutrients as their small size may allow them access to various plant surfaces and transport channels [48]. A study on the two different sizes of particles (43 nm and 1.1 μm diameter) in *Vicia faba* L. [49] indicated that nanosized particles could transport effectively in the leaf interior through stomatal pores [50, 51]. The well-characterized ZnO NPs were tested as nanopriming agents in promoting seed germination and vigor of the finger millet by the seed germination test. The results revealed that ZnO NPs significantly improved the seed germination and vigor of the finger millet at 100 and 500 mg/L concentrations. Seed treatment with ZnO NPs at 500 mg/L provided the highest vigor (1.7 fold), followed by 100 mg/L (1.4 fold), compared to the control. The seedling's fresh weight also significantly improved at 100 (1.3 fold) and 500 (1.5 fold) mg/L, whereas the dry weight of the seedling was increased at 100 (1.5 fold) and 500 (2.2 fold) mg/L. Herein, the concentration of ZnO NPs at 500 mg/L was

found to be significant for enhancing plant growth-related parameters, but a higher concentration of 1000 mg/L caused a negative effect on seed germination and plant growth.

ZnO NPs might have affected plant growth by regulating the hormone biosynthesis of plants [52], particularly auxins and gibberellins (GAs) (increasing the expression of GA-related genes BnGA20ox, BnGA3ox, and BnCPS during germination) [53]. These hormones increase reserve food breakdown in the seed, which can help augment seed germination and vigor [54]. The ZnO NP treatment reduced seed dormancy by downregulating abscisic acid (decreasing the expression of abscisic acid-related genes BnCYP707A1, 3, and 4) [53]. Another possible reason for faster seed germination could be the generation of reactive oxygen species (ROS) that result from the entry of ZnO NPs in the area between the cell membrane and the intracellular space of the seed coat parenchyma. Elevated levels of ROS in seeds increased ion penetration as well as water and oxygen absorption, both of which are required for faster seed germination [30]. Rawashdeh et al. [55] found that lettuce seeds (*Lactuca sativa* L.) treated with ZnO NPs had to have a higher Zn content than the control. In *Allium cepa* L., seed treatment with ZnO NPs at 800 mg L^{-1} significantly enhanced seed vigor by 56% compared to the control, while at higher concentration (3200 mg L^{-1}), seed germination reduced to 11% [56]. ZnO NPs (25 mg/100 ml) considerably increased the germination percentage in *Vigna mungo* L. by 111.3 compared to the control [57]. *Pennisetum glaucum* (L.) R. Br. seeds treated with ZnO NPs showed 60% more seed germination than the control [58]. ZnO NPs at 100 mg L^{-1} were also effective in rice [59] and maize [60] seed germination and dry matter accumulation. At 500 mg L^{-1}, ZnO NPs improved seed germination, seedling growth, and antioxidant enzymes in *Capsicum chinense* Jacq. [61] and *Portulaca oleracea* L. [62]. ZnO NPs priming reduced ROS and MDA accumulation in maize and wheat under cobalt stress [63] and drought stress [64], respectively. Improved seed germination and vigor of the finger millet in the present experiment were due to the stimulating effect of ZnO NPs by modulating hormone biosynthesis either directly or through the production of ROS.

5. Conclusion

The present study gives a successful and reproducible protocol of ecofriendly green synthesis of ZnO NPs and highlights its application in improving seedling growth parameters of finger millet under *in vitro* conditions. It quickly entered the plant cell and supported the development of the plant's biomass. ZnO NP cell wall deposition alters the metabolic activities of plants and activates their defense mechanisms. Therefore, crop improvement has been greatly influenced by its application to plants with the best consideration. According to the study mentioned above, seed treatment with lower doses of ZnO NPs (100 mg/L and 500 mg/L) is a successful strategy for promoting seedling development in *E. coracana*. In the future, applying green synthesized ZnOs may improve seed germination and plant growth promotion of other crops. Besides, these NPs could also be used as a readily absorbable form of micronutrient, boosting the successful establishment of crops under stress conditions and improving crop productivity in farmers' fields.

Data Availability

The data can be obtained from the corresponding author upon request.

Ethical Approval

This article does not contain any studies with human participants or animals performed by any of the authors.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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References

[1] A. P. Ramos, M. A. E. Cruz, C. B. Tovani, and P. Ciancaglini, "Biomedical applications of nanotechnology," *Biophysical reviews*, vol. 9, no. 2, pp. 79–89, 2017.

[2] T. Aziz, A. Ullah, A. Ali et al., "Manufactures of biodegradable and bio-based polymers for bio-materials in the pharmaceutical field," *Journal of Applied Polymer Science*, vol. 139, no. 29, pp. 1–21, 2022.

