Ishfaq A *et al.* (2025)
Notulae Botanicae Horti Agrobotanici Cluj-Napoca
Volume 53, Issue 2, Article number 14419
DOI:10.15835/nbha53214419
Research Article

Sustainable agriculture through nano-priming: Evaluating the role of biogenic manganese oxide nanoparticles in mitigating wastewater-induced stress in wheat (*Triticum aestivum*)

Aneeza ISHFAQ[1], Sabir HUSSAIN[1], Tanvir SHAHZAD[1], Muhammed SHAHID[2], Faisal MAHMOOD[1]*, Syed Raza HUSSAIN[1], Irfan HAIDRI[1], Imran KHAN[3], Muhammad Ahsan ASGHAR[4], Muhammad Fuad ANSHORI[5], Mahmoud F. SELEIMAN[6]

[1] *Government College University Faisalabad, Department of Environmental Science, Faisalabad, 38000, Pakistan; aneeza.201703601@gcuf.edu.pk; sabir.hussain@gcuf.edu.pk; tanvirshahzad@gcuf.edu.pk; faisalmahmood@gcuf.edu.pk (*corresponding author); raza.hussain.starfarm@gmail.com; irfanhaidri.2017010933@gcuf.edu.pk*
[2] *Government College University Faisalabad, Department of Bioinformatics & Biotechnology, Faisalabad, 38040, Pakistan; mshahid@gcuf.edu.pk*
[3] *University of Agricultural Faisalabad, Department of Agronomy, Faisalabad, Pakistan; drimran@uaf.edu.pk*
[4] *Agricultural Institute, Centre for Agricultural Research, Department of Biological Resources, ELKH, Brunzvik St. Martonvásár, 2462, Hungary; ahsanasghar3853@gmail.com*
[5] *Hasanuddin University, Department of Agronomy, Makassar, 90245, Indonesia; faud.anshori@unhas.ac.id*
[6] *King Saud University, College of Food and Agriculture Sciences, Department of Plant Production, P.O. Box 2460, Riyadh 11451, Saudi Arabia; mseleiman@ksu.edu.sa*

Abstract

Nanoparticles have potential to mitigate the harmful effects of wastewater in agricultural crop, reducing crop contamination and improving wheat growth and development. However, no information has been reported concerning the impact of seed priming of biogenic MnO-NPs on the germination and physiochemical parameters of wheat crop under wastewater stress condition. The current study examined the impact of primed seed with biosynthesized manganese oxide nanoparticles (MnO-NPs) from *Bacillus flexus* on wheat growth under wastewater stress. This study was conducted in a pot experiment. Wheat seeds were primed with different concentrations of MnO-NPs (0, 20,40, 60, 80, and 100 mg L^{-1}). Primed seeds were sown in wastewater-polluted soil. The pot experiment was conducted for 30 days. The soil in the pots was kept consistently moist, with moisture levels at about 60-70% of the soil's maximum water-holding capacity. Control included seeds without MnO-NP priming. The wastewater contained contaminants such as heavy metals and organic pollutants. The result showed that 80 mg L^{-1}MnO-NP treatment showed a more positive impact in combating wastewater stress. MnO-NPs at 80 mg L^{-1} positively influenced wheat seed germination parameters up to 57% and growth attributes by 63.75% compared to the control treatment. Moreover, seed priming with MnO-NPs at 80 mg L^{-1} improved gas exchange attributes by 67%, increased chlorophyll contents by 53.5%, and enhanced antioxidant enzyme activities: 38% for superoxide dismutase (SOD), 61% for ascorbate peroxidase (APX), 65% for catalase (CAT), and 55% for peroxidase (POD). Meanwhile, hydrogen peroxide(H_2O_2), malondialdehyde (MDA), and electrolyte leakage (EL) were decreased by 51%, 69%, and 52%

Received: 17 Feb 2025. Received in revised form: 04 Apr 2025. Accepted: 25 Jun 2025. Published online: 29 Jun 2025.
From Volume 49, Issue 1, 2021, Notulae Botanicae Horti Agrobotanici Cluj-Napoca journal uses article numbers in place of the traditional method of continuous pagination through the volume. The journal will continue to appear quarterly, as before, with four annual numbers.

respectively. These results demonstrate that primed seed with MnO-NPs can significantly enhance wheat's germination and biochemical attributes under wastewater stress, providing a promising approach for improving crop resilience and productivity in wastewater polluted conditions.

Keywords: biochemicals attributes; cereal crop; manganese oxide nanoparticles; wastewater reuse; wheat

Introduction

Due to global water scarcity, wastewater is much needed for agricultural irrigation reuse (Al-Suhaibani *et al.*, 2021; Seleiman *et al.*, 2021). Generally, this wastewater is used to irrigate the field crops near the cities. The improper disposal of such wastewater leads to water, air, and soil pollution. This problem is even more severe in developing countries, where wastewater is utilized for various purposes, including irrigation (Haidri *et al.*, 2023; Rubab *et al.*, 2023). Untreated or inadequately treated wastewater may negatively impact soil, plants, ecosystems, and human beings. The quality of irrigation water plays a crucial role in farming practices, directly influencing the soil's physical and chemical properties and the overall crop yield. Using low-quality irrigation water, such as wastewater or saline-sodic water, reduces crop yield and contributes to soil deterioration (Hashem and Qi, 2021). Estimates suggest that water demand will rise by approximately 30% by 2040 (Kumar and Lee, 2012). It is estimated that the world produces over 359 billion cubic meters (95 trillion gallons) of wastewater annually (Hashmi *et al.*, 2023). There are many sources of wastewater, i.e., industries, household activities, etc. Household activities contribute significantly to wastewater generation, especially in urban areas (Ahmed *et al.*, 2022; Fu *et al.*, 2022; Irfan *et al.*, 2023).

Rapid industrialization, urbanization, and increasing population are putting tremendous pressure on natural resource utilization and ecosystem deterioration (Barros Ruas *et al.*, 2022; Zhang *et al.*, 2022). Wastewater contains large amounts of salts and heavy metals which may affect the physiological processes, particularly in salt-sensitive crops, and lead to lower crop yield *(Li et al.*, 2022; Mustafa *et al.*, 2023). Moreover, there are many studies, that show that heavy metals hinder the metabolic process of the crops. Especially, heavy metals like Cd, Cu, Zn, Pb, and Hg can replace the central atom of chlorophyll and magnesium (Anderson *et al.*, 2022; Liu *et al.*, 2023). This replacement interferes with chlorophyll's ability to absorb light, hindering photosynthesis in stressed plants. Photosynthesis involves the absorption of light energy, which is then converted into biochemical energy (Shebl *et al.*, 2019; Haidri *et al.*, 2024). Recently, nanomaterials have gained significant attention across various fields (Ahmad *et al.*, 2023; Seleiman *et al.*, 2023). Previous studies have proven that nanoparticles (NPs) are highly effective in reducing the risks posed by abiotic stresses in plants (Ye *et al.*, 2019; Al-Selwey*et al.*, 2023; Shafqat *et al.*, 2023). Bacterial synthesis of nanoparticles is often considered more efficient than plant-based synthesis for several reasons: control over synthesis conditions, higher yield, and cost-effectiveness. Silver nanoparticles (AgNPs) were found to mitigate the toxic effects of lead in two genotypes of *Vigna radiata.* The results showed that 25 mg L^{-1} of AgNPs was the optimal concentration, yielding the best results in both genotypes (Chen *et al.*, 2022). Another study showed that selenium nanoparticles SeNPs at 10 mg L^{-1} have alleviated the harmful effects of drought and heat stresses on wheat (*Triticum aestivum* L.) seedlings (Omar *et al.*, 2023). Similarly, due to their multiple functions, reports showed that NPs have been used in wastewater reclamation. Previous studies showed that MnNPs and ZnNP have been used to remove pollutants from wastewater (Harsh *et al.*, 2023). Biosynthesized MnNPs used for wastewater treatment demonstrated a 79% removal of COD (Chemical Oxygen Demand) from the wastewater (Ishfaq *et al.*, 2023). Biosynthesized ZnNPs used for wastewater treatment demonstrated a 76.5% removal of COD (Chemical Oxygen Demand) from the wastewater (Haidri *et al.*, 2023).

Crops are a significant source of essential nutrients for animals and humans (Rubab *et al.*, 2023). Important minerals like iron (Fe) with manganese (Mn) must be absorbed, and this depends upon soil properties along with the presence of microbes and the soil's nutrient concentrations (Mpanga *et al.*, 2022). Plant development depends on the germination of seeds and after that establishment of seedlings (Heidari and Kahrizi, 2018). The nutritious composition of seeds may be enhanced by using nano-priming methods with micronutrients like zinc and magnesium, according to earlier research (Ishfaq *et al.*, 2023, 2025). Primed seed with nanoparticles has increased attention in today's research to improve crop yield (Gao *et al.*, 2024). Primed seeds often show better tolerance to various stresses, including heavy metal stress. They may have improved antioxidant defenses and detoxification mechanisms. According to the research, seed priming enhances phenolic accumulation, nutrient uptake, photosynthetic activity, net assimilation rate, leaf area index, relative water content (RWC), chlorophyll (Chl) content, and antioxidant enzyme activities (Imran *et al.*, 2021; Ahmad *et al.*, 2024). Recently, reports exposed that nano-priming improved the morpho-physiological attributes of plants under stress conditions. For example, chili (*Capsicum annum* L.) seeds priming with metal oxide nanoparticles (zinc, titanium, and silver) enhanced seedling growth processes (Kumar *et al.*, 2020).

Wheat (*Triticum aestivum* L.) is an essential crop for 35 percent of the world's population. As per (Wheat and Glutens 1996), it is acknowledged as the principal cereal crop globally. According to a recent survey, it is the second most farmed crop after maize, with a global production of 749 million tons (Shafqat *et al.*, 2023). So, Numerous items that support the cattle, as well as poultry feed industries, are made from wheat. Pakistan is among the nations where a scarcity of water represents an issue. Water scarcity and irrigation with wastewater are two more serious problems associated with wheat cultivation (Rhaman *et al.*, 2022; Irfan *et al.*, 2023). Therefore, this research aimed to evaluate the role of MnO-NPs in maintaining plant development and mitigating the negative impacts of wastewater. Specifically, we implemented seed priming with MnO-NPs to enhance seed germination and developmental performance. Seed priming accelerates germination, enabling dormant seeds to germinate more rapidly and uniformly. This method helps plants become more resilient to various environmental stresses, including those induced by wastewater (Sytar *et al.*, 2019). The current research offers a new method for mitigating the negative impacts on wheat crops using biosynthesized MnO-NPs as seed priming. Therefore, this study was conducted with the following objectives; i) to mitigate the negative impact of wastewater stress on wheat by using the biogenic MnO-NPs, ii) to improve the germination and biochemical attributes of wheat under wastewater stress.

