American Journal of Potato Research (2024) 101:322–335
https://doi.org/10.1007/s12230-024-09961-7

# Impact of Metal Oxide Nanoparticles on Potato (*Solanum tuberosum* L.) Tuber Yield in Hydroponics

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Accepted: 15 July 2024 / Published online: 1 August 2024


**Abstract**
The application of phyto-nanotechnology in horticulture is a sustainable tool for agriculture due to its cost-effectiveness and eco-friendly properties. Essential micro-elements have been used as nano-fertilizer to enhance crop production. Fine-tuning of the nanoparticle dose is also recognized as the critical factor determining their impact on plant growth. Therefore, this study aimed to investigate the effects of iron (Fe) and manganese (Mn) oxide nanoparticles (NPs) on potato plants physiological and biochemical changes under the hydroponic conditions. Observations revealed that the plant growing in the adapted hydroponics media (supplemented with 4.0 mg $L^{-1}$ $Fe_3O_4$ NPs and 1.0 mg $L^{-1}$ $MnO_2$ NPs instead of original Fe and Mn salt respectively) improved various physiological and biochemical parameters, total biomass, and tuber yield compared to the untreated control. The growth-promoting impact of metal oxide NPs (hereafter refers as MONPs) simultaneously induced the activity of various antioxidant enzymes (SOD, CAT, POD) and contributed to the adequate reduction in malondialdehyde (MDA) and hydrogen peroxide ($H_2O_2$) content relative to the untreated control plants. This indicated that the application of MONPs could improve the potato yield per plant via modulating the plant antioxidant machinery. In addition, the application of MONPs as nano-nutrient appreciably improved the photosynthetic efficiency of plants via modulating the photosynthetic pigment content like Chl a, Chl b, total Chl, ratio Chl a/b, carotenoids as well as soluble sugar. The SEM-EDX elemental mapping also showed a slightly higher content of metals ions (Fe, Mn, and Ca) in the root and shoot tissues, however, the TEM analysis also confirmed absorption as well as transportation of MONPs in the root tissues growing in the presence of MONPs. This study opened the opportunity of utilizing MONPs as nano-nutrient in a hydroponic condition for development of pathogen-free potato tuber.

**Keywords** Hydroponics · Nano-nutrient · Metal oxide nanoparticle · Antioxidant enzymes · Photosynthetic pigments · Pathogen-free tuber

## Introduction

Nanoscience has made a significant impact on agriculture by enhancing crop production, improving disease detection and management, monitoring soil health, boosting seed germination and growth, and optimizing post-harvest management. It is essential to explore potential of NPs in agriculture due to their potential to revolutionize crop production and sustainability. The NPs can enhance nutrient delivery, minimize fertilizer loss, and boost plant resilience against stressors, leading to increased crop yield and efficiency. Their unique properties, such as small size and large surface area, allow better absorption and targeted delivery of nutrients, reducing environmental impact. Understanding nanoparticles' interactions with plants can also help to develop innovative agricultural practices, lower fertilizer usage, and decrease costs. Earlier studies have reported that the application of nanoparticles (NPs) as nano-nutrients on the plant lead to the accelerated germination rates, improved tolerance to abiotic and biotic stresses, and enhanced nutrient metabolism efficiency (Mushinskiy et al. 2018). The Metal Oxide nanoparticles (MONPs) have also been applied as a nano-nutrient or nano-fertilizer to counter limited microelement uptake and management of micronutrient deficiency in crops, all with a lower environmental impact compared

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to traditional components. These nano-fertilizers exhibited promoting impact on the overall better growth and yield of crops including potatoes (Janmohammadi et al. 2016; Bradfield et al. 2017).

The potato (*Solanum tuberosum* L.) is globally recognized as a vital vegetable crop. It ranks as the third most important staple food crop worldwide, following rice and wheat (https://www.fao.org/faostat/2023). Potato is a significant source of starch, protein, vitamins, and essential minerals such as potassium and calcium. The demand for potatoes in agriculture has significantly increased in recent years compared to other agronomic crops. However, the potato farming face substantial challenges primarily in the form of abiotic stresses, including drought, salinity, high temperature and deficiencies in vital micronutrients. The primary concerns in potato farming revolve around disparities in nutrient supply, inadequate water management, and diminishing soil fertility (Handayani et al. 2019). Notably, potato tubers represent the ultimate agricultural product that grown inside the soil. Therefore, the soil nutrients directly impact tuber yield. Fine tuning of the macronutrients and micronutrients are essential for a wide range of physiological and biochemical homeostasis within plant cell (Bindraban et al. 2015). Researchers and breeders have already focused on increasing potato tuber yield to meet the increasing demand for food as well as maintenance of the nutritional quality through innovative and sustainable technology. Additionally, integrating NPs into hydroponic systems can improve nutrient solutions and overall plant health, promoting sustainable and productive farming. Present-day hydroponic method aims to cost-effective cultivation of robust and disease-free potato (Woznicki et al. 2021). The synergy between hydroponic mediums and MONPs have been established as an effective nutrient delivery system, contributing to enhanced plant growth and development (Maluin et al. 2021).

Essential microelements have been used as nano-fertilizers in agriculture to boost crop yields and promote environmental sustainability (Bindraban et al. 2015). Notably, iron (Fe) and manganese (Mn) are vital micronutrients for plant growth serving as an essential-cofactors in photosynthesis and various metabolic enzymatic reactions. Additionally, these metal ions also play a crucial role in the structure and function of antioxidant enzymes like superoxide dismutase (SOD) catalase (CAT) etc., protecting against various kind of reactive oxygen species (ROS) and free radicals (Diedrick 2010; Millaleo et al. 2010; Mousavi et al. 2011). Numerous studies have been done on the application of Mn oxide (MnO) and Fe oxide (FeO) NPs as potential nano-fertilizer on crops such as rice, mung bean, eggplant, cherry radish, wheat, spinach, pumpkin, and soybeans and peanut growth and development (Pradhan et al. 2013; Elmer et al. 2016; Rui et al. 2016; Dimkpa et al. 2018). Zhou et al. (2023) reported that nano zero-valent iron (nZVI) emerged as highly effective in reducing Cd accumulation in rice by adsorbing Cd ions and enhancing iron plaque formation on roots leading to mitigates Cd uptake and enhances plant growth by modulating gene expression related to Cd transport, phytohormones, and phyto-chelatin. Shakoor et al. (2022) reported the application of iron-based nanoparticles ($Fe_3O_4$) on cherry radish (*Raphanus sativus* L.) increase in iron content by 58%, zinc by 37%, vitamin C by 48%, crude protein by 67%, and essential amino acids like phenylalanine, leucine, and isoleucine by 11–14%. Application of FeO NPs to Hoagland media has also been shown to improve the growth of spinach, pumpkin, and soybeans, while ZnO NPs have also exhibited its positive impact on tobacco growth and development via increased photosynthetic activity and antioxidant enzyme function (Zhu et al. 2008; Ghafariyan et al. 2013; Jeyasubramanian et al. 2016; Tirani et al. 2019). Al-juthery et al. (2019) reported that the application of nano-chelated zinc oxide (ZnO) and calcium oxide (CaO) to the soil not only decreased the time needed for tuber induction but also boosted tuber yield. However, the precise mechanism underlying the relationship between tuber yield and biochemical changes resulting from the application of nano-nutrients in Hoagland media has not yet been fully elucidated.

This study aimed to analyse the impact of biogenic Fe and Mn oxide NPs as nano-nutrient in hydroponic media on potato physiological and biochemical characteristics as well as tuber yield. The study also aimed to elucidate the impact of NPs on increased antioxidant enzyme activities and photosynthetic efficiency in plants. These effects have the potential to enhance plant growth and increase tuber yield by reducing the levels of ROS within plant cells. Understanding these mechanisms can provide valuable insights for developing advanced agricultural practices that leverage nanotechnology for better crop management and productivity.