[3] A. Sirelkhatim, S. Mahmud, A. Seeni et al., "Review on zinc oxide nanoparticles: antibacterial activity and toxicity mechanism," *Nano-Micro Letters*, vol. 7, no. 3, pp. 219–242, 2015.

[4] M. Oves, M. A. Rauf, M. O. Ansari et al., "Graphene decorated zinc oxide and curcumin to disinfect the methicillin-resistant *Staphylococcus aureus*," *Nanomaterials*, vol. 10, no. 5, pp. 1004–1017, 2020.

[5] M. Anjum, M. Oves, R. Kumar, and M. A. Barakat, "Fabrication of ZnO-ZnS@ polyaniline nanohybrid for enhanced photocatalytic degradation of 2-chlorophenol and microbial contaminants in wastewater," *International Biodeterioration & Biodegradation*, vol. 119, pp. 66–77, 2017.

[6] D. H. Abdelkader, W. A. Negm, E. Elekhnawy, D. Eliwa, B. N. Aldosari, and A. S. Almurshedi, "Zinc oxide nanoparticles as potential delivery carrier: green synthesis by Aspergillus Niger endophytic fungus, characterization, and in vitro/in vivo antibacterial activity," *Pharmaceuticals*, vol. 15, no. 9, pp. 1057–1117, 2022.

[7] M. Ahmar Rauf, M. Oves, F. Ur Rehman, A. Rauf Khan, and N. Husain, "Bougainvillea flower extract mediated zinc oxide's nanomaterials for antimicrobial and anticancer activity," *Biomedicine & Pharmacotherapy*, vol. 116, pp. 108983–109012, 2019.

[8] J. D. Onubun, J. L. Konne, and G. A. Cookey, "Green synthesis of zinc oxide and hydrogenated zinc oxide catalysts," *Material Sciences: An Indian Journal*, vol. 15, no. 4, pp. 1–12, 2017.

[9] P. Dasta, A. Pratap Singh, and A. Pratap Singh, "Zinc oxide nanoparticle as a heterogeneous catalyst in generation of biodiesel," *Materials Today Proceedings*, vol. 52, pp. 751–757, 2022.

[10] D. Maity, U. Gupta, and S. Saha, "Biosynthesized metal oxide nanoparticles for sustainable agriculture: next-generation nanotechnology for crop production, protection and management," *Nanoscale*, vol. 14, no. 38, pp. 13950–13989, 2022.

[11] R. A. de Jesus, G. C. de Assis, R. J. de Oliveira et al., "Environmental remediation potentialities of metal and metal oxide nanoparticles: mechanistic biosynthesis, influencing factors, and application standpoint," *Environmental Technology & Innovation*, vol. 24, pp. 101851–101921, 2021.

[12] A. Hussain, M. Oves, M. F. Alajmi et al., "Biogenesis of ZnO nanoparticles using Pandanus odorifer leaf extract: anticancer and antimicrobial activities," *RSC Advances*, vol. 9, no. 27, pp. 15357–15369, 2019.

[13] H. M. Fouda and M. R. Sofy, "Effect of biological synthesis of nanoparticles from Penicillium chrysogenum as well as traditional salt and chemical nanoparticles of zinc on Canola plant oil productivity and metabolic activity," *Egyptian Journal of Chemistry*, vol. 65, pp. 1-2, 2022.

[14] M. H. Hajian, M. Ghorbanpour, F. Abtahi, and J. Hadian, "Differential effects of biogenic and chemically synthesized silver-nanoparticles application on physiological traits, antioxidative status and californidine content in California poppy (Eschscholzia californica Cham)," *Environmental Pollution*, vol. 292, pp. 118300–118316, 2022.

[15] S. Begum, A. Zahid, T. Khan, N. Z. Khan, and W. Ali, "Comparative analysis of the effects of chemically and biologically synthesized silver nanoparticles on biomass accumulation and secondary metabolism in callus cultures of Fagonia indica," *Physiology and Molecular Biology of Plants*, vol. 26, no. 8, pp. 1739–1750, 2020.

[16] M. Girilal, A. Mohammed Fayaz, L. K. Elumalai, A. Sathiyaseelan, J. Gandhiappan, and P. T. Kalaichelvan, "Comparative stress physiology analysis of biologically and chemically synthesized silver nanoparticles on Solanum lycopersicum L," *Colloid and Interface Science Communications*, vol. 24, pp. 1–6, 2018.