Materials and Methods

Biosynthesis and characterization of MnO-NPs

It was reported that Ishfaq *et al.* (2023) that manganese oxide (MnO) nanoparticles were synthesized using *Bacillus flexus* with 10 mM $MnCl_2.4H_2O$. Bacteria cultured in 100 mL nutrient broth on a rotary shaker (120 rpm, 28 °C) for 24 hours turned from yellow to dark brown upon adding 0.1 mM $MnCl_2$. After shaking for 24 hours, centrifugation (700 rpm, 10 min), and oven drying (24 hours), MnO-NPs were obtained. There are some common analytical instruments and techniques used for characterizing nanoparticles: Scanning Electron Microscopy (SEM), X-ray Diffraction (XRD), Fourier Transform Infrared Spectroscopy (FTIR), Ultraviolet-Visible Spectroscopy (UV-Vis). These nanoparticles (MnO-NPs) were subsequently applied to wheat plants against wastewater stress conditions. The UV-visible spectrometer detected a peak at 325.23 nm, indicating the biosynthesis of MnO NPs in Figure 1A). SEM results showed that MnO nanoparticles are agglomerated flakes with spherical particles (Figure 1D). XRD analysis revealed that the MnO-NPs have an average size of 21 to 30 nm (Figure 1B). Furthermore, FTIR of MnO-NPs was examined the strongest peak at 3397.55 cm^{-1} was observed due to the broad bonded N-H/O-H stretching group of alcohol and amine. The peaks at 1725.41 cm^{-1} and 1532.05 cm^{-1} represented the C-O group of aldehydes and N-O stretching group of nitro compounds, respectively. The peaks at 1230.24 cm^{-1} were due to the C-O group of ether stretching (Figure 1C).

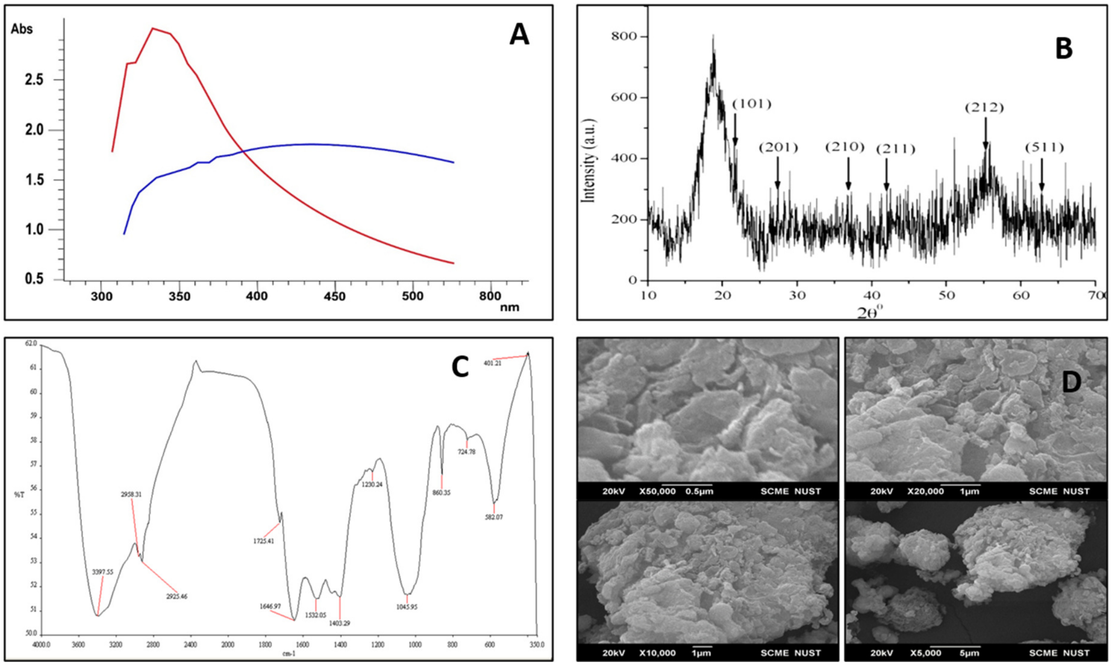

Figure 1. Characterization of MnO-NPs: UV–VIS spectroscopy (A), XRD (B), FTIR (C), and SEM (D) (Ishfaq *et al.*, 2023)

Sample collection of wastewaters

Samples of wastewater were collected from the industrial drainage near Sargodha Road, Faisalabad, Pakistan (31°25′15″ N 73°5′21″ E). This area is known for its industrial activities and wastewater outlets, including textile mills, dyeing units, tanneries, and metalworking factories. These samples were centrifuged at 1000 RPM to remove particles and stored at 4 °C to analyze their physical and chemical properties. Wastewater's physicochemical characteristics were examined according to the standard methods of Arshad and Shakoor (2017)(Table 1).

Table 1. Analysis of different parameters of wastewater

Parameters	Value
Color removal	0.14
E.C(dS m^{-1})	5.4
PH	8.6
COD (mg L^{-1})	371
TDS (mg L^{-1})	3403
Sulfates (mg L^{-1})	761
Phosphates (mg L^{-1})	345
Manganese (mg L^{-1})	15

Seed priming with MnO-NPs

Wheat seeds (Akbar-2019) were sterilized using sodium hypochlorite for 2 minutes. Wheat seeds were splashed with deionized water to clear the residue. For seed priming, a 100 mL solution was prepared with diverse concentrations of MnO-NPs (0, 20, 40, 60, 80, and 100 mg L^{-1}). Initially, nanoparticles were dispersed via mechanical stirring and ultrasonic agitation (100 W, 40 kHz) using sonicators in distilled water for approximately 30 minutes to prevent particle aggregation (Arikan *et al.*, 2022). 100 mL of the resultant solution was used to soak 400 wheat seeds of varying sizes. After soaking, seeds were dried to their initial moisture content and stored 4 °C pot experiment.

Experimental conditions

The pot study was conducted in October 2024 under natural environmental conditions (67 ± 5% humidity, 28/22 °C Day/night temperature, respectively, and light intensity PPFD, 23 μmol m-2s^{-1}) at the garden of GCUF, Faisalabad, Pakistan (31.4° N, 73.06° E). Soil samples were collected from an agricultural field. Surface soil, within the depth range of 0 to 15 cm, was collected in triplicate through an augur. These soil samples were randomly placed in polythene bags. Soil samples were assorted to create composite samples with uniform properties. The soil was sieved to remove stones and other contaminants. Soil's physicochemical characteristics were examined according to the standard methods of Kumar and Rao (2017) before filling in pots (Table 2). Every pot was filled with 5kg of soil. A total numberof 36 pots were used for this experiment. Pots were divided into two groups; the 1st was irrigated with distilled water, while the 2nd was irrigated with wastewater. In the control group of distilled and wastewater, plants were irrigated with distilled water and wastewater with zero concentration of MnO-NPs. There was a total of five treatments of MnO nanoparticles: T1= 20 mg L^{-1} MnO nanoparticles, T2 = 40 mg L^{-1} MnO nanoparticles, T3 = 60 mg L^{-1} MnO nanoparticles, T4 = 80 mg L^{-1} MnO nanoparticles, T5 = 100 mg L^{-1} MnO nanoparticles. 10 primed seeds were planted in individual pots. The plants were thinned after germination and 4 plants were kept per pot. These 4 plants were then utilized for assessing germination parameters. The plants were harvested after 30 days of sowing. Roots and shoots of plants were taken from each pot washed with distilled water and then stored at 4°C for further analysis.

Table 2. Studies of all physiochemical properties of soil

Parameter	**Unit**	**Reading**
Texture	-	Clay loam
E.C.	dS m^{-1}	7.9
pH	-	2.8
Nitrogen	%	0.6
Phosphorous	Mg L^{-1}	11.8
Potassium	Mg L^{-1}	150
Organic matter	%	0.79

Germination parameters

At 2nd day of sowing, the germination rate of the wheat seeds was examined using the methodology of Lahuta *et al.* (2022). Five days after sowing, plumule, and radical lengths were measured. The methodology of Abou-Zeid *et al.* (2021) was used to determine the length of the shoot and roots. The seedling vigor was calculated using the following equations:

Vigor index = Germination% x Seedling length (Root + Shoot)

Plant physical parameters

After 30 days of sowing, plant biomass and growth parameters were examined by following the protocol of Mazhar *et al.* (2023). From each pot, the average shoot and root length, fresh root, and shoot weight were calculated.

Gas exchange parameters

Using the methods described by Macedo *et al.* (2021), the Infrared Gas Analyzer (ADC-225-MK3 + WA-161-MK3A Hoddesdon, UK) was used to assess gas exchange parameters such as net photosynthetic rate, stomatal conductance, transpiration rate, and water usage efficiency. These measurements were taken before the one week of harvest.

Chlorophyll contents

The chlorophyll content, which includes chlorophyll a, chlorophyll b, and carotenoids, was estimated by using the methodology of Lichtenthaler and Buschmann (2001). 0.5 grams of leaf material were ground in

10 ml of 80% (v/v) acetone. Then, the extract was separated by centrifugation at 10,000 rpm for 10 minutes. The supernatant was gathered and kept in refrigerate at -4 °C. According to Lichtenthaler and Buschmann (2001), a spectrophotometer (IRMECO U2020) was utilized to measure the amount of chlorophyll at three distinct wavelengths (663, 645, and 480).

$$\text{Total Chl.} = [20.2(OD_{645}) - 8.02(OD_{663})] \times v/w \times 1/1000$$
$$\text{Chl. a} = [12.7(OD_{663}) - 2.69(OD_{645})] \times v/1000 \times w$$
$$\text{Chl. b} = [22.9(OD_{645}) - 4.68(OD_{663})] \times v/1000 \times w$$
$$\text{A Car. } (\mu g/g\ FW) = OD_{480} + (0.114 \text{ x } OD_{663}) \times (0.638 \times OD_{645})$$

where Car = A Car/Em 100% × 100, emission = Em 100% = 2500, OD = absorbance at respective wavelength, V = volume of the extract (mL), and W = weight of the fresh leaf tissue (g).

Estimation of proline and phenolic content

Proline concentration in wheat plants was measured using the Troll and Lindsley (1955) method. 10 ml of 30% sulpho-salicylic acid was used to grind fresh leaf (0.5 g), and filter paper was used to filter the mixture. Glacial acetic acid and acid ninhydrin (2 ml) were combined with the filtrate solution. A water bath was used to heat the resultant mixture to 100 °C for 60 minutes. The absorbance of the resulting solution was examined by a spectrophotometer (IRMECO U2020) at 520 wavelengths.

Phenolic concentration in wheat plants was measured using the Julkunen-Tiitto (1985) method. Fresh leaves (0.5 g) were crushed with 5 ml of the enzyme extract in 80% (v/v) acetone. After that, the mixture solution was centrifuged at 10,000 rpm for 10 minutes. 1 ml of Folin-Ciocalteu's phenol reagent and 2 ml of distilled water were added to produce 0.1 ml of the supernatant. Afterwards, 5 milliliters of sodium carbonate were added to the mixture solution, and distilled water was used to increase the volume to 10 milliliters. At 750 nm, the absorbance of the solution was measured with a spectrophotometer.

Estimation of antioxidants

Through spectroscopic evaluation, various enzymatic antioxidant content such as SOD, POD, APX, and CAT in wheat plants were examined. Maehly (2006) method was used to measure SOD and POD content. Fresh samples of leaves were washed using distilled water. From these, a fresh leaf of 0.5 g was ground in 0.05 M phosphate buffer (pH 7.8). The centrifugation was done at 4000 rpm for four minutes at 4 °C; the cooled temperature was set at 4 °C. Finally, the supernatant was collected and subsequently, the spectrophotometer was utilized for the SOD and POD activity measurements. To score the CAT activity, the Cohen *et al.* (1996) method was used. An experimental solution that comprised 3 mL in 1 L of H_2O_2, which was approximately 100 mg mL^{-1}, 100 mL of sample (3000 mM) was prepared. At 240 nm, the absorbance of the solution was measured with a spectrophotometer. To assess the activity of APX enzymatic activity the procedure originally described Amako *et al.* (1994) was applied.