## Materials and Methods

### Synthesis and Suspension of Metal Oxide Nanoparticles

The MONPs were synthesized using green synthesis method with help of beetroot (*Beta vulgaris* L.) leaf aqueous extract as reported from our laboratory earlier (Joshi et al. 2022). Iron Chloride salts ($FeCl_3.6H_2O$ and $FeCl_2.4H_2O$) and $KMnO_4$ were used as precursors for the synthesis of $Fe_3O_4$ and $MnO_2$ NPs. The synthesized MONPs were characterized using various techniques as reported earlier from

our laboratory (Joshi et al. 2022). However, earlier study focused mainly on the synthesis and characterization of these MONPs and their impact on in vitro tuberization in potato. The current study builds upon our past study by highlighting the impact of MONPs on potato tuberization in a hydroponic environment. The $Fe_3O_4$ and $MnO_2$ NPs with average particle sizes of approximately 10.6 ± 2.24 nm and 6.3 ± 1.17 nm, respectively, were used for this study.

The stock solutions of MONPs were prepared by dispersing suitable quantity of NPs in sterilized double distilled water, followed by ultrasonic sonication for 30 min at 25 ºC using a bath Sonicator (Ultrasonic Cleaner, LABMAN™, Scientific Instruments, India). It is worth noting that all MONPs stock solutions were freshly prepared just before application in the hydroponic condition.

## Plant Material, Variety and Treatment Conditions

Potato tubers *ver.* 'Kufri Jyoti 'obtained from the Central Potato Research Institute (CPRI) Shimla India were used in the experiments. The plants were proliferated in culture tubes on Murashige and Skoog (MS medium) medium containing 3.0% (w/v) sucrose and 0.8% (w/v) plant agar (Hi-Media). The cultures were maintained at 16-hour light & 8-hour dark photoperiod cycle at 22 ± 2°C. The in-vitro grown plantlets (5–7 cm in height) were further used for the hydroponic experiments.

Modified Hoagland medium (Hoagland and Arnon 1950) was used for the potato growth and tuber development (Supplementary Table 1). In order to determine the optimal concentrations of MONPs ($Fe_3O_4$ and $MnO_2$ NPs), we conducted a screening process by substituting Fe ($FeSO_4.7H_2O$-EDTA) and Mn ($MnCl_2.4H_2O$) salts in the Hoagland medium with varying MONP concentrations (0 to 6 $mg^{-1}$). The in-vitro grown plantlets were incubated in Pyrex glass culture tubes (25 mm × 150 mm) filled with the modified Hoagland medium, maintaining a 16-hour light & 8-hour dark photoperiod cycle at 22 ± 2 °C and 70% relative humidity. The liquid nutrient media was replenished in every 4 days while maintaining the pH at 5.8 and an electrical conductivity (EC) of 1000 µS $cm^{-1}$ (Portable A1 TDS & EC meter). Various plant growth parameters including physiological changes such as leaf count, stem length, root length, fresh and dry weights, tuber induction period, tuber numbers, tuber size, and the overall yield, were observed until reaching full maturity of plant (65 ± 4 days).

## Evaluation of Biochemical Profile of Plant

Biochemical analysis was conducted using plant tissues (leaves and tubers) growing in a hydroponic environment in the presence of MONPs. The antioxidant enzyme such as superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD) along with ROS indicators such as malondialdehyde (MDA) and hydrogen peroxide ($H_2O_2$) were evaluated (Gupta et al. 1993; Aebi 1984; Chance and Maehly 1955). Additionally, the photosynthetic pigments, including chlorophylls and carotenoids, along with the total soluble sugar content were also estimated (Arnon and Whatley 1949; Dubois et al. 1956).

### Antioxidant Enzyme Assay

The leaves and tubers were collected from the MONPs-treated and untreated control plants growing under hydroponic medium. The tissues were homogenised in extraction buffer (100 mM sodium phosphate buffer (pH 7.0) supplemented with 0.1% (w/v) polyvinyl pyrrolidone) and homogenates were centrifuged at 15,000 g for 20 min at 4 °C. The clear supernatants were quantified for proteins using a protein-dye binding assay (Bradford 1976).

This supernatant was used to quantify changes in various antioxidant enzymes (SOD, CAT, and POD). The SOD activity was determined by measuring the inhibition of the photochemical reduction of nitro blue tetrazolium (NBT) at a wavelength of 560 nm using a spectrophotometer (UV-1800, Shimadzu, Japan) (Gupta et al. 1993). The CAT activity was quantified using the method described by Aebi in 1984, with slight modifications. The reduction in absorbance of the reaction mixture was measured at 240 nm using spectrophotometer. The POD activity was determined using the method as described by Chance and Maehly (1955). Briefly, POD catalyse the conversion of $H_2O_2$ and pyrogallol into purpurogallin. The change in absorbance was monitored at 430 nm at 20-second intervals for a duration of up to 15 min. One unit of peroxidase enzyme activity was equivalent to the conversion of 1.0 mg of purpurogallin from $H_2O_2$ and pyrogallol in 20 s.

### Estimation of MDA and $H_2O_2$ Content

The MDA content was determined using thio-barbituric acid (TBA) method (Heath et al. 1968). The absorbance of the supernatant was recorded at 532 and 600 nm via a spectrophotometer (UV-1800, Shimadzu, Japan). Concentration of the MDA was calculated using the formula given below:

Concentration of MDA (mM) = (Absat532 nm - Absat600 nm)/155 $mM^{-1}$ $cm^{-1}$.

Absorbance coefficient of extinction = 155 $mM^{-1}$ $cm^{-1}$.

Hydrogen peroxide ($H_2O_2$) was estimated following the standard protocol as reported (Velikova et al. 2000). The $H_2O_2$ content was quantified via standard curve and expressed as µmol $g^{-1}$ FW. The in-situ detection of $H_2O_2$

was also performed with the leaf staining using 3, 3'- diaminobenzidine (DAB) (Daudi and O'Brien 2012).

#### Estimation of Photosynthetic Pigments and Total Soluble Sugar

The photosynthetic pigments in the MONPs treated and untreated control plants leaf samples were quantified according to method as reported (Arnon and Whatley 1949). The phenol-sulphuric acid method was used to estimate total soluble sugar contents (Dubois et al. 1956).

### Evaluation of Metal Oxide Nanoparticles Uptake by Plant Tissues

#### Scanning Electron Microscopy (SEM) and Energy Dispersive X-ray (EDX) Spectrum Analysis

Comparative analysis of Fe, Mn, and Ca metal ions in the MONPs treated and untreated root, and shoot samples were quantified by SEM-EDX Spectrum analysis software (Nova Nanosem 450) (Joshi et al. 2022; Shankramma et al. 2016).

#### Transmission Electron Microscopy (TEM) of root

The root tissues were taken for the microscopic analysis in order to observe the entry and transport through the root. The tissues were prepared for TEM analysis by fixing in 2% of paraformaldehyde followed by ethanol series as per standard protocol (Yuan and Xu 2017). The NPs inside the root samples were visualized using the TEM (TEM, Morgagni 268D) at 200 kV accelerating potential.

### Statistical Analysis

The data were analysed with the one-way analysis of variance (ANOVA) using SPSS software (version Statistics V26). Tukey's t-test at $P < 0.05$ was used for multiple comparisons of the mean (Tukey 1977).