[17] S. Jadoun, N. P. S. Chauhan, P. Zarrintaj et al., "Synthesis of nanoparticles using microorganisms and their applications: a review," *Environmental Chemistry Letters*, vol. 20, no. 5, pp. 3153–3197, 2022.

[18] N. Arshi, Y. Prashanthi, T. N. Rao, F. Ahmed, S. Kumar, and M. Oves, "Biosynthesis of ZnO nanostructures using Azadirachta indica leaf extract and their effect on seed germination and seedling growth of tomato: an eco-friendly approach," *Journal of Nanoelectronics and Optoelectronics*, vol. 15, no. 11, pp. 1412–1422, 2020.

[19] R. Singh, C. Hano, G. Nath, and B. Sharma, "Green biosynthesis of silver nanoparticles using leaf extract of Carissa carandas L. and their antioxidant and antimicrobial activity against human pathogenic bacteria," *Biomolecules*, vol. 11, no. 2, pp. 299–310, 2021.

[20] S. S. Sana, H. Li, Z. Zhang et al., "Recent advances in essential oils-based metal nanoparticles: a review on recent developments and biopharmaceutical applications," *Journal of Molecular Liquids*, vol. 333, pp. 115951–116015, 2021.

[21] S. Hisiger and M. Jolicoeur, "Analysis of Catharanthus roseus alkaloids by HPLC," *Phytochemistry Reviews*, vol. 6, no. 2-3, pp. 207–234, 2007.

[22] H. Mumivand, P. Khanizadeh, M. R. Morshedloo et al., "Improvement of growth, yield, seed production and phytochemical properties of Satureja khuzistanica jamzad by foliar application of boron and zinc," *Plants*, vol. 10, no. 11, pp. 2469–2515, 2021.

[23] S. Yeboah, J. Asibuo, P. Oteng-Darko et al., "Impact of foliar application of zinc and magnesium aminochelate on bean physiology and productivity in Ghana," *International Journal of Agronomy*, vol. 2021, Article ID 9766709, 1–9 pages, 2021.

[24] N. Natasha, M. Shahid, I. Bibi et al., "Zinc in soil-plant-human system: a data-analysis review," *Science of the Total Environment*, vol. 808, pp. 152024–152121, 2022.

[25] S. Praharaj, M. Skalicky, S. Maitra et al., "Zinc biofortification in food crops could alleviate the zinc malnutrition in human health," *Molecules*, vol. 26, no. 12, pp. 3509–3517, 2021.

[26] G. Jat, S. Sharma, R. Meena et al., "Amelioration of zinc deficiency in blackgram (Vigna mungo L.) through soil applied zinc in Typic Haplustepts soil of Rajasthan," *Journal of Environmental Biology*, vol. 42, no. 6, pp. 1554–1559, 2021.

[27] P. Kumari, P. Kajla, and S. Naudiyal, "Finger millet (eleusine coracana L.)—properties and health benefits," *Handbook of Cereals, Pulses, Roots, and Tubers*, CRC Press, Boca Raton, FL. USA, pp. 157–172, 2021.

[28] A. A. Powell, J. M. Thornton, and J. A. Mitchell, "Vigour differences in brassica seed and their significance to emergence and seedling variability," *The Journal of Agricultural Science*, vol. 116, no. 3, pp. 369–373, 1991.

[29] E. A. Ibrahim, "Seed priming to alleviate salinity stress in germinating seeds," *Journal of Plant Physiology*, vol. 192, pp. 38–46, 2016.

[30] S. H. Nile, M. Thiruvengadam, Y. Wang et al., "Nano-priming as emerging seed priming technology for sustainable agriculture—recent developments and future perspectives," *Journal of Nanobiotechnology*, vol. 20, no. 1, pp. 1–31, 2022.

[31] D. Mishra, M. K. Chitara, and P. Chaturvedi, "Study of phytochemicals, antioxidant activity and antimicrobial properties of Catharanthus roseus (L.) G. Don," *Emergent Life Sciences Research*, vol. 8, no. 1, pp. 75–79, 2022.

[32] S. M. Lam, J. C. Sin, A. Z. Abdullah, and A. R. Mohamed, "Degradation of wastewaters containing organic dyes photocatalysed by zinc oxide: a review," *Desalination and Water Treatment*, vol. 41, no. 1-3, pp. 131–169, 2012.

[33] AOSA, "Seed vigor testing handbook. Association of official seed analysts," *Contribution*, vol. 32, 1983.

[34] S. Azizi, R. Mohamad, A. Bahadoran et al., "Effect of annealing temperature on antimicrobial and structural properties of bio-synthesized zinc oxide nanoparticles using flower extract of Anchusa italica," *Journal of Photochemistry and Photobiology B: Biology*, vol. 161, pp. 441–449, 2016.