Estimation of electrolyte leakage

Thalhammer *et al.* (2014) procedure used to calculate electrolyte leakage (EL). The reagent used in this experiment is 8 milliliters of distilled water to wet a 5 mm plant sample. Then, by interacting twice with a water bath kept at 32 °C for 2 hours, it remained. The sample's starting electrical conductance (EC1) has been calculated. After that, the sample was put in an autoclave for 20 minutes at 121 ºC to cleanse the remaining electrolytes. When at T = 25 °C, the sample's second electrical conductance (EC2) was recorded in the experiment. For this, the equation for EL was used as follows:

$$EL = (EC1/EC2) \times 100$$

Estimation of hydrogen peroxide and malondialdehyde content

By following the method of Eisenberg (1943), fresh leaves (50 mg) were ground with phosphate buffer (50 mM, pH 6.5) to quantify H_2O_2. The amount of H_2O_2 present was measured by centrifuging a mixture containing 3 mL of enzyme extract and 1 mL of titanium sulfate in 20% (v/v) H_2SO_4 at 6000 rpm for 15 minutes. The absorbance of this solution was measured by spectrophotometer at 410 nm.

A method described by Davey *et al.* (2005) was used to measure the quantity of MDA. 5 mL of trichloroacetic acid (TCA) at a 0.1% concentration was combined with one gram of leaf extract. For five minutes, the solution was centrifuged at 10,000 rpm for 10 minutes. 1 mL of the supernatant was combined with 20% TCA (4 mL) and 0.5% thiobarbituric acid (TBA). After 30 minutes at 95 °C in the water bath, the mixed solution was allowed to cool to room temperature. The mixture's absorbance was measured at 532 nm using the spectrophotometer after 10 minutes of centrifugation at 10,000 rpm.

Estimation of Mn concentration in wheat

The method Bhat *et al.* (2010) was followed to estimate manganese concentration in wheat. The dried sample of plant shoot and root (0.5g) was splashed with distilled water and taken in the flask. The samples were mixed with concentrated nitric and perchloric acids ($HClO_4$-HNO_3). They were added at 1:3 and kept for 24 hours. The mixed solution was boiled until all the samples were dissolved. To ensure the quality of the sample result, a blank solution is maintained as a control. The amount of Mn was measured using atomic absorption spectroscopy (Hitachi, Model 7JO-8024; Tokyo, Japan).

Statistical analysis

The statistical analysis was performed using Statistix 8.1 to conduct a two-way analysis of variance (ANOVA). Statistical design was performed in complete randomized design (CRD). The experiment was conducted in triplicates. To determine whether there were any statistically significant variations between the means of the different treatments, the 95% confidence level Fisher's Least Significant Difference (LSD) test was utilized. The results are presented as mean values ± standard deviation (SD). A significant variation is indicated by treatments with different letters.

Results

Germination parameters of seed

Results displayed that in wastewater, seed priming at the concentration of 80 mg L^{-1} led to a significant increase of 40% in germination percentage, 70% enhancement in radical length, 40% growth in Plumule length, and 70% rise in seedling vigor index (SVI), compared to the control treatment (Figure 2A-D). Similarly, primed seeds with MnO nanoparticles (NPs) at varying concentrations showed variable germination percentages; however, maximum germination was observed at 80 mgL^{-1} In distilled water, seed priming at the concentration of 80 mg L^{-1} led to a significant increase of 44% in germination percentage, 75% enhancement in radical length, 44% growth in plumule length, and 72% rise in Seedling Vigor Index (SVI), as well as the control treatment. When the concentration increased to 100 mg L^{-1}, both germination and growth showed a decline due to the possibility of toxicity.

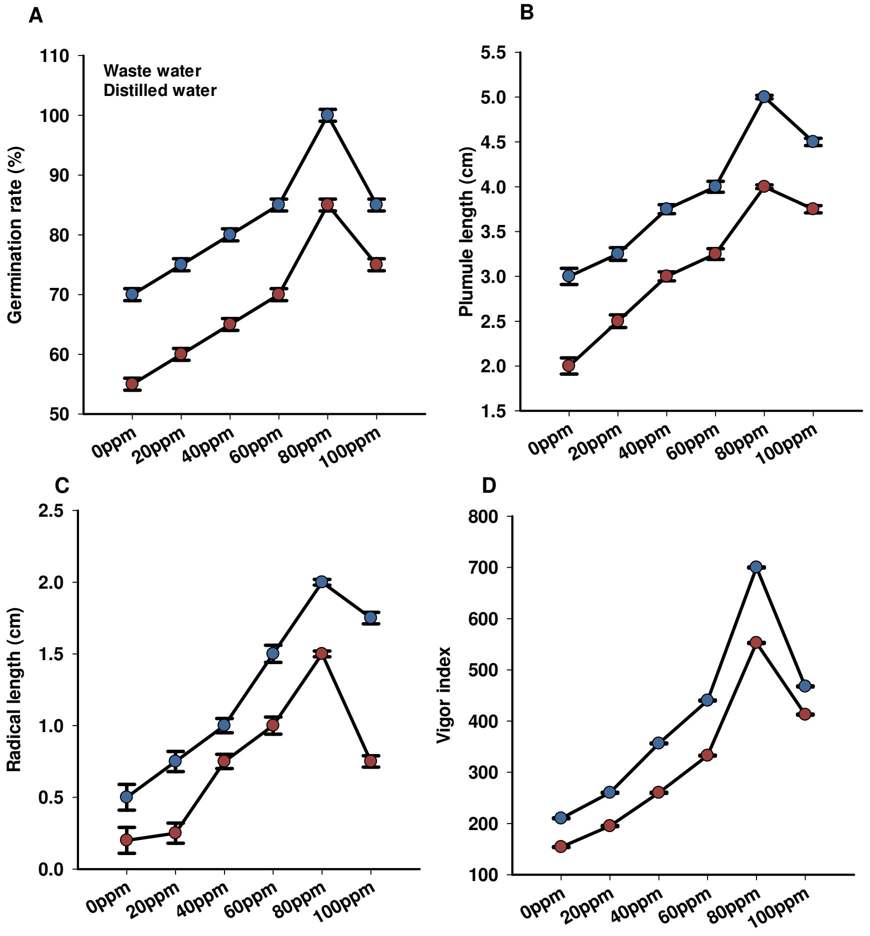

Figure 2. Effect of seed priming with different concentrations of manganese oxide nanoparticles on germination attributes of wheat seedlings (mean ± standard error; n = 4)
GR germination rate(A), shoot length (B), Root length (C), vigour index(D)

Plant physical growth attributes

The study evaluated the effects of primed seed with varying concentrations of MnO-NPs on wheat growth under wastewater stress by measuring shoot and root heights, as well as fresh shoot and root biomass (Figure 3A-D). Results showed a significant reduction in shoot and root height, fresh weight (FM), and dry weight (DM) due to the imposition of wastewater stress. However, seed priming with MnO-NPs enhanced the physical growth parameters of wheat plants. The treatments (wastewater and distilled) where seeds were primed with 80 mg L^{-1} MnO-NPs showed the highest development in plant heights; nevertheless, the distilled water treatment showed an increase in plant height in comparison to the wastewater-stressed treatment. Applying the MnO-NPs concentration (80 mg L^{-1}) in distilled water treatment resulted in a significant increase of 52% in shoot height, 58% in root height, 74% in shoot biomass, and 74% in root biomass as well as to the control (distilled water without nanoparticles). Similarly, under wastewater stress conditions, MnO-NP concentration led to a substantial enhancement of 43% in shoot height, 55% in root height, 72% in shoot biomass, and 72% in root biomass as well as to the control (wastewater without nanoparticles). It was observed that wheat growth attributes showed a significant increase up to 80 mg L^{-1} concentration of MnO-NPs under wastewater stress conditions, however, 100 mg L^{-1} of MnO-NP concentration showed a decline in growth. These findings conclude that using MnO-NPs improves wheat plants' growth parameters only up to a specific concentration, after that an increase in concentration results in a negative impact on wheat growth.

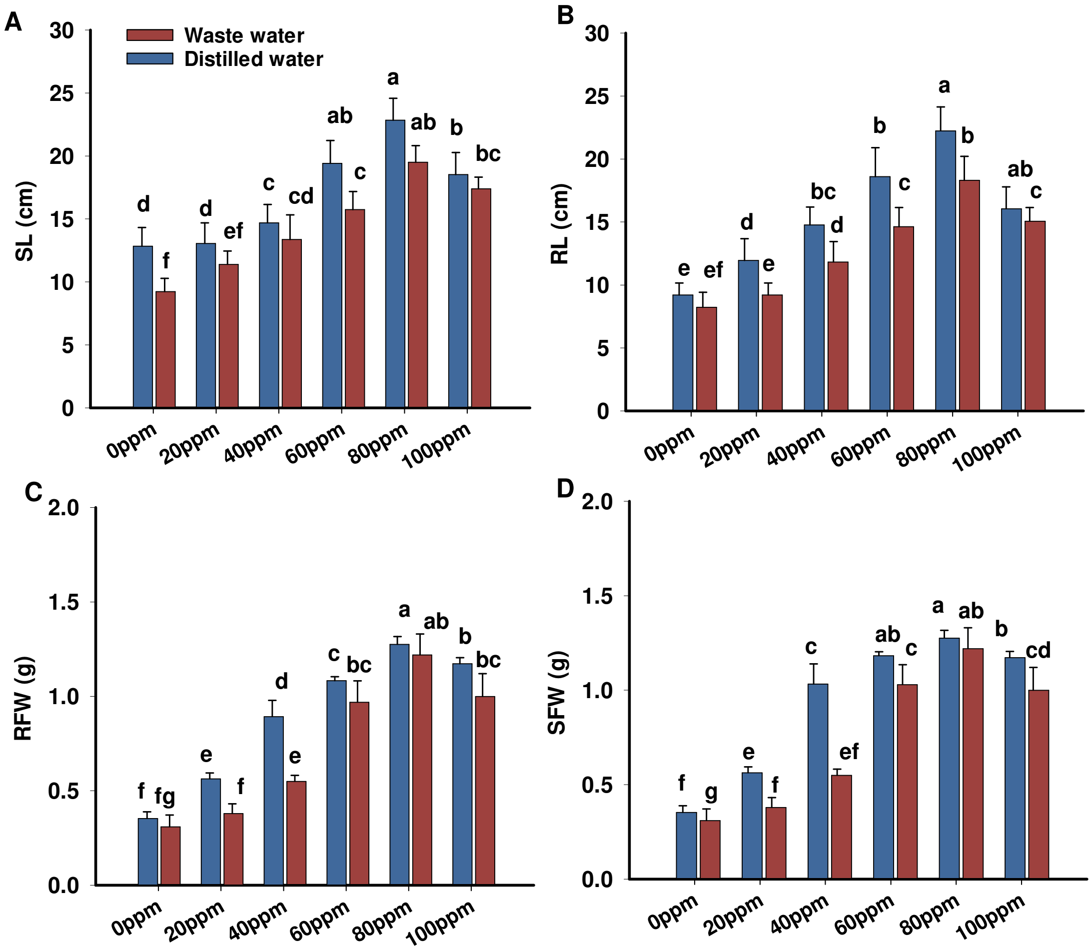

Figure 3. Effect of seed priming with different concentrations of manganese oxide nanoparticles on growth attributes of wheat under water-stress condition (mean ± standard error; n = 4)

Bars labeled with identical letters for both water-stressed and non-stressed scenarios indicate no significant difference. (A) shoot length, (B) root length, (C) Root fresh weight, (D) Shoot fresh weight

Photosynthetic pigments

Figure 4A-D showed that the seed priming of MnO NPs significantly increased chlorophyll a, chlorophyll b, and carotenoid contents. MnO NPs significantly increased the photosynthetic pigments by 53.5% up to 80 mg L^{-1}. After the threshold, at 100 mg L^{-1} photosynthetic pigment contents were decreased.