## Results

### Impact of MONPs on Potato

The impact of MONPs on potato plants were evaluated via measuring various plant growth parameters upon 35 days of transplantation in hydroponic medium. The tuber yield was also estimated after 65 days upon maturity of the plant. The result exhibited that the application of 4.0 mg $L^{-1}$ $Fe_3O_4$ NPs and 1.0 mg $L^{-1}$ of $MnO_2$ NPs found to be efficient in inducing various growth parameters, including leaf count, stem length, root length, as well as the fresh and dry weight of the plants (Fig. 1; Table 1). Additionally, the application of MONPs effectively reduced the time required for tuber induction and a subsequent increase in tuber yield was observed. Notably, the tubers were observed within $46 \pm 2$ days of initiating the cultures in the presence of MONPs, while untreated plants took $65 \pm 3$ days to show tuber growth. In addition, the presence of MONPs also resulted in higher numbers of tubers per plant, increased tuber mass, and an improved average tuber yield (see Fig. 2; Table 2). It is worth mentioning that the $MnO_2$ NPs treatment exhibited the highest levels of plant growth and tuber yield compared to plants treated with $Fe_3O_4$ NPs. Supplementation of either of MONPs in the liquid medium contributed to a more substantial potato yield, along with enhanced tuber mass and number.

### Quantification in the Change of ROS Removal Antioxidant Enzyme

The antioxidant enzyme activities in potato leaf tissues and tuber tissue at developmental stages. An increase in antioxidant enzymes activity (SOD, CAT, and POD) was observed in leaf as well as tuber tissues of plants grown in the presence of MONPs (4.0 mg $L^{-1}$ $Fe_3O_4$ and 1.0 mg $L^{-1}$ $MnO_2$ NPs), compared to untreated control plant. The results revealed a significant increase in SOD activity in plant with an increase of up to 0.68% for $Fe_3O_4$ NPs and 1.16% for $MnO_2$ NPs when compared to untreated leaf tissues (Fig. 3a). Similarly, tubers also exhibited elevated SOD enzyme activities as compared to untreated control tubers (Fig. 3a).

Furthermore, the CAT activity also exhibited a significant increase in MONPs treated leaf and tuber tissues compared to their respective controls. The enzyme activity increased by up to 1.44% for $Fe_3O_4$ NPs and 1.3% for $MnO_2$ NPs in hydroponically grown leaves, and by 1.6% for $Fe_3O_4$ NPs and 1.4% for $MnO_2$ NPs in tuber tissue, all relative to untreated tissues (Fig. 3b).

Additionally, peroxidase (POD) activity also enhanced in the presence of MONPs compared to untreated controls. Treatment of $Fe_3O_4$ NPs and $MnO_2$ NPs under hydroponic conditions induced a POD activity up to1.4% and 1.5% in leaf tissue and 1.5% and 1.6% in tuber tissue, respectively, when compared to control tissues (Fig. 3c).

### Assessment of MDA and $H_2O_2$ Content

The MDA and $H_2O_2$ content in the potato leaf and tubers grown in presence of MONPs displayed a declining trend when compared to untreated tissues (Fig. 4a and b). Additionally, we examined $H_2O_2$ accumulation in the leaves following MONPs treatment using 3, 3'-diaminobenzidine

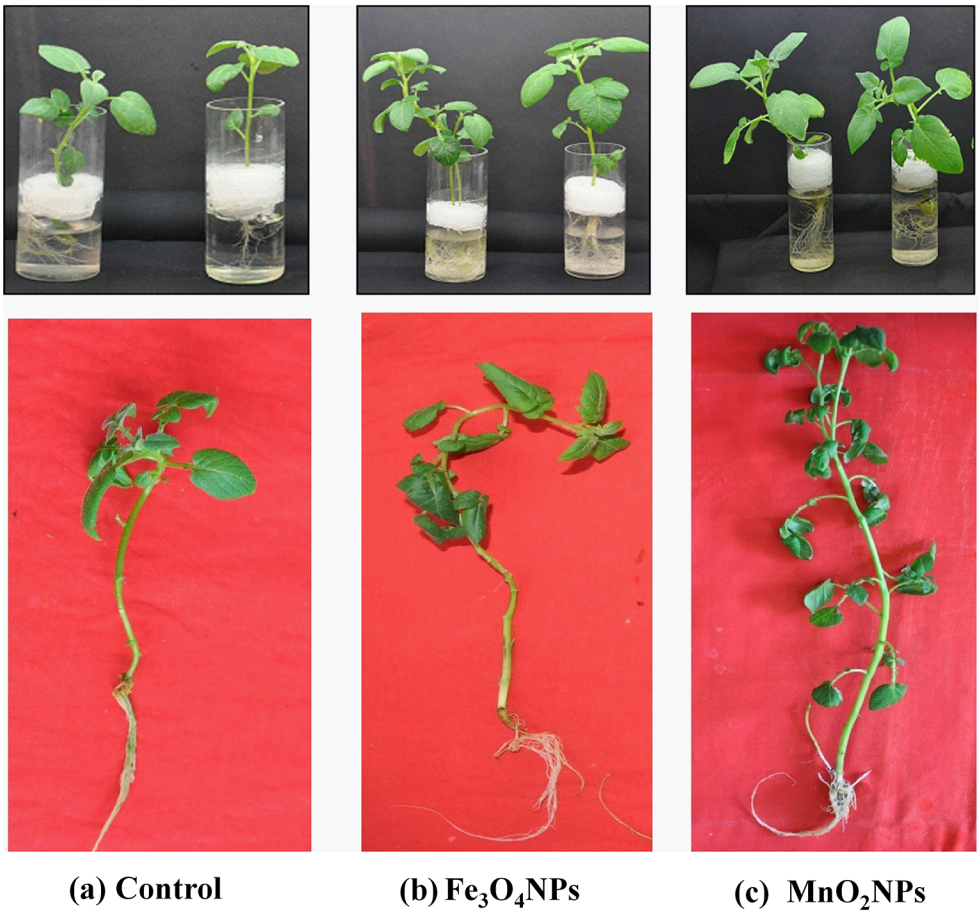


**Fig. 1** Impact of metal oxide nanoparticles growth and development of potato plants. The application of NPs in the hydroponic condition resulted in positive change of various growth parameter such as leaf count, stem length, root length observed after 35 days. (**a**) Control plant; (**b**) Plant grown in presence of $Fe_3O_4$ NPs (4.0 mg $L^{-1}$) and (**c**) in presence of $MnO_2$ NPs (1.0 mg $L^{-1}$)

(DAB) staining. In this process, Fe-containing proteins interacted with DAB, resulting in oxidation when exposed to $H_2O_2$, leading to the development of dark brown spots on the leaves. This stain precipitate served as a valuable indicator for detecting the presence and distribution of $H_2O_2$ in plant cells. Loss of membrane integrity and the induction of cell damage are common causes of oxidative stress in plants. However, the application of MONPs in the hydroponic reduced $H_2O_2$ accumulation in the leaves due to the elevated antioxidant enzyme activity in MONPs-treated plant leaves (Fig. 4c). Our findings indicated that leaves treated with MONPs exhibited lower $H_2O_2$ accumulation in comparison to untreated leaf tissues.

## Estimation of Photosynthetic Pigments and Soluble Sugar Contents

The plant treated with MONPs exhibited elevated photosynthetic pigments in leaves, including chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids, in comparison to the untreated control. Notably, the application of 1.0 mg $L^{-1}$ $MnO_2$ NPs induced a significant increase in various photosynthetic pigments compared to $Fe_3O_4$ NPs at concentrations of 4.0 mg $L^{-1}$ (Table 3). The application of MONPs in the Hoagland medium showed positive impact on the plant's photosynthetic efficiency via increasing chlorophyll pigments in the treated potato plants.