[35] J. Santhoshkumar, S. V. Kumar, and S. Rajeshkumar, "Synthesis of zinc oxide nanoparticles using plant leaf extract against urinary tract infection pathogen," *Resource-Efficient Technologies*, vol. 3, no. 4, pp. 459–465, 2017.

[36] P. Rai-Kalal, R. S. Tomar, and A. Jajoo, "H_2O_2 signaling regulates seed germination in ZnO nanoprimed wheat (*Triticum aestivum* L.) seeds for improving plant performance under drought stress," *Environmental and Experimental Botany*, vol. 189, pp. 104561–104612, 2021.

[37] M. Ajaib, M. Ishtiaq, K. H. Bhatti et al., "Inventorization of traditional ethnobotanical uses of wild plants of drgdna," *PLoS One*, vol. 16, no. 7, pp. e0255010–e0255018, 2021.

[38] M. Khan, P. Ware, and N. Shimpi, "Synthesis of ZnO nanoparticles using peels of Passiflora foetida and study of its activity as an efficient catalyst for the degradation of hazardous organic dye," *SN Applied Sciences*, vol. 3, no. 5, pp. 528–617, 2021.

[39] A. Jayachandran, A. Tr, and A. S. Nair, "Green synthesis and characterization of zinc oxide nanoparticles using Cayratia pedata leaf extract," *Biochemistry and Biophysics Reports*, vol. 26, pp. 100995–100998, 2021.

[40] A. Chaudhary, N. Kumar, R. Kumar, and R. K. Salar, "Antimicrobial activity of zinc oxide nanoparticles synthesized from Aloe vera peel extract," *SN Applied Sciences*, vol. 1, no. 1, pp. 136–139, 2019.

[41] T. Khalafi, F. Buazar, and K. Ghanemi, "Phycosynthesis and enhanced photocatalytic activity of zinc oxide nanoparticles toward organosulfur pollutants," *Scientific Reports*, vol. 9, no. 1, pp. 6866–6910, 2019.

[42] W. Muhammad, N. Ullah, M. Haroon, and B. H. Abbasi, "Optical, morphological and biological analysis of zinc oxide nanoparticles (ZnO NPs) using Papaver somniferum L," *RSC Advances*, vol. 9, no. 51, pp. 29541–29548, 2019.

[43] S. Yedurkar, C. Maurya, and P. Mahanwar, "Biosynthesis of zinc oxide nanoparticles using Ixora coccinea leaf extract-a green approach," *Open Journal of Synthesis Theory and Applications*, vol. 05, no. 01, pp. 1–14, 2016.

[44] M. Ebadi, M. R. Zolfaghari, S. S. Aghaei et al., "A bio-inspired strategy for the synthesis of zinc oxide nanoparticles (ZnO NPs) using the cell extract of cyanobacterium Nostoc sp. EA03: from biological function to toxicity evaluation," *RSC Advances*, vol. 9, no. 41, pp. 23508–23525, 2019.

[45] M. S. Geetha, H. Nagabhushana, and H. N. Shivananjaiah, "Green mediated synthesis and characterization of ZnO nanoparticles using Euphorbia Jatropa latex as a reducing agent," *Journal of Science: Advanced Materials and Devices*, vol. 1, no. 3, pp. 301–310, 2016.

[46] N. Sedefoglu, Y. Zalaoglu, and F. bozok, "Green synthesized ZnO nanoparticles using ganoderma lucidum: characterization and in vitro nanofertilizer effects," *Journal of Alloys and Compounds*, vol. 918, pp. 165695–165716, 2022.

[47] Y. A. Selim, M. A. Azb, I. Ragab, and M. H M Abd El-Azim, "Green synthesis of zinc oxide nanoparticles using aqueous extract of Deverratortuosa and their cytotoxic activities," *Scientific Reports*, vol. 10, no. 1, pp. 3445–3449, 2020.

[48] K. Neme, A. Nafady, S. Uddin, and Y. B. Tola, "Application of nanotechnology in agriculture, postharvest loss reduction and food processing: food security implication and challenges," *Heliyon*, vol. 7, no. 12, pp. 085399–e8612, 2021.

[49] T. Eichert, A. Kurtz, U. Steiner, and H. E. Goldbach, "Size exclusion limits and lateral heterogeneity of the stomatal foliar uptake pathway for aqueous solutes and water-suspended nanoparticles," *Physiologia Plantarum*, vol. 134, no. 1, pp. 151–160, 2008.