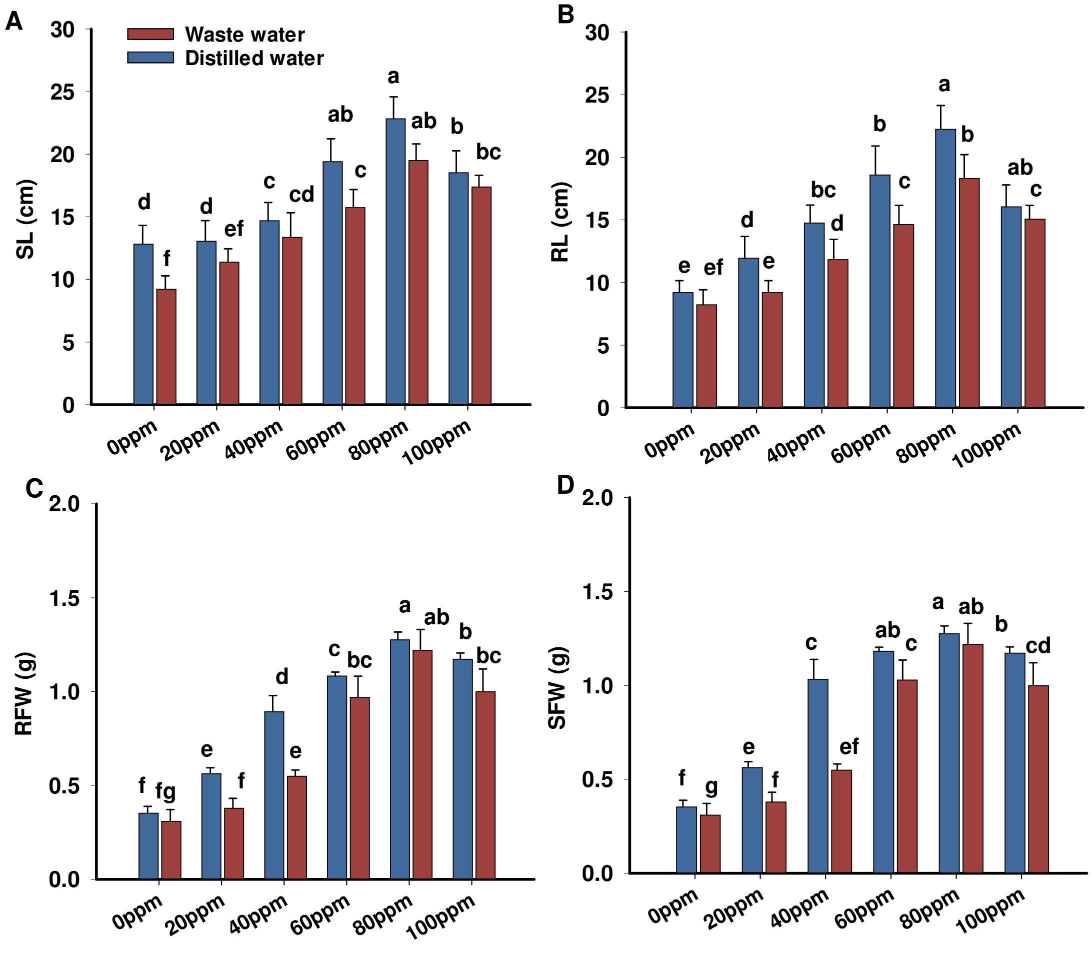

Figure 4. Effect of seed priming with different concentrations of manganese oxide nanoparticles on chlorophyll content of wheat under water-stress condition (mean ± standard error; n = 4)
Bars labeled with identical letters for both water-stressed and non-stressed scenarios indicate no significant difference. (A) Chlorophyll a, (B) chlorophyll b ,(C) total chlorophyll content, (D) carotenoids

Gas exchange parameters

When compared to the control in distilled water, the results in Figure 5(A-D) demonstrated that seed priming with manganese oxide nanoparticles (MnO-NPs) at concentrations up to 80 mg L^{-1} significantly improved gas exchange parameters, such as stomatal conductance, net photosynthesis rate, water use efficiency, and transpiration rate, by roughly 78%, 63%, 58%, and 68%, respectively.

Moreover, in wastewater application, seed priming with MnO-NPs at a concentration of 80 mgL^{-1} resulted in a substantial enhancement of 58%, 61%, 56%, and 58% in gas exchange parameters compared to the control.

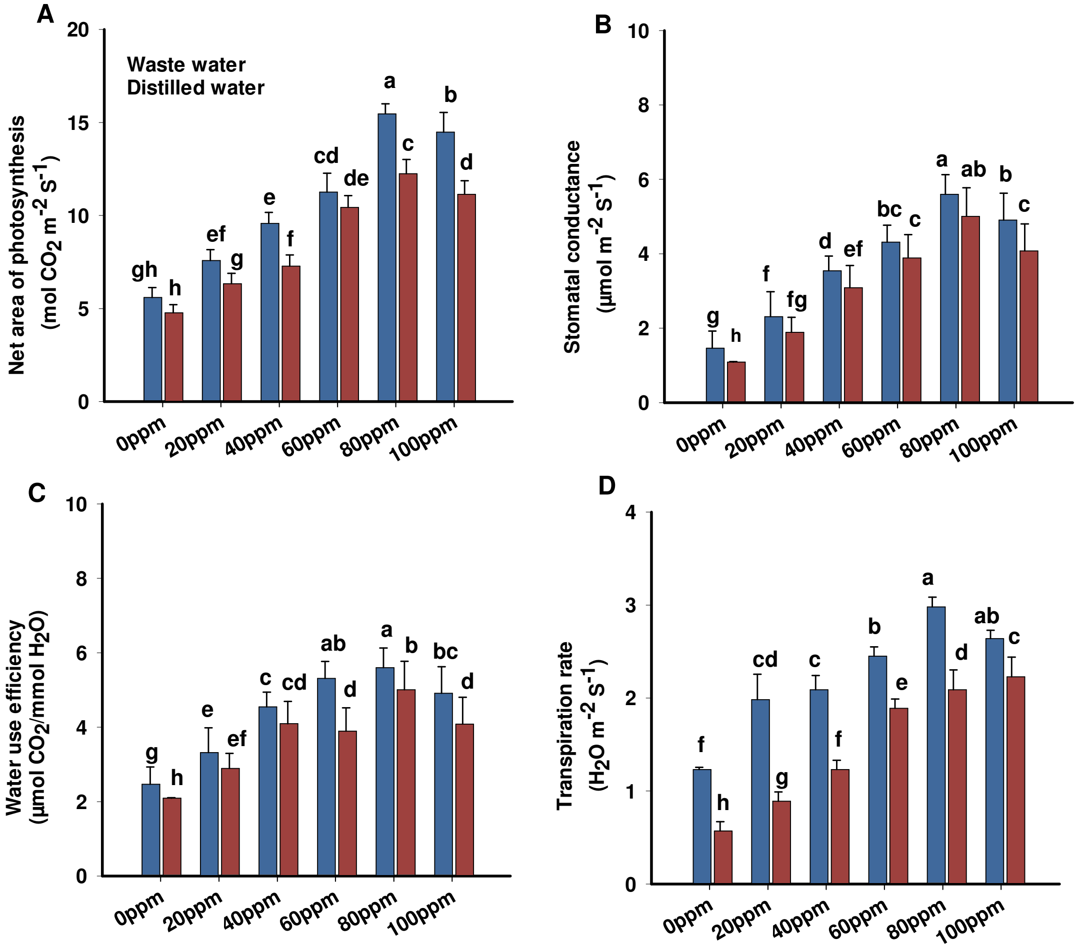

Figure 5. Effect of seed priming with different concentrations of manganese oxide nanoparticles on gas exchange parameters of wheat under water-stress condition (mean ± standard error; n = 4)

Bars labeled with identical letters for both water-stressed and non-stressed scenarios indicate no significant difference. (A) net area of photosynthesis, (B) stomatal conductance, (C) water use efficiency, (D) transpiration rare

Biochemical parameters

Figure 6 and 7 depicted the impact of priming with varying concentrations of MnO-NPs on wheat plant antioxidants (CAT, POD, SOD, APX), hydrogen peroxide, malondialdehyde, and electrolyte leakage in the presence of wastewater-induced stress.

The results indicated a significant reduction in antioxidants (CAT, POD, SOD, APX), due to the influence of wastewater stress as compared to distilled water (Figure 6 (A-D)).

Notably, the highest increase in plant antioxidants was observed in plants that were primed with 80 mg L^{-1} MnO-NPs. Specifically, in distilled water, the application of MnO-NPs concentration (80 mg L^{-1}) led to a substantial rise of 40.9%, 58%, 72%, and 63% in SOD, POD, CAT, and APX respectively as compared to the control.