Furthermore, the application of MONPs also resulted in an increase of the total soluble sugar in the leaf and tuber tissues compared to respective untreated controls. In particular, the total soluble sugar content in the leaf sample increased up to 0.7% and 1.02% with the treatment of 4.0 mg $L^{-1}$ $Fe_3O_4$ NPs and 1.0 mg $L^{-1}$ $MnO_2$ NPs respectively, when compared to the untreated control tissues (Fig. 5). The higher concentration of soluble sugar in the MONPs treated tissues could be attributed to the enhanced photosynthetic efficiency observed in the treated plants (Kalal and Jajoo 2021).

## SEM-EDX and TEM Observation

The SEM-EDX spectra and EDX mapping analysis revealed the elemental composition of root and shoot sample treated with MONPs under hydroponic conditions. The SEM-EDX analysis of MONPs treated root and shoot samples indicated a slightly higher content of Fe, Mn, and Ca elements when compared to untreated tissues (Fig. 6a and b). These findings signify the plant's capacity to absorb and transport

**Table 1** Effect of metal oxide nanoparticles on various growth parameters of potato plants grown under hydroponic medium

| Treat-ments | Total No. of plants | No. of plants developed to maturity | No. of leaves per plants | Average stem length (cm) | Average root length (cm) | Fresh weight of plant (g) | Dry weight of plant (g) |
|---|---|---|---|---|---|---|---|
| **$Fe_3O_4$ NPs (mg $L^{-1}$)** | | | | | | | |
| 0.0 | 45 | 33b | 27.33 ± 1.45a | 16.33 ± 1.20c | 10.23 ± 0.32b | 1.47 ± 0.066b | 0.11 ± 0.066b |
| 1.0 | 45 | 24c | 23.23 ± 1.11b | 20.45 ± 0.22b | 12.24 ± 0.32a | 1.09 ± 0.011b | 0.012 ± 0.069b |
| 2.0 | 45 | 34b | 28.21 ± 1.23a | 22.23 ± 0.32b | 11.24 ± 0.32b | 1.49 ± 0.062a | 0.12 ± 0.066b |
| **4.0** | **45** | **38a** | **29.33 ± 0.33a** | **28.23 ± 0.45a** | **14.24 ± 0.32a** | **1.89 ± 0.023a** | **0.148 ± 0.061a** |
| 6.0 | 45 | 20d | 14.22 ± 1.12c | 18.45 ± 0.99c | 6.66 ± 0.577c | 0.999 ± 0.053c | 0.011 ± 0.063b |
| **$MnO_2$NPs (mg $L^{-1}$)** | | | | | | | |
| 0.0 | 45 | 35b | 27.33 ± 1.45b | 16.33 ± 1.20d | 10.23 ± 0.32c | 1.47 ± 0.066b | 0.11 ± 0.066c |
| 0.5 | 45 | 33b | 28.45 ± 1.33b | 25.23 ± 1.2c | 12.45 ± 0.32b | 1.49 ± 0.062b | 0.21 ± 0.034b |
| **1.0** | **45** | **40a** | **31.33 ± 3.48 a** | **32.33 ± 2.184a** | **16.45 ± 0.577a** | **2.54 ± 0.066a** | **0.629 ± 0.065a** |
| 1.5 | 45 | 23c | 25.32 ± 1.23c | 28.32 ± 1.11b | 12.23 ± ± 0.32b | 1.42 ± 0.023bc | 0.22 ± 0.056b |
| 2.0 | 45 | 21d | 18.21 ± 1.21d | 20.23 ± 1.2c | 8.76 ± 0.32d | 1.23 ± 0.021d | 0.14 ± 0.021c |

Hydroponic media: (without Fe and Mn salt + respective metal oxide NPs). Values are mean ± SE of three repeated experiments. Number of plantlets inoculated per treatment: 15; Average plant growth parameters were calculated per treatment containing 45 plantlets. Mean values followed by a different letter within a row indicate significant differences ($P < 0.05$) according to Tukey's test.Bold values highlight the most effective treatment, achieved with 4.0 mg $L^{-1}$iron oxide NPs and 1.0 mg $L^{-1}$ manganese dioxide NPs, demonstrating statistically significant improvement in plant growth parameters at $p < 0.05$

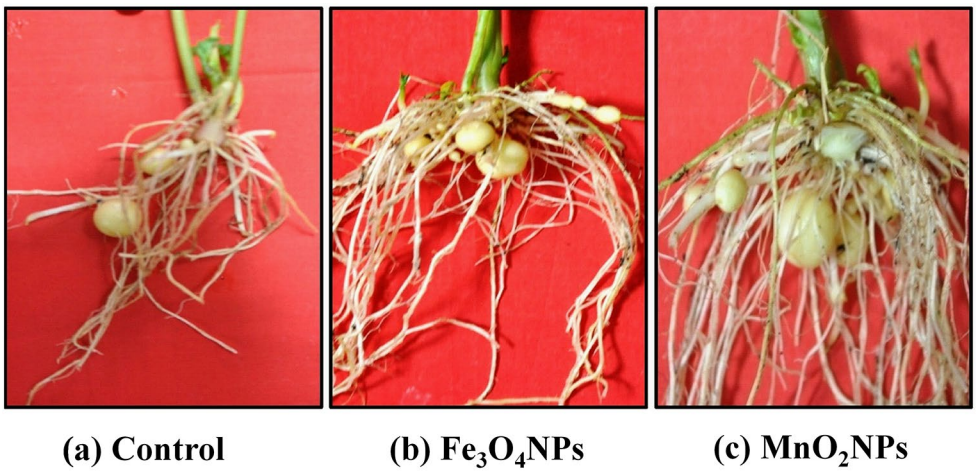


**Fig. 2** Impact of metal oxide nanoparticles on tuber yield Early tuber induction and enhanced yield were observed upon the application of MONPs after 65 days. (**a**) Tuberization in untreated plants; (**b**) Tuberization in presence of $Fe_3O_4$ NPs (4.0 mg $L^{-1}$) and (**c**) in presence of $MnO_2$ NPs (1.0 mg $L^{-1}$)

MONPs. To corroborate these results, EDX elemental mapping (Supplementary Figs. 1 and 2) was also done. The EDX mapping allowed for the estimation of $Ca^{+2}$ ion content, facilitating a comparative assessment of $Ca^{+2}$ levels in MONPs treated and untreated potato tissues. This analysis clearly demonstrated that the treatment with MONPs led to an increase in the content of $Ca^{+2}$ ions within the plant cells when compared to untreated plant tissues (Fig. 6a, b, and Supplementary Figs. 1 and 2).

The TEM analysis also exhibited the presence of electron-dense patches in the roots treated with MONPs. These patches, comprised of either single MONPs or clusters, were observed within various root regions, including the cell wall, epidermis, intercellular spaces, and vacuoles. This observation provides clear evidence of the transport and internalization of both $Fe_3O_4$ NPs (Fig. 7d-f) and $MnO_2$ NPs (Fig. 7g-i) in the NPs treated root tissues. Notably, the TEM images also demonstrated the ability of MONPs to