[50] Y. Kumar, T. Singh, and R. Raliya, "Nanofertilizers and their role in sustainable agriculture," *Annals of Plant and Soil Research*, vol. 23, no. 3, pp. 238–255, 2021.

[51] Z. Hua, S. Ma, Z. Ouyang, P. Liu, H. Qiang, and X. Guo, "The review of nanoplastics in plants: detection, analysis, uptake, migration and risk," *TrAC, Trends in Analytical Chemistry*, vol. 158, pp. 116889–116912, 2023.

[52] A. Mardi, H. Mohajjel Shoja, and E. Mohajel Kazemi, "Comparative study of growth responses, photosynthetic pigment content, and gene expression pattern in tobacco plants treated with ZnO nano and ZnO bulk particles," *Journal of Nanoparticle Research*, vol. 24, no. 10, pp. 208–218, 2022.

[53] A. M. El-Badri, M. Batool, C. Wang et al., "Selenium and zinc oxide nanoparticles modulate the molecular and morpho-physiological processes during seed germination of Brassica napus under salt stress," *Ecotoxicology and Environmental Safety*, vol. 225, pp. 112695–112713, 2021.

[54] D. Sharma, S. Afzal, and N. K. Singh, "Nanopriming with phytosynthesized zinc oxide nanoparticles for promoting germination and starch metabolism in rice seeds," *Journal of Biotechnology*, vol. 336, pp. 64–75, 2021.

[55] R. Y. Rawashdeh, A. M. Harb, and A. M. AlHasan, "Biological interaction levels of zinc oxide nanoparticles; lettuce seeds as case study," *Heliyon*, vol. 6, no. 5, pp. 039833–e4010, 2020.

[56] A. Tymoszuk and J. Wojnarowicz, "Zinc oxide and zinc oxide nanoparticles impact on in vitro germination and seedling growth in Allium cepa L," *Materials*, vol. 13, no. 12, pp. 2784–2816, 2020.

[57] K. V. Pavani, M. Beulah, and G. U. Sai-Poojitha, "The effect of zinc oxide nanoparticles (ZnO NPs) on Vigna mungo L. seedling growth and antioxidant activity," *Nanoscience and Nanotechnology - Asia*, vol. 10, no. 2, pp. 117–122, 2020.

[58] M. Rafique, R. Tahir, S. S. A. Gillani et al., "Plant-mediated green synthesis of zinc oxide nanoparticles from Syzygium Cumini for seed germination and wastewater purification," *International Journal of Environmental Analytical Chemistry*, vol. 102, no. 1, pp. 23–38, 2022.

[59] F. Wu, Q. Fang, S. Yan, L. Pan, X. Tang, and W. Ye, "Effects of zinc oxide nanoparticles on arsenic stress in rice (Oryza sativa L.): germination, early growth, and arsenic uptake," *Environmental Science and Pollution Research*, vol. 27, no. 21, pp. 26974–26981, 2020.

[60] P. D. Itroutwar, G. Kasivelu, V. Raguraman, K. Malaichamy, and S. K. Sevathapandian, "Effects of biogenic zinc oxide nanoparticles on seed germination and seedling vigor of maize (Zea mays)," *Biocatalysis and Agricultural Biotechnology*, vol. 29, pp. 101778–101785, 2020.

[61] J. Israel García-López, R. H. Lira-Saldivar, F. Zavala-García et al., "Effects of zinc oxide nanoparticles on growth and antioxidant enzymes of Capsicum chinense," *Toxicological and Environmental Chemistry*, vol. 100, no. 5-7, pp. 560–572, 2018.

[62] E. Iziy, A. Majd, M. R. Vaezi-Kakhki, T. Nejadsattari, and S. Kazemi Noureini, "Effects of zinc oxide nanoparticles on enzymatic and nonenzymatic antioxidant content, germination, and biochemical and ultrastructural cell characteristics of Portulaca oleracea L," *Acta Societatis Botanicorum Poloniae*, vol. 88, no. 4, pp. 1–14, 2019.

[63] A. Salam, A. R. Khan, L. Liu et al., "Seed priming with zinc oxide nanoparticles downplayed ultrastructural damage and improved photosynthetic apparatus in maize under cobalt stress," *Journal of Hazardous Materials*, vol. 423, pp. 127021–127117, 2022.

[64] A. Azmat, Y. Tanveer, H. Yasmin et al., "Coactive role of zinc oxide nanoparticles and plant growth promoting rhizobacteria for mitigation of Synchronized effects of heat and drought stress in wheat plants," *Chemosphere*, vol. 297, pp. 133982–134024, 2022.