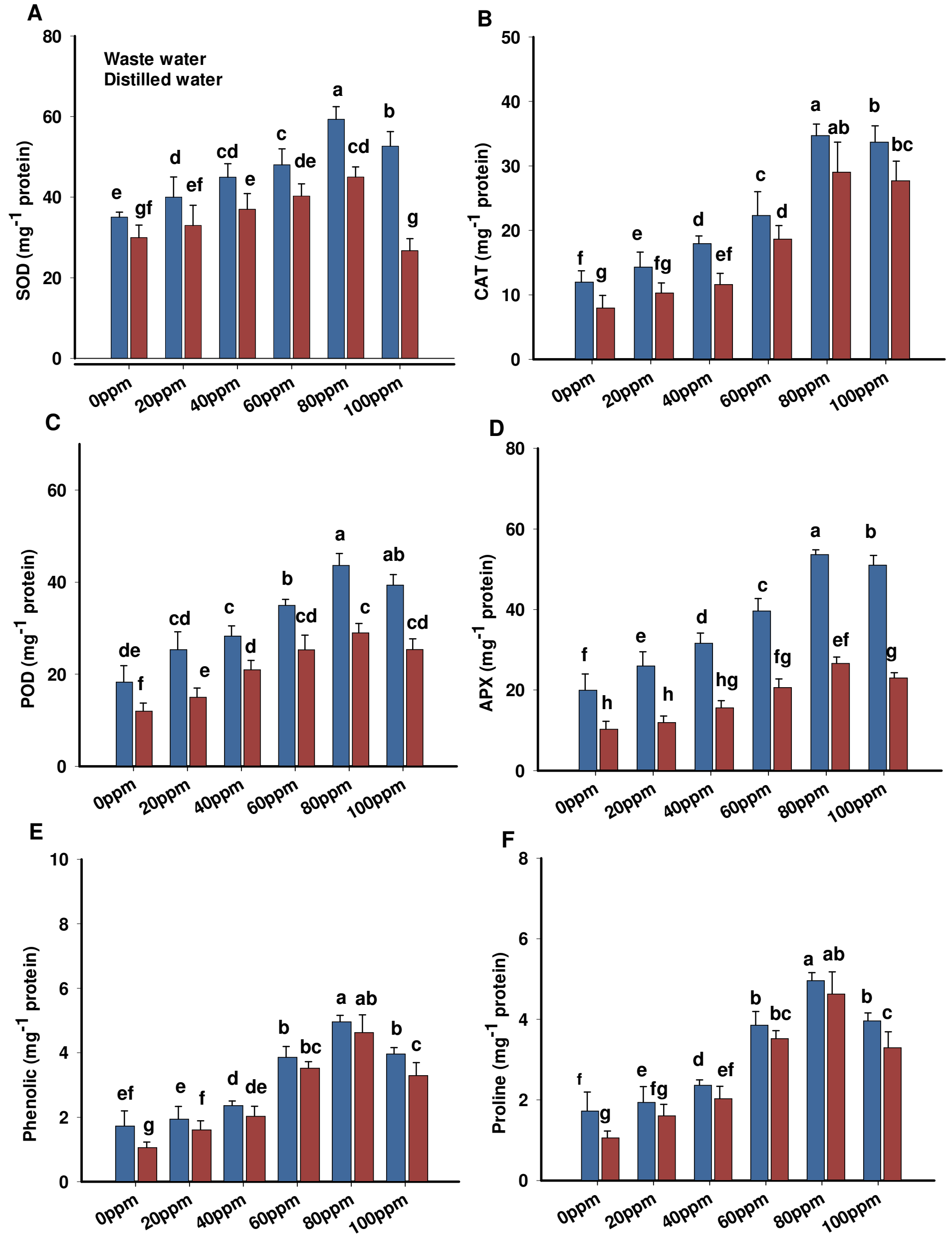

Figure 6. Effect of seed priming with different concentrations of manganese oxide nanoparticles on antioxidants of wheat under water-stress condition (mean ± standard error; n = 4)
Bars labeled with identical letters for both water-stressed and non-stressed scenarios indicate no significant difference. (A) superoxide dismutase, (B) catalase, (C) peroxidase, (D) ascorbate peroxidase, (E) phenolic, (F) proline

Likewise, under wastewater-induced stress condition, the same concentration of MnO-NPs resulted in a noteworthy enhancement of 38%, 55%, 65%, and 61% in SOD, POD, CAT, and APX respectively as well as to the control. The attributes of wheat antioxidants demonstrated improvement in the presence of wastewater stress up to a concentration of 80 mg L^{-1} of MnO-NPs, after which antioxidant attributes slightly declined at 100 mg L^{-1} of MnO-NPs. These findings suggest that the application of MnO-NPs may offer enhancement to the antioxidant's parameters up to a specific level under wastewater-induced stress conditions. In distilled water and wastewater, the seed priming of MnO-NPs concentration (80 mg L^{-1}) resulted in a significant decrease of 63.3% and 57.3% respectively in oxidative stress parameters (Hydrogen Peroxide, Malondialdehyde, and Electrolyte Leakage) as compared to the control (Figure 7A-C). When seeds were subjected to priming with MnO-NPs at a concentration of 80 mgL^{-1} in both distilled water and wastewater, there was a notable boost in proline and phenolic content. Specifically, in distilled water, proline increased by 54.3% and phenolic content by 77.3%. In wastewater, the increase was even more pronounced, with proline showing a 52.3% rise and phenolic content experiencing a substantial increase of 65.3% compared to the control (Figure 6 E,F).

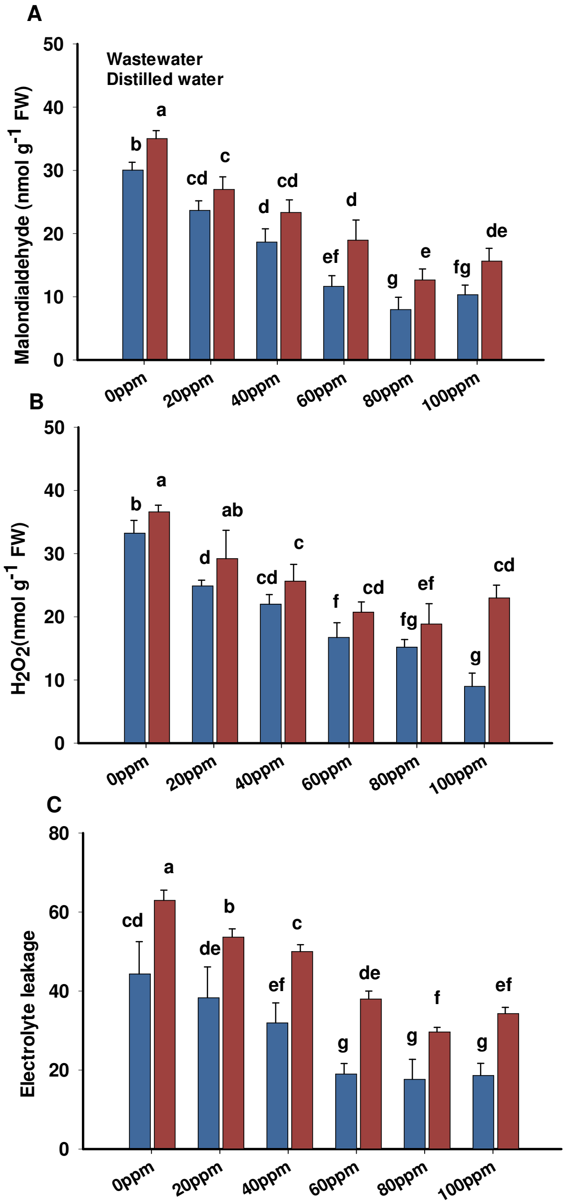

Figure 7. Effect of seed priming with different concentrations of manganese oxide nanoparticles on oxidative parameters of wheat under water-stress conditions (mean ± standard error; n = 4)

Bars with the same letters specified for water-stressed and non-stressed conditions do not differ significantly. (A) malondialdehyde, (B) hydrogen peroxide, (C) electrolyte leakage

Uptake of manganese concentration in roots and shoots

Figure 8 (A-B) depicted the effect of primed seed using various concentrations of MnO-NPs on manganese uptake by wheat plants under wastewater-induced stress condition. In distilled water and wastewater, the seed priming of MnO-NPs concentration (80 mg L^{-1}) resulted in a significant increase of 18% and 21% respectively in the shoot of wheat in contrast to the control. Similarly, in deionized water and wastewater, the seed priming of MnO-NPs concentration (80 mg L^{-1}) resulted in a significant increase of 21% and 22% respectively in the root in contrast to the control.

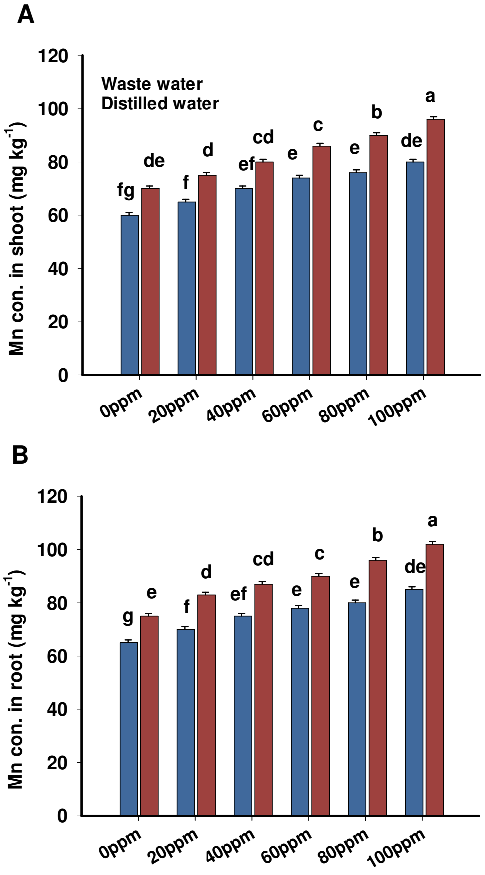

Figure 8. Effect of seed priming with different concentrations of manganese oxide nanoparticles on manganese concentration of wheat under water-stress condition (mean ± standard error; n = 4)

Bars with the same letters specified for water-stressed and non-stressed conditions do not differ significantly. (A) manganese concentration in the shoot, (B) manganese concentration in the root

Correlation analysis

Pearson correlation analysis was shown between the uptake of manganese in the root and shoot of plants to gas exchange parameters, physical parameters, and physicochemical parameters (Figure 9). The analysis showed that the higher concentration of manganese in root and shoot showed a strong negative correlation with gas exchange parameters, physical growth parameters, photosynthetic pigments, and antioxidant attributes. The higher concentration of manganese in wheat plants showed a positive correlation with MDA, H_2O_2, and EL concentration.

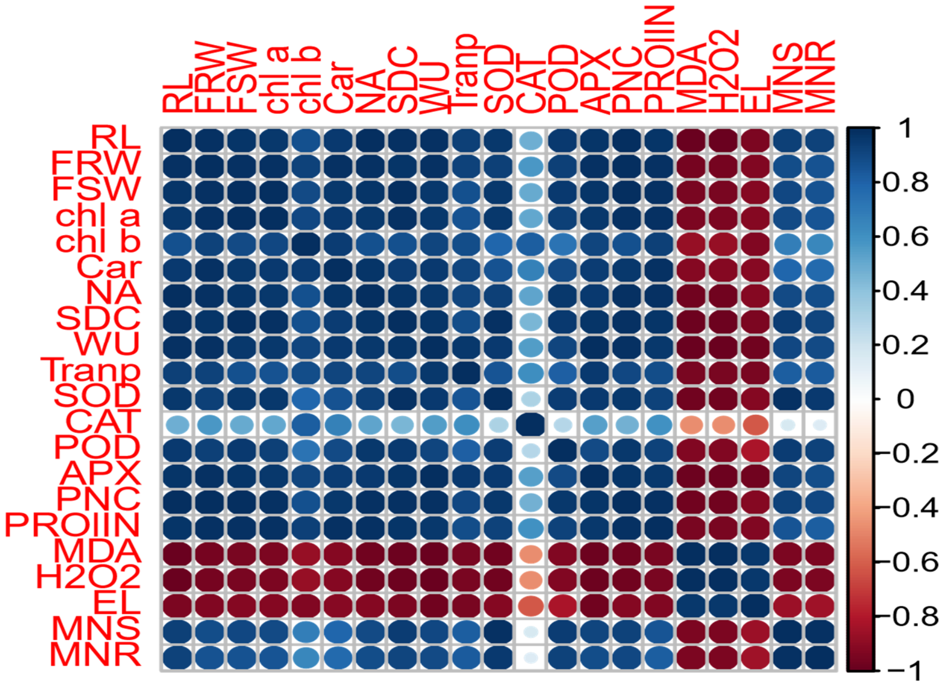

Figure 9. Person correlation analysis between the 21 main parameters on studied wheat plants. The red color shows negative correlations, while the red color shows negative correlations

Abbreviations: MnR, manganese in the root; Mn S, manganese in the shoot; CHL, chlorophyll content; CAR, carotenoids; RL, root length; SL, shoot length; SFW, shoot fresh weight; RFW, root fresh weight, APX, ascorbate peroxidase; CAT, catalase; POD, peroxidase; SOD, superoxide dismutase; MDA, malondialdehyde; H_2O_2, hydrogen peroxide; EL, electrolyte leakage; Transp, Transpiration rate; Res, respiration rate and WU, water use efficiency; NA, net area of photosynthesis; SDC, stomatal conductance; proin, proline; PNC, phenolic