**Table 2** Effect of metal oxide nanoparticles on various growth parameters of potato tubers developed under hydroponic medium

| Treatments | Total No. of plants | No. of plants develop to maturity | No. of stolon inducing | Av. Tuber size (mm) | Av. Tuber wt. (g) | Tuber induction period | Tuber number per plant | Total tubers yield (no.) |
|---|---|---|---|---|---|---|---|---|
| **$Fe_3O_4$NPs (mg $L^{-1}$)** | | | | | | | | |
| 0.0 | 45 | 35a | 48d | 2.45 ± 0.11c | 1.26 ± 1.22 c | 63-65c | 1.43 ± 0.43 c | 25.2 ± 0.36 c |
| 1.0 | 45 | 24b | 70b | 2.99 ± 0.21c | 1.34 ± 1.123b | 56-58b | 2.54 ± 0.45b | 32.23 ± 0.99b |
| 2.0 | 45 | 34a | 60c | 2.65 ± 0.2c | 1.28 ± 1.11bc | 57-59bc | 2.45 ± 0.54b | 34.23 ± 0.88b |
| **4.0** | **45** | **36a** | **76a** | **4.12 ± 0.10a** | **2.93 ± 1.14a** | **50-52a** | **3.99 ± 0.45a** | **38.2 ± 0.16a** |
| 6.0 | 45 | 20c | 40d | 3.23 ± 0.21b | 2.35 ± 1.11b | 59-60bc | 2.23 ± 0.11b | 26.34 ± 0.23c |
| **$MnO_2$NPs ( mg $L^{-1}$)** | | | | | | | | |
| 0.0 | 45 | 35a | 48d | 2.45 ± 0.11c | 1.26 ± 1.22c | 63-65d | 1.33 ± 0.54c | 25.2 ± 0.36c |
| 0.5 | 45 | 33a | 60c | 2.99 ± 0.13b | 2.28 ± 1.11c | 53-55c | 2.45 ± 0.43b | 30.2 ± 0.23b |
| **1.0** | **45** | **38a** | **95a** | **7.41 ± 0.4a** | **3.82 ± 1.04a** | **45-46a** | **4.23 ± 0.11a** | **40.2 ± 0.14a** |
| 1.5 | 45 | 23b | 63b | 2.33 ± 0.23c | 2.35 ± 1.11b | 53-55bc | 2.23 ± 0.45b | 29.32 ± 0.2 |
| 2.0 | 45 | 21c | 56c | 1.99 ± 0.4 cd | 1.23 ± 1.11 cd | 56-58c | 1.23 ± 0.34c | 24.2 ± 0.36c |

Hydroponic media: (without Fe and Mn salt + respective metal oxide NPs). Values are mean ± SE of three repeated experiments. Number of plantlets inoculated per treatment: 15; Average tuber yield was calculated per treatment containing 45 plantlets. The values are represented as the mean ± SE of three replicates. Mean values followed by a different letter within a row indicate significant differences ($P < 0.05$) according to Tukey's test. Bold values highlight the most effective treatment, achieved with 4.0 mg $L^{-1}$ iron oxide NPs and 1.0 mg $L^{-1}$ manganese dioxide NPs, demonstrating statistically significant improvement in potato tuber biomass and yield at $p < 0.05$

penetrate the roots and enter xylem cells, marking the initial direct confirmation of MONPs uptake by potato plant roots while preserving the root tissue's morphology. However, the untreated root samples displayed no presence of electron-dense patches and clusters in the TEM images (refer to Fig. 7a-c).

## Discussion

### Impact of MONPs on Plant Growth and Tuber Yield

The supplementation of $MnO_2$ and $Fe_3O_4$ NPs in the hydroponics medium resulted in improved plant growth and increased tuber number, size and mass; ultimately contributing to enhanced potato yield in hydroponic conditions. Existing literatures also report change in various physiological, biochemical, and molecular parameters in plants when exposed to MONPs. Evidence from earlier studies indicates that the application of MONPs exhibit positive impact on growth in peanut, soybean, wheat, onion, *Cyamopsis tetragonoloba*, and tobacco by modulating antioxidant levels (Tirani et al. 2019; Prasad et al. 2012; Ramesh et al. 2014; Raskar and Laware 2014; Raliya and Tarafdar 2013). Application of ZnO NPs has been shown to enhance the physiological status of wheat, particularly by improving photosynthetic performance (Kalal and Jajoo 2021). In another study, the combined application of ZnO and FeO NPs on the plant has been reported to increase plant height, root growth, and carrot yield in *Daucus carota* (Elizabath et al. 2017). Additionally, the application of nano silicon fertilizer, complete nano-fertilizer, nano-chelated Zn, and nano-chelated Ca oxide has been shown to reduce the time required for tuber induction and enhance tuber yield (Janmohammadi et al. 2016; Al-juthery et al. 2019). The application of MONPs might improve photosynthetic efficiency via increasing photosynthetic pigments. Additionally, this also induced the ROS scavenging antioxidant enzyme as well as the expression of $Ca^{2+}$ ion regulatory genes (*CDPK* and *CAM1*), along with the tuber-inducing gene referred as lipoxygenase (*LOX*). Synergistic outcome of these biochemical and molecular changes potentially leads to increase in plant dry and mass tuber yield (Joshi et al. 2022; Marmiroli et al. 2015; Mijweil and Abboud 2018). This study also demonstrated that application MONPs as a nano-nutrient in hydroponic increased plant growth and tuber yield. The possible mechanism behind the enhanced growth parameters and tuber yield is attributed to increased nutrient uptake, translocation, and metabolism of nutrient, as observed in *Daucus carota* with application of ZnO and FeO NPs (Elizabath et al. 2017).

### Alteration in the Biochemical Profile

Activity of antioxidant enzymes in particular the superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD) was found to increase in leaves and tuber tissues of plants grown in MONPs supplemented liquid medium. Kouhi et al. (2015) has also reported an increase SOD enzyme activity in *Brassica napus* when exposed to lower doses of ZnO NPs. The CAT and POD are also recognized as ROS scavenging enzymes responsible for detoxifying $H_2O_2$ (Sharma et al. 2012; Ma et al. 2015). Exposure to even lower doses of ZnO NPs has been shown to increase CAT enzyme activity

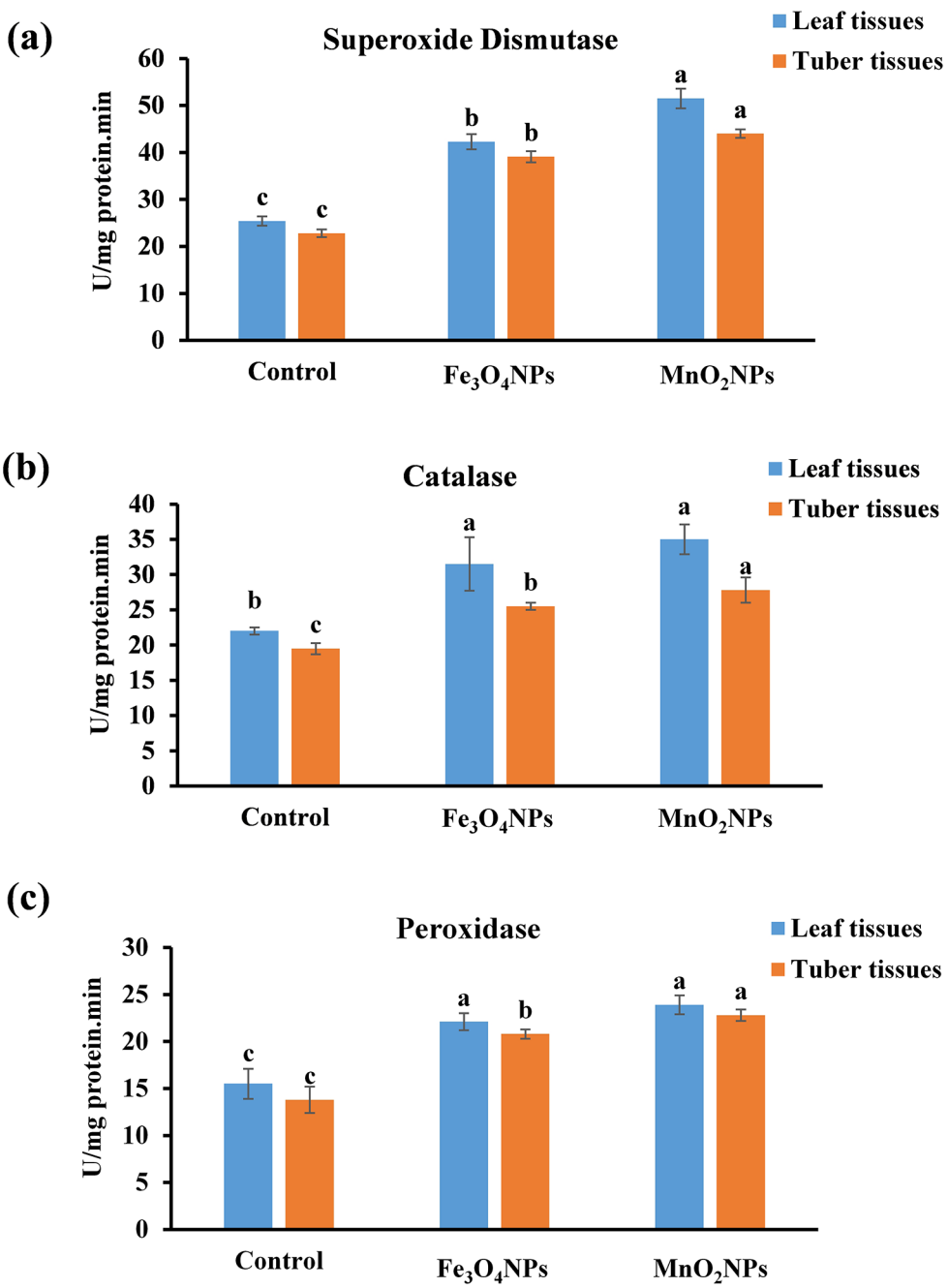


**Fig. 3** Antioxidant enzymes activities in plant tissues growing in the presence of MONPs. (**a**) SOD; (**b**) CAT, and (**c**) POD activities determined in the leaves and tuber tissues of treated and untreated control plants. The values are represented as the mean ± SE (standard errors of the means) of three replicates and different lowercase letters above bars indicate statistically significant differences among the treatments at $p < 0.05$ according to Tukey's test

in *Prosopis juliflora* and *Spirodela polyrhiza* plants (Hernandez-Viezcas et al. 2011; Hu et al. 2013). Additionally, POD enzyme activity increased in *Glycine max* and *Cucumis sativus* plants treated with CuO NPs (Kim et al. 2012; Castiglione et al. 2016). Application of $Fe_3O_4$ NPs induced antioxidant enzymes (SOD, CAT, APX, and GPX) in wheat. Furthermore, Fe and Mn ions serve as co-factors for scavenging enzymes and play a crucial role in maintaining cellular redox balance in plant cells (Rout et al. 2015).

Various report published on the application of NPs in plants activates signalling pathways maintaining the redox balance of plant cells via regulating cellular $Ca^{+2}$ homeostasis (Boudsocq et al. 2005; Mirzajani et al. 2014; Marmiroli et al. 2015; Joshi et al. 2022). Studies on NPs clearly indicate that application of NPs in optimal concentration maintains cellular redox potential. Earlier studies have also suggested MONPs enhance antioxidant enzyme activity in plants via mechanisms including ROS production and scavenging, modulation of gene expression, and signalling pathways. These nanoparticles balance oxidative stress, boosting enzymes like CAT, SOD, APX and POD. In present study, the application of MONPs also altered cellular $Ca^{+2}$ ions

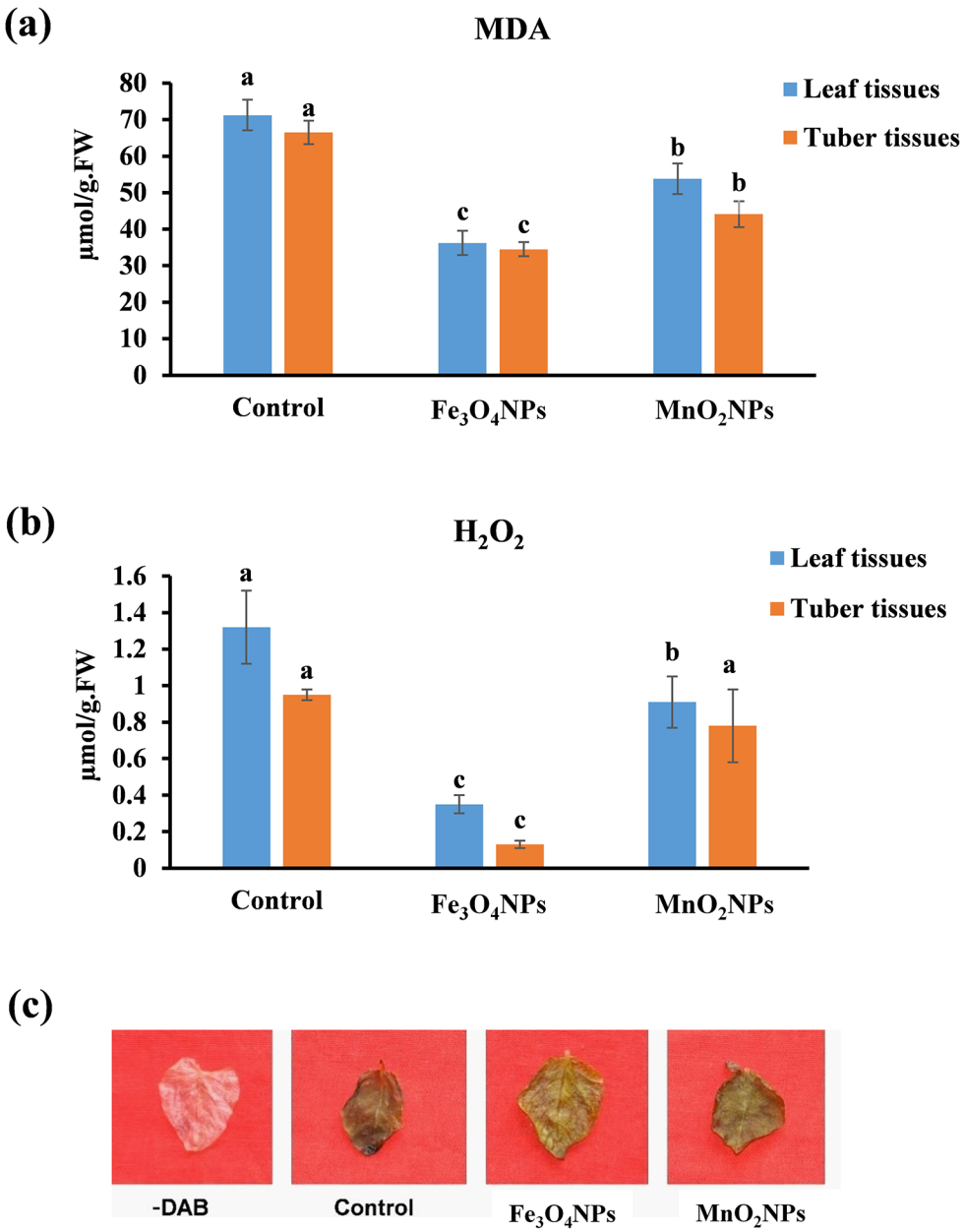


**Fig. 4** Estimation of malondialdehyde (MDA) and hydrogen peroxide ($H_2O_2$) content in the plant tissues. (**a**) MDA; (**b**) $H_2O_2$ content, and (**c**) DAB staining of leaves growing in presence of MONPs. The dark brown spot indicated distributions of $H_2O_2$ in the entire leaf. The values are represented as the mean ± SE of three replicates and different lowercase letters above bars indicate statistically significant differences among the treatments at $p < 0.05$ according to Tukey's test

confirmed by EDX analysis, inducing plant defence mechanisms by maintaining a balance between the oxidative and reductive states of the cell through increased antioxidant enzyme activity in potato.