Discussion

MnO-NPs have small-sized particles that are easily absorbed by wheat plant seeds during seed priming (Murgueitio-Herrera *et al.*, 2022). The wheat plant absorbs its manganese directly from the seed primed, the NPs mediated by MnO (Ahsan *et al.*, 2022). This could be kind of a stimulant for the growth and development of wheat and fight the manganese deficiency at the same time (Siddique *et al.*, 2021). The presence of MnO-NPs shows a considerable increase in radicle and plumule, which was profoundly significant. It can be attributed to manganese's function, which cannot be ignored, in the production of hormones that are called gibberellins and auxins (Noreen *et al.*, 2018). Previous researchers found that manganese is a great facilitator in the metabolism of protein and carbohydrates which in turn leads to a successful germination and assimilation of green synthesized nanoparticles (MnO-NP) (Konate *et al.*, 2017). Given the high sensitivity of manganese to seedling emergence and development, putting it directly into the seed priming solution could therefore lead to better root development at the early stages (Broadley *et al.*, 2012; Jiang *et al.*, 2023; Shafqat *et al.*, 2024). Plant morphological attributes were reduced in the control treatment where no application of MnO NPs was applied. Plant photosynthetic processes may be reduced as a result of compromised water transport and absorption mechanisms, which could explain the decline in plant height (Tourky *et al.*, 2023). Primed seed with MnO NPs improved the morphological attributes of wheat due to manganese being a dynamic micronutrient and increased the accessibility of nutrients against stress (Kasote *et al.*, 2021; Haidri *et al.*, 2023). The latest research on MnO-NPs suggested that improved wheat germination and morphological attributes can be ensured from the stressed plants when exposed to wastewater (Asmat-Campos *et al.*, 2023). As well this seed priming also has an effect on plant's metabolic processes, which leads to better seedling development and more vigor in seedlings (Shivay and Prasad, 2014).

Chlorophyll is positively correlated with photosynthetic rate (Song *et al.*, 2022). Wastewater stress notably impacts the chlorophyll levels in leaves, helping as a prominent indicator of toxicity in plants (Verma *et al.*, 2023). The photosynthetic parameters and chlorophyll content of wheat were increased at 80 mgL^{-1} of MnO-NPs with seed priming, this concentration provided an optimal environment for the plants to absorb and utilize the manganese oxide nanoparticles. It leads to enhanced growth and performance of the wheat plant (Paparella *et al.*, 2015). However, at 100 mg L^{-1} of nanoparticles concentration, toxicity may have been induced causing adverse effects on the plants. This higher concentration may have been too much for the metabolism of the wheat plant to process or tolerate (Oliveira *et al.*, 2022). The result of the study aligns with Kasote *et al.* (2021) showed that seed priming of MnO-NPs increased photosynthetic parameters levels in wheat. The observed improvement in these growth parameters might be due to the significant accumulation of water and nutrients in wheat plant (Mazhar *et al.*, 2023).

The current study indicated that, in wastewater conditions, primed seed improved wheat antioxidant levels to 80 mg L^{-1} of MnO-NPs. Under wastewater conditions, ROS of wheat decreased at 80 mg L^{-1} of MnO-NPs with primed seed. Examining antioxidant attributes revealed how wheat plants protect themselves against abiotic stress from outside environmental elements such as drought, sewage, and weather (Yang *et al.*, 2023). Results showed that the plants antioxidant system becomes healthier by applying biogenic MnO-NPs under wastewater. The biogenic nanoparticles possess intrinsic antioxidant properties. These intrinsic antioxidant properties of nanoparticles may directly scavenge ROS, reducing oxidative stress in wheat plants (Liu *et al.*, 2021). The reduction in ROS increased the antioxidant enzymes like SOD, POD, CAT, and APX to function more effectively. The seed priming of MnO-NPs may increase the structural integrity of plant cell membranes. Because less oxidative stress is produced, this increase lessens membrane damage from wastewater stress. The increase in enzymatic antioxidant activity in wheat plants might be because the nanoparticles may modulate plant stress signaling pathways (Mughal et al., 2025). Results are inconsistent with (Hussain *et al.*, 2019), they used seed priming of silicon nanoparticles at 0, 300, 600, 900, and 1200 mg L^{-1} under cd stress. These found that applying silicon nanoparticles on wheat plants in the presence of cadmium stress promotes the activity of antioxidant mechanisms within the plant, leading to enhanced stress tolerance to salinity. Khepar *et al.* (2023) used ferrous sulfide NPs (FeS-NPs) and manganese sulfide NPs (MnS-NPs) as nano-priming agents for pathological, and antioxidative defense parameters of rice. For eight hours, a concentration of 35 µg/mL enhanced the germination indicators while decreasing the phytopathological. Additionally, it was shown that FeS-NPs and MnS-NPs were efficient nano-priming agents for encouraging the germination of rice seeds that were naturally contaminated with fungi (Yan *et al.*, 2022).

The uptake of Mn nanoparticles by biological systems is influenced by several factors, including particle size, surface charge, and the presence of coatings or functional groups (Perfileva and Krutovsky, 2024). MnNPs are primarily taken up by cells through endocytosis. The efficiency of uptake can vary depending on the cell type and the physicochemical properties of NPs. For instance, smaller particles with a positive surface charge are generally taken up more efficiently. Green synthesis of nanoparticles is more economical and scalable compared to traditional methods, as it uses inexpensive, natural materials and minimizes environmental negative impact (Song *et al.*, 2022). This approach supports large-scale applications and aligns with sustainable practices, making it a viable and more friendly solution for agriculture. Seeds are usually stored in rooms under natural environmental conditions for nearly a year before sowing next year in conventional agriculture (Pathirana and Carimi, 2022). During this time, the seeds may naturally deteriorate. However, this aging process can cause a decline in seed germination rates, sometimes falling below the suggested threshold of 80% set by seed companies, which can negatively influence crop output and farmer income (Guo *et al.*, 2015). Nanoparticle-mediated wastewater treatment offers farmers a sustainable way to reuse water and boost plant health, reducing environmental negative impacts and increasing crop yields. Various techniques have been employed to address this issue, including priming (Ferreira *et al.*, 2022; Javaid *et al.*, 2022). However, seed priming with polyethylene glycol can be costly and may not be economically viable for large-scale applications due to problems (Singh *et al.*, 2020). Primed seed with nanoparticles presents an alternative solution that can be adopted for large-scale crops. Also, due to its bactericidal properties, the seed-priming solution contains no environmental hazard, and therefore, it is economical and eco-friendly for agriculture and urban farming systems. It is an affordable and practical way that is beneficial for crops, both increasing their yields as well as protecting them against biotic stress, like wastewater.

Conclusions

This research showed MnO-NPs as a viable means of treatment that could be applied to wheat seed and ultimately help to alleviate stress from wastewater exposure on wheat plants. Especially at the MnO-NPs concentration of 80 mg/L, the following development indicators showed the enhancement of the growth attributes such as biomass, shoot, and root length, as well as gas exchange parameters. Enhanced photosynthetic activity and increased antioxidant levels were observed as significant physiological improvements in the plants. Furthermore, the use of nanoparticles that make nanopriming environmentally friendly emphasizes its promise as a sustainable agriculture approach.

Authors' Contributions

Conceptualization, AI, SH and FM.; methodology, AI, TS, and IH; Software, AI, IK; Validation, SRH and MFS; Formal Analysis, AI, SH; Resources, MAA, SRH.; investigation, MFA, SH and FM writing - original draft preparation, AI, IH, SRH and MS.; writing, review and editing, TS, IK and AI; Data Curation, SH ad FM; Visualization, SRH, IK; Supervision, FM; Project Administration, MFS.; Funding Acquisition, MAA.

All authors have read and agreed to the published version of the manuscript.

Acknowledgements

Ongoing Research Funding Program, (ORF-2025-751), King Saud University, Riyadh, Saudi Arabia.

Data Availability

Other data could be made available upon request to the corresponding author.

Conflict of Interests

The authors declare no conflicts of interest

References

Abou-Zeid HM, Ismail GSM, Abdel-Latif SA (2021). Influence of seed priming with ZnO nanoparticles on the salt-induced damages in wheat (*Triticum aestivum* L.) plants. Journal of Plant Nutrition 44(5):629-643. *https://doi.org/10.1080/01904167.2020.1849288*

Ahmad A, Tola E, Alshahrani TS, Seleiman MF (2023). Enhancement of morphological and physiological performance of zea mays l. under saline stress using ZnO nanoparticles and 24-epibrassinolide seed priming. Agronomy 13(3):771. *https://doi.org/10.3390/agronomy13030771*

Ahmad S, Khan Sehrish A, Hussain A, Zhang L, Alomrani S, Ahmad A, … Sarker PK (2024). Salt stress amelioration and nutrient strengthening in spinach (*Spinacia oleracea* L.) via biochar amendment and zinc fortification: seed priming versus foliar application. Scientific Reports 14(1):15062. *https://doi.org/10.1038/s41598-024-65834-3*

Ahmed SF, Mehejabin F, Momtahin A, Tasannum N, Faria NT, Mofijur M, … Mahlia TMI (2022). Strategies to improve membrane performance in wastewater treatment. Chemosphere 306:135527. *https://doi.org/10.1016/j.chemosphere.2022.135527*

Ahsan H, Shahid M, Imran M, Mahmood F, Siddique MH, Ali HM, … Ayyub M (2022). Photocatalysis and adsorption kinetics of azo dyes by nanoparticles of nickel oxide and copper oxide and their nanocomposite in an aqueous medium. PeerJ 10:e14358. *https://doi.org/10.7717/peerj.14358*

Al-Selwey WA, Alsadon AA, Ibrahim AA, Labis JP, Seleiman MF (2023). Effects of zinc oxide and silicon dioxide nanoparticles on physiological, yield, and water use efficiency traits of potato grown under water deficit. Plants 12(1):218. *https://doi.org/10.3390/plants12010218*

Al-Suhaibani N, Seleiman MF, El-Hendawy S, Abdella K, Alotaibi M, Alderfasi A (2021). Integrative effects of treated wastewater and synthetic fertilizers on productivity, energy characteristics, and elements uptake of potential energy crops in an arid agro-ecosystem. Agronomy 11(11):2250. *https://doi.org/10.3390/agronomy11112250*

Amako K, Chen GX, Asada K (1994). Separate assays specific for ascorbate peroxidase and guaiacol peroxidase and for the chloroplastic and cytosolic isozymes of ascorbate peroxidase in plants. Plant and Cell Physiology 35(3):497-504. *https://doi.org/10.1093/oxfordjournals.pcp.a078621*

Anderson A, Anbarasu A, Pasupuleti RR, Manigandan S, Praveenkumar TR, Kumar JA (2022). Treatment of heavy metals containing wastewater using biodegradable adsorbents: A review of mechanism and future trends. Chemosphere 295:133724. *https://doi.org/10.1016/j.chemosphere.2022.133724*

Arikan B, Ozfidan-Konakci C, Yildiztugay E, Turan M, Cavusoglu H (2022). Polystyrene nanoplastic contamination mixed with polycyclic aromatic hydrocarbons: Alleviation on gas exchange, water management, chlorophyll fluorescence and antioxidant capacity in wheat. Environmental Pollution 311:119851. *https://doi.org/10.1016/j.envpol.2022.119851*

Arshad M, Shakoor A (2017). Irrigation water quality. Water International 12(1-2):145-160.