## Photosynthetic Pigments and Total Soluble Sugar Content Treated Plants

Application of suitable content of MONPs played an important role in maintaining the photosynthetic efficiency of potato plants via increasing the total chlorophyll content consisting with previous findings (Ghafariyan et al. 2013; Falco et al. 2015). Similarly, the application of FeO NPs to soybean and barley plants in a liquid nutrient medium resulted in higher chlorophyll content in leaves, attributed to the enhanced expression of photosynthetic pathway-related genes (Pradhan et al. 2013; Ghafariyan et al. 2013; Tombuloglu et al. 2019). $MnO_2$ NPs were also found to increase photosynthetic efficiency in *Vigna radiate* (Pradhan et al. 2013). Reports on wheat under in vitro conditions has shown that the application of Zn-CuO NPs and nano-priming of ZnO NPs enhanced the content of photosynthetic pigments (Taran et al. 2017; Latef et al. 2017; Kalal and Jajoo 2021; Singh et al. 2021). Increased photosynthetic activity in treated plants might be due to increased chlorophyll, carotenoid, and other pytopigment levels in plants.

The total soluble sugar content in MONPs treated plants may be attributed to the enhanced photosynthetic efficiency

**Table 3** Effects of metal oxide nanoparticles on photosynthetic-pigments contents in potato

| Phytopigments | Untreated | $Fe_3O_4$ NPs | $MnO_2$ NPs |
|---|---|---|---|
| Chlorophyll-a (mg/g of FW) | 0.64 ± 0.024c | 1.2 ± 0.09b | **1.8 ± 0.10a** |
| Chlorophyll-b (mg/g of FW) | 0.21 ± 0.01c | 0.34 ± 0.01b | **0.44 ± 0.02a** |
| Total Chlorophyll-a + b (mg/g of FW) | 0.85 ± 0.03c | 1.54 ± 0.10b | **2.2 ± 0.11a** |
| Ratio a/b | 3.067 ± 0.027c | 3.5 ± 0.15ab | **4.07±0.28a** |
| Carotenoids (mg/g of FW) | 0.32 ± 0.014c | 0.41 ± 0.01ab | **0.43±0.02a** |

The photosynthetic-pigments contents including the chlorophyll a, chlorophyll b, total chlorophyll a + b, Chl a/b and carotenoids. The values are presented as the mean ± SE of three replicates. Mean values followed by a different letter within a row indicate significant differences ($P < 0.05$) according to Tukey's test. Mean values followed by a different letter within a row indicate significant differences) according to Tukey's test. Bold values highlight the most effective treatment, achieved with 1.0 mg $L^{-1}$ manganese dioxide NPs concerning 4.0 mg $L^{-1}$ of iron oxide NPs and untreated plants, demonstrating statistically significant improvement on plant photopigments at $p < 0.05$

of potato. Reports indicated that the application of FeO NPs increased sugar accumulation in tobacco plants (Alkhatib et al. 2019). ZnO NPs have also been shown to improve plant growth and development by increasing cellular proteins and carbohydrate content due to enhanced photosynthetic efficiency (Bandyopadhyay et al. 2015; Wang et al. 2016; Kalal and Jajoo 2021). In present study, the higher sugar contents positively correlated with the increased photosynthetic efficiency of MONPs-treated plants.

## Nanoparticles Uptake Evaluation

The SEM-EDX analysis, along with corresponding EDX-mapping data of MONPs-treated root and shoot samples, confirmed the absorption and transportation of $Fe_3O_4$ and $MnO_2$ NPs by the plants. Likewise, elemental quantification of Fe, Mn, and Zn ions in tomato roots, potato micro-tubers,

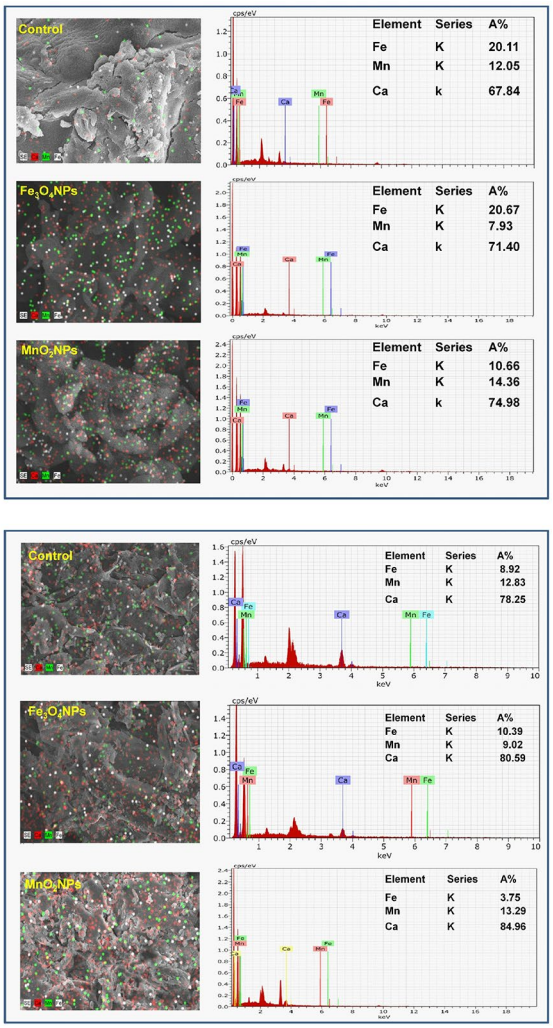


**Fig. 6** Energy Dispersive X-ray spectroscopy (EDX) mapping and elemental data analysis of MONPs treated plat tissues. EDX spectra indicated % quantity of metals (Fe, Mn and Ca) with the MONPs treated and untreated plant tissues. (**a**) SEM-EDX mapping and elemental analysis of root tissue. (**b**) SEM-EDX mapping and elemental analysis of shoot tissue

and *L. usitatissimum* plants was conducted. Earlier reports

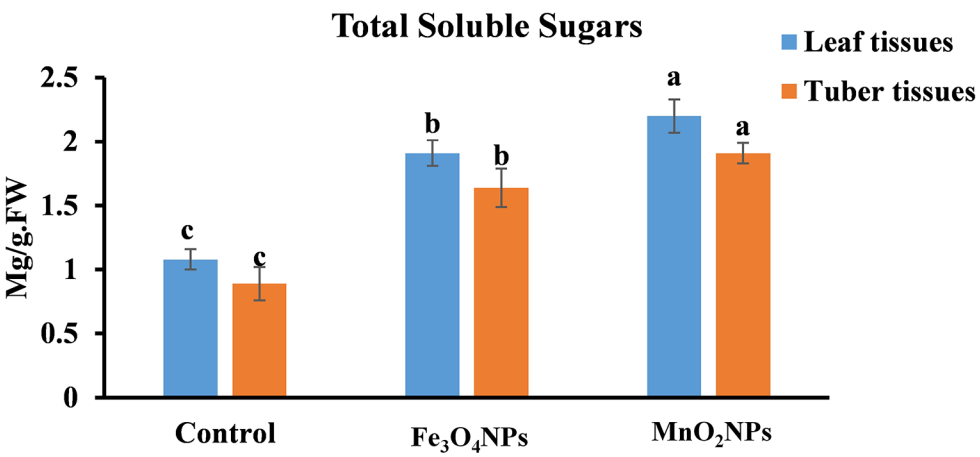


**Fig. 5** Estimation of total soluble sugar content in the plant tissues growing in presence of MONPs. The values are represented as the mean ± SE of three replicates and different lowercase letters above bars indicate statistically significant differences among the treatments at $p < 0.05$ according to Tukey's test