Asmat-Campos D, López-Medina E, Gil-Rivero E, Villena-Zapata L, Carreño-Ortega A (2023). Effect of concentration of biosynthesized zinc oxide nanoparticles on the growth and development of *Lycopersicon esculentum*. Biocatalysis and Agricultural Biotechnology 52:102832. *https://doi.org/10.1016/j.bcab.2023.102832*

Bhat R, Kiran K, Arun AB, Karim AA (2010). Determination of mineral composition and heavy metal content of some nutraceutically valued plant products. Food Analytical Methods 3:181-187. *https://doi.org/10.1007/s12161-009-9107-y*

Broadley M, Brown P, Cakmak I, Rengel Z, Zhao F (2012). Function of nutrients: micronutrients: In: Marschner's Mineral Nutrition of Higher Plants 191-248. *https://doi.org/10.1016/B978-0-12-384905-2.00007-8*

Cai X, Zhu Q, Zeng Y, Zeng Q, Chen X, Zhan Y (2019). Manganese oxide nanoparticles as MRI contrast agents in tumor multimodal imaging and therapy. International Journal of Nanomedicine 14:8321-8344. *https://doi.org/10.2147/ijn.s218085*

Chen F, Aqeel M, Maqsood MF, Khalid N, Irshad MK, Ibrahim M, … Hashem M (2022). Mitigation of lead toxicity in Vigna radiata genotypes by silver nanoparticles. Environmental Pollution 308:119606. *https://doi.org/10.1016/j.envpol.2022.119606*

Cohen G, Kim M, Ogwu V (1996). A modified catalase assay suitable for a plate reader and for the analysis of brain cell cultures. Journal of Neuroscience Methods 67(1):53-56. *https://doi.org/10.1016/0165-0270(96)00011-8*

Davey MW, Stals E, Panis B, Keulemans J, Swennen RL (2005). High-throughput determination of malondialdehyde in plant tissues. Analytical Biochemistry 347(2):201-207. *https://doi.org/10.1016/j.ab.2005.09.041*

de Barros Ruas R, Costa LMS, Bered F (2022). Urbanization driving changes in plant species and communities-A global view. Global Ecology and Conservation 38:e02243. *https://doi.org/10.1016/j.gecco.2022.e02243*

Eisenberg G (1943). Colorimetric determination of hydrogen peroxide. Industrial & Engineering Chemistry Analytical Edition 15(5):327-328. *https://doi.org/10.1021/i560117a011*

Ferreira RB, Parreira MR, de Arruda FV, Falcão MJA, de Freitas Mansano V, Nabout JC (2022). Combining ecological niche models with experimental seed germination to estimate the effect of climate change on the distribution of endangered plant species in the Brazilian Cerrado. Environmental Monitoring and Assessment 194(4):283.

https://doi.org/10.1007/s10661-022-09897-7

Fu H, Niu J, Wu Z, Xue P, Sun M, Zhu H, Cheng B (2022). Influencing factors of stereotypes on wastewater treatment plants-Case study of 9 wastewater treatment plants in Xi'an, China. Environmental Management 70(3):526-535. *https://doi.org/10.1007/s00267-022-01663-2*

Gao J, Liu Y, Zhao D, Ding Y, Gao L, Su X, Song K, He X (2024). CeO_2NP priming enhances the seed vigor of alfalfa (*Medicago sativa*) under salt stress. Frontiers in Plant Science 14:1264698. *https://doi.org/10.3389/fpls.2023.1264698*

Guo G, Wen Q, Zhu J (2015). The impact of aging agricultural labor population on farmland output: from the perspective of farmer preferences. Mathematical Problems in Engineering 2015:1-7. *https://doi.org/10.1155/2015/730618*

Haidri I, Ishfaq A, Shahid M, Hussain S, Shahzad T, Shafqat U, … Mahmood F (2024). Enhancement of antioxidants' enzymatic activity in the wheat crop by *Shewanela* sp. mediated zinc oxide nanoparticles against heavy metals contaminated wastewater. Journal of Soil Science and Plant Nutrition 24:7068-7089. *https://doi.org/10.1007/s42729-024-02025-z*

Haidri I, Shahid M, Hussain S, Shahzad T, Mahmood F, Hassan MU, … Rezk AAS (2023). Efficacy of biogenic zinc oxide nanoparticles in treating wastewater for sustainable wheat cultivation. Plants 12(17):3058. *https://doi.org/10.3390/plants12173058*

Harsh H, Moghal AAB, Rasheed RM, Almajed A (2023). State-of-the-art review on the role and applicability of select nano-compounds in geotechnical and geoenvironmental applications. Arabian Journal for Science and Engineering 48(4):4149-4173. *https://doi.org/10.1007/s13369-022-07036-5*

Hashem MS, Qi X (2021). Treated wastewater irrigation - A review. Water 13(11):1527. *https://doi.org/10.3390/w13111527*

Hashmi SI, Hewage HTSA, Visvanathan C (2023). Cleaner production auditing for plastic recycling industry in Pakistan: A baseline study. Chemosphere 337:139338. *https://doi.org/10.1016/j.chemosphere.2023.139338*

Heidari H, Kahrizi D (2018). Effect of water stress and contaminated water on seed germination traits and early growth in maize (*Zea mays*). Environmental Engineering and Management Journal 17(1):35-42.

Hussain A, Rizwan M, Ali Q, Ali S (2019). Seed priming with silicon nanoparticles improved the biomass and yield while reduced the oxidative stress and cadmium concentration in wheat grains. Environmental Science and Pollution Research 26:7579-7588. *https://doi.org/10.1007/s11356-019-04210-5*

Imran K, Seleiman MF, Chattha MU, Jalal RS, Mahmood F, Hassan FAS, … Hassan MU (2021). Enhancing antioxidant defense system of mung bean with a salicylic acid exogenous application to mitigate cadmium toxicity. Notulae Botanicae Horti Agrobotanici Cluj-Napoca 49(2):12303. *https://doi.org/10.15835/nbha49212303*

Irfan M, Aslam H, Maqsood A, Tazeen SK, Mahmood F, Shahid M (2023). Changes in plant microbiome in response to abiotic stress. Plant Microbiome for Plant Productivity and Sustainable Agriculture 37:99-119. *https://doi.org/10.1007/978-981-19-5029-2_5*

Ishfaq A, Haidri I, Shafqat U, Khan I, Iqbal M, Mahmood F, Hassan MU (2025). Impact of biogenic zinc oxide nanoparticles on physiological and biochemical attributes of pea (*Pisum sativum* L.) under drought stress. Physiology and Molecular Biology of Plants 31:11-26. *https://doi.org/10.1007/s12298-024-01537-3*

Ishfaq A, Shahid M, Nawaz M, Ibrar D, Hussain S, Shahzad T (2023). Remediation of wastewater by biosynthesized manganese oxide nanoparticles and its effects on development of wheat seedlings. Frontiers in Plant Science 14:1263813. *https://doi.org/10.3389/fpls.2023.1263813*

Janeiro CAN, Arsénio AM, Brito R, Van Lier JB (2020). Use of (partially) treated municipal wastewater in irrigated agriculture; potentials and constraints for sub-Saharan Africa. Physics and Chemistry of the Earth, Parts a/B/C 118:102906. *https://doi.org/10.1016/j.pce.2020.102906*

Javaid MM, Mahmood A, Alshaya DS, AlKahtani MDF, Waheed H, Wasaya A, … Shahid M (2022). Influence of environmental factors on seed germination and seedling characteristics of perennial ryegrass (*Lolium perenne* L.). Scientific Reports 12:9522. *https://doi.org/10.1038/s41598-022-13416-6*

Jiang Y, Zhou P, Ma T, Adeel M, Shakoor N, Li Y, Li M, Guo M, Rui Y (2023). Effects of two Mn-based nanomaterials on soybean antioxidant system and mineral element homeostasis. Environmental Science and Pollution Research 30:18880-18889. *https://doi.org/10.1007/s11356-022-23559-8*

Julkunen-Tiitto R (1985). Phenolic constituents in the leaves of northern willows: methods for the analysis of certain phenolics. Journal of Agricultural and Food Chemistry 33(2):213-217. *https://doi.org/10.1021/jf00062a013*

Kasote DM, Lee JHJ, Jayaprakasha GK, Patil BS (2021). Manganese oxide nanoparticles as safer seed priming agent to improve chlorophyll and antioxidant profiles in watermelon seedlings. Nanomaterials 11(4):1016. *https://doi.org/10.3390/nano11041016*

Khepar V, Ahuja R, Sidhu A, Samota MK (2023). Nano-sulfides of Fe and Mn efficiently augmented the growth, antioxidant defense system, and metal assimilation in rice seedlings. ACS Omega 8(33):30231-30238. *https://doi.org/10.1021/acsomega.3c03012*

Konate A, He X, Zhang Z, Ma Y, Zhang P, Alugongo GM, Rui Y (2017). Magnetic (Fe3O4) nanoparticles reduce heavy metals uptake and mitigate their toxicity in wheat seedling. Sustainability 9(5):790. *https://doi.org/10.3390/su9050790*

Kumar GD, Raja K, Natarajan N, Govindaraju K, Subramanian, K. S. (2020). Invigouration treatment of metal and metal oxide nanoparticles for improving the seed quality of aged chilli seeds (*Capsicum annum* L.). Materials Chemistry and Physics 242:122492. *https://doi.org/10.1016/j.matchemphys.2019.122492*

Kumar Reddy D H, Lee SM (2012). Water pollution and treatment technologies. Journal of Environmental & Analytical Toxicology 2(5):2-4. *https://doi.org/10.4172/2161-0525.1000e103*

Kumar S, Rao R (2017). Protocols for soil sampling, soil and water analysis. Technological Intervention Productivity 37:1-15.

Lahuta LB, Szablińska-Piernik J, Głowacka K, Stałanowska K, Railean-Plugaru V, ... Buszewski B (2022). The effect of bio-synthesized silver nanoparticles on germination, early seedling development, and metabolome of wheat (*Triticum aestivum* L.). Molecules 27(7):2303. *https://doi.org/10.3390/molecules27072303*

Li M, Chen N, Shang H, Ling C, Wei K, Zhao S, Zhou B, Jia F, Ai Z, Zhang L (2022). An electrochemical strategy for simultaneous heavy metal complexes wastewater treatment and resource recovery. Environmental Science & Technology 56(15):10945-10953. *https://doi.org/10.1021/acs.est.2c02363*

Lichtenthaler HK, Buschmann, C. (2001). Chlorophylls and carotenoids: Measurement and characterization by UV-VIS spectroscopy. Current Protocols in Food Analytical Chemistry 1(1):F4-3. *https://doi.org/10.1002/0471142913.faf0403s01*

Liu Y, Cui W, Li W, Xu S, SunY, Xu G, Wang F (2023). Effects of microplastics on cadmium accumulation by rice and arbuscular mycorrhizal fungal communities in cadmium-contaminated soil. Journal of Hazardous Materials 442:130102. *https://doi.org/10.1016/j.jhazmat.2022.130102*

Liu Y, Xiao Z, Chen F, Yue L, Zou H, Wang Z (2021). Metallic oxide nanomaterials act as antioxidant nanozymes in higher plants: Trends, meta-analysis, and prospect. Science of the Total Environment 780:146578. *https://doi.org/10.1016/j.scitotenv.2021.14657 8*

Macedo F, da CO, Daneluzzi GS, Capelin D, de Oliveira RF (2021). Equipment and protocol for measurement of extracellular electrical signals, gas exchange and turgor pressure in plants. MethodsX 8:101214. *https://doi.org/10.1016/j.mex.2021.101214*

Maehly AC (2006). The assay of catalases and peroxidases. In: Methods of Biochemical Analysis 357-424. *https://doi.org/10.1002/9780470110171.ch14*

Mazhar MW, Ishtiaq M, Maqbool M, Ullah F, Sayed SRM, Mahmoud EA (2023). Seed priming with iron oxide nanoparticles improves yield and antioxidant status of garden pea (*Pisum sativum* L.) grown under drought stress. South African Journal of Botany 162:577-587. *https://doi.org/10.1016/j.sajb.2023.09.047*

Mpanga IK, Sserunkuma H, Tronstad R, Pierce M, Brown JK (2022). Soil health assessment of three semi-arid soil textures in an Arizona vineyard irrigated with reclaimed municipal water. Water 14(18):2922. *https://doi.org/10.3390/w14182922*

Mughal UA, Farid M, Sarfraz W, Abdel-Maksoud MA, Malik A, Abbas M, ... Khalid N (2025). Enhancing phytoremediation in chamomile (*Matricaria chamomilla* L.) using moringa extract and zero-valent iron nanoparticles. South African Journal of Botany 77:14-25. *https://doi.org/10.1016/j.sajb.2024.11.02 0*

Murgueitio-Herrera E, Falconí CE, Cumbal L, Gómez J, Yanchatipán K, Tapia A, ... Toulkeridis T (2022). Synthesis of iron, zinc, and manganese nanofertilizers, using Andean blueberry extract, and their effect in the growth of cabbage and lupin plants. Nanomaterials 12(11):1921. *https://doi.org/10.3390/nano12111921*

Mustafa S, Mahmood F, Shafqat U, Hussain S, Shahid M, Batool F, ... Shahzad T (2023). The biosynthesis of nickel oxide nanoparticles: An eco-friendly approach for azo dye decolorization and industrial wastewater treatment. Sustainability 15(20):14965. *https://doi.org/10.3390/su15201496 5*

Nordberg M, Nordberg GF (2022). Metallothionein and cadmium toxicology-Historical review and commentary. Biomolecules 12(3):360. *https://doi.org/10.3390/biom12030360*

Noreen S, Akhter MS, Yaamin T, Arfan M (2018). The ameliorative effects of exogenously applied proline on physiological and biochemical parameters of wheat (*Triticum aestivum* L.) crop under copper stress condition. Journal of Plant Interactions 13(1):221-230. *https://doi.org/10.1080/17429145.2018.1437480*

Oliveira KS, de Mello Prado R, Checchio MV, Gratão PL (2022). Interaction of silicon and manganese in nutritional and physiological aspects of energy cane with high fiber content. BMC Plant Biology 22(1):374. *https://doi.org/10.1186/s12870-022-03766-8*

Omar AA, Heikal YM, Zayed EM, Shamseldin SAM, Salama YE, Amer KE, … Mohamed AH (2023). Conferring of drought and heat stress tolerance in wheat (*Triticum aestivum* L.) genotypes and their response to selenium nanoparticles application. Nanomaterials 13(6):998. *https://doi.org/10.3390/nano13060998*

Paparella S, Araúj SS, Rossi G, Wijayasinghem M, Carbonera D, Balestrazzi A (2015). Seed priming: state of the art and new perspectives. Plant Cell Reports 34:1281-1293. *https://doi.org/10.1007/s00299-015-1784-y*

Pathirana R, Carimi F (2022). Management and utilization of plant genetic resources for a sustainable agriculture. Plants 11(15):2038. *https://doi.org/10.3390/plants11152038*

Perfileva AI, Krutovsky KV (2024). Manganese nanoparticles: Synthesis, mechanisms of influence on plant resistance to stress, and prospects for application in agricultural chemistry. Journal of Agricultural and Food Chemistry 72(14):7564-7585. *https://doi.org/10.1021/acs.jafc.3c07350*

Rhaman MS, Tania SS, Imran S, Rauf F, Kibria MG, Ye W, … Murata Y (2022). Seed priming with nanoparticles: An emerging technique for improving plant growth, development, and abiotic stress tolerance. Journal of Soil Science and Plant Nutrition 22(4):4047-4062. *https://doi.org/10.1007/s42729-022-01007-3*

Rubab S, Mahmood F, Shahzad T, Shahid M, Hussain S (2023). Reduction of hexavalent chromium in wastewater and three varying agricultural soils using a bacterial strain *Bacillus* sp. SR18 isolated from a contaminated field. Pakistan Journal of Agricultural Sciences 60(2):293-302. *https://doi.org/10.21162/pakjas/23.22*

Seleiman MF, Ahmad A, Battaglia ML, Bilal HM, Alhammad BA, Khan N (2023). Zinc oxide nanoparticles: A unique saline stress mitigator with the potential to increase future crop production. South African Journal of Botany 159:208-218. *https://doi.org/10.1016/j.sajb.2023.06.009*

Seleiman MF, Al-Suhaibani N, El-Hendawy S, Abdella K, Alotaibi M, Alderfasi A (2021). Impacts of long- and short-term of irrigation with treated wastewater and synthetic fertilizers on the growth, biomass, heavy metal content, and energy traits of three potential bioenergy crops in arid regions. Energies 14(11):3037. *https://doi.org/10.3390/en14113037*

Shafqat U, Hussain S, Shahzad T, Shahid M, Mahmood F (2023). Elucidating the phytotoxicity thresholds of various biosynthesized nanoparticles on physical and biochemical attributes of cotton. Chemical and Biological Technologies in Agriculture 10(1):1-15. *https://doi.org/10.1186/s40538-023-00402-x*

Shafqat U, Yasin MU, Shahid M, Hussain S, Shahzad T, Mahmood F, Ishfaq A, Nawaz M, Shah AN, Ali HM (2024). Evaluating the impact of biogenic nanoparticles and pesticide application in controlling Cotton Leaf Curl Virus Disease (CLCuD) in Cotton (*Gossypium hirsutum* L.). Chemical and Biological Technologies in Agriculture 11:149. *https://doi.org/10.1186/s40538-024-00656-z*

Shebl A, Hassan AA, Salama DM, Abd El-Aziz ME, Abd Elwahed MSA (2019). Green synthesis of nanofertilizers and their application as a foliar for *Cucurbita pepo* L. Journal of Nanomaterials 2019(1):3476347. *https://doi.org/10.1155/2019/3476347*

Shivay YS, Prasad R (2014). Effect of source and methods of zinc application on corn productivity, nitrogen and zinc concentrations and uptake by high quality protein corn (*Zea mays*). Egyptian Journal of Biology 16:72-78. *https://doi.org/10.4314/ejb.v16i1.10*

Siddique K, Shahid M, Shahzad T, Mahmood F, Nadeem H, Rehman M, … Kamal T (2021). Comparative efficacy of biogenic zinc oxide nanoparticles synthesized by *Pseudochrobactrum* sp. C5 and chemically synthesized zinc oxide nanoparticles for catalytic degradation of dyes and wastewater treatment. Environmental Science and Pollution Research 28:28307-28318. *https://doi.org/10.1007/s11356-021-12575-9*

Singh VK, Singh R, Tripathi S, Devi RS, Srivastava P, Singh P, Kumar A, Bhadouria R (2020). Seed priming: State of the art and new perspectives in the era of climate change. Climate Change and Soil Interactions 143-170. *https://doi.org/10.1016/B978-0-12-818032-7.00006-0*

Song K, Zhao D, Sun H, Gao J, Li S, Hu T, He X (2022). Green nanopriming: responses of alfalfa (*Medicago sativa* L.) seedlings to alfalfa extracts capped and light-induced silver nanoparticles. BMC Plant Biology 22(1):323. *https://doi.org/10.1186/s12870-022-03692-9*

Song X, Mo F, Yan M, Zhang X, Zhang B, Huang X, … Li YR (2022). Effect of smut infection on the photosynthetic physiological characteristics and related defense enzymes of sugarcane. Life 12(8):1201.

https://doi.org/10.3390/life12081201

Sytar O, Kumari P, Yadav S, Brestic M, Rastogi A (2019). Phytohormone priming: regulator for heavy metal stress in plants. Journal of Plant Growth Regulation 38:739-752. *https://doi.org/10.1007/s00344-018-9886-8*

Thalhammer A, Hincha DK, Zuther E (2014). Measuring freezing tolerance: electrolyte leakage and chlorophyll fluorescence assays. Plant Cold Acclimation: Methods and Protocols 15-24. *https://doi.org/10.1007/978-1-4939-0844-8_3*

Troll W, Lindsley J (1955). A photometric method for the determination of proline. Journal of Biological Chemistry 215(2):655-660.

Verma A, Gupta A, Gaharwar US, Rajamani P (2023). Effect of wastewater on physiological, morphological and biochemical levels and its cytotoxic potential on *Pisum sativum*. International Journal of Environmental Science and Technology 1-18. *https://doi.org/10.1007/s13762-023-04941-6*

Wheat D, Glutens BW (1996). Improvement of durum wheat pastamaking and breadmaking qualities. Cereal Chemistry 73(2):155-166.

Yan X, Chen S, Pan Z, Zhao W, Rui Y, Zhao L (2022). AgNPs-triggered seed metabolic and transcriptional reprogramming enhanced rice salt tolerance and blast resistance. ACS Nano 17(1):492–504. *https://doi.org/10.1021/acsnano.2c09181*

Yang H, Fang R, Luo L, Yang W, Huang Q, Yang C, Hui W, … Wang J (2023). Potential roles of melatonin in mitigating the heavy metals toxicity in horticultural plants. Scientia Horticulturae 321:112269. *https://doi.org/10.1016/j.scienta.2023.112269*

YasmeenT, Ali Q, Islam F, Noman A, Akram MS, Javed, M. T. (2014). Biologically treated wastewater fertigation induced growth and yield enhancement effects in *Vigna radiata* L. Agricultural Water Management 146:124-130. *https://doi.org/10.1016/j.agwat.2014.07.025*

Ye Y, Medina-Velo IA, Cota-Ruiz K, Moreno-Olivas F, Gardea-Torresdey JL (2019). Can abiotic stresses in plants be alleviated by manganese nanoparticles or compounds? In Ecotoxicology and Environmental Safety 184:109671. *https://doi.org/10.1016/j.ecoenv.2019.109671*

Zhang H, Xu F, Xue J, Chen S, Wang J, Yang Y (2020). Enhanced removal of heavy metal ions from aqueous solution using manganese dioxide-loaded biochar: Behavior and mechanism. Scientific Reports 10:6067. *https://doi.org/10.1038/s41598-020-63000-z*

Zhang X, Han L, Wei H, Tan X, Zhou W, … Qian Y (2022). Linking urbanization and air quality together: A review and a perspective on the future sustainable urban development. Journal of Cleaner Production 346:130988. *https://doi.org/10.1016/j.jclepro.2022.130988*

Zhang Y, Shen Y (2019). Wastewater irrigation: past, present, and future. Wiley Interdisciplinary Reviews: Water *6*(3):e1234. *https://doi.org/10.1002/wat2.1234*