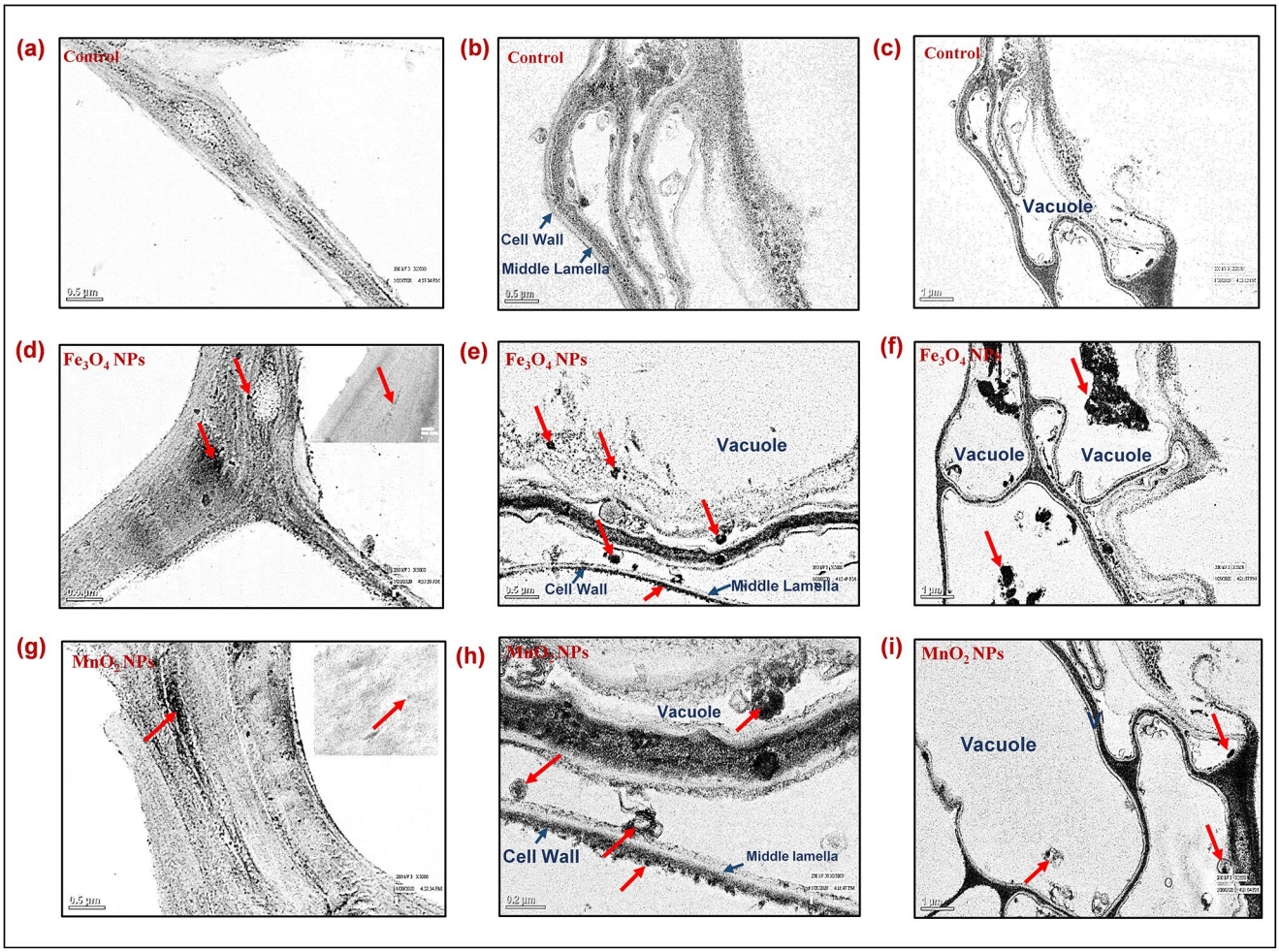


**Fig. 7** Transmission Electron Microscopy (TEM) images of root tissues. (**a-c**) Absence of NPs in the untreated control root tissues. (**d-f**) $Fe_3O_4$ NPs (4.0 mg $L^{-1}$) treated root shows free and aggregated clump of NPs in cell wall, middle lamella, and the vacuole. (**g-i**) $MnO_2$ NPs (1.0 mg $L^{-1}$) treated root shows free and aggregated clump of NPs in cell wall, middle lamella, and vacuole. The red arrows indicate the localization of MONPs in the root tissue

have also indicated that the application of NPs ($Fe_3O_4$/$MnO_2$ in potato and ZnO NPs in *L. usitatissimum*) increased the concentration of $Ca^{+2}$ ions in plant cells, as analysed by SEM-EDX elemental mapping (Singh et al. 2021; Joshi et al. 2022).

TEM was also performed to visualize NPs in roots grown in hydroponic in the presence of MONPs. The darker patches observed in the TEM images of treated root samples correspond to the presence of the applied nanoparticles (Red arrows in Fig. 7d-i), however, no dark patches were exhibited in the untreated control root tissues (Fig. 7a-c). The darker regions in the TEM images also represented the electron-dense NPs accumulation in the root tissues. The contrast between the NPs and the surrounding cellular structures allows for their visualization using TEM. The TEM images also suggested that NPs could penetrate roots and enter xylem vessels, providing direct evidence of NPs uptake in potato plant roots. The images revealed extensive adherence of NPs to the root epidermal surface, potentially through mechanical attachment or diffusion, as observed with FeO, $TiO_2$, ZnO, $CeO_2$, and CuO NPs on the roots of capsicum, rice, corn, and wheat, respectively (Lin et al. 2008; Wild et al. 2009; Zhou et al. 2011; Zhao et al. 2012; Deng et al. 2017; Yuan et al. 2018). In potato root cells, no specific pattern in the intracellular distribution of MONPs was observed; they often appeared in outer root layers in agglomerate form, similar to observations in wheat roots (Deng et al. 2017).

## Conclusion

Application of metal oxide nanoparticles as $Fe_3O_4$ and $MnO_2$ in the hydroponic condition significantly enhanced the growth and development as well as tuber yield in potato. The uptake of MONPs enabled the plant to elevate cellular

$Ca^{+2}$, maintaining the redox status, increased photosynthetic activity and other metabolic processes crucial for tuber induction. The SEM-EDX and TEM analyses also confirmed the absorption and distribution of NPs throughout plant tissues, highlighting their potential for transformative applications in agriculture. Moreover, the study also introduces an innovative facet by demonstrating the application of eco-friendly $Fe_3O_4$ and $MnO_2$ NPs for enhanced nutrient delivery, antioxidant activity, and photosynthetic efficiency in hydroponic potato cultivation. These advancements not only support pathogen-free tuber production but also promise increased productivity and profitability for potato growers and farmers engaged in sustainable agricultural practices. While the findings underscore substantial benefits for hydroponic potato farming, further study into field applications is necessary to refine NPs usage and verify their efficacy across diverse agricultural settings. Conclusively, integrating $Fe_3O_4$ and $MnO_2$ NPs into hydroponic systems represents a promising innovative approach in enhancing crop yield, improving plant health, and fortifying global food security, eventually advancing agricultural practices for the benefit of farmers and consumers worldwide.

**Supplementary Information** The online version contains supplementary material available at https://doi.org/10.1007/s12230-024-09961-7.

**Acknowledgements** Authors are thankful to the Centre for Advanced Research (CAR) at Dr. Harisingh Gour University, Sagar, Madhya Pradesh, India. We also acknowledge the use of Sophisticated Analytical Instrumentation Facility (SAIF), All India Institute of Medical Sciences (AIIMS), New Delhi for the TEM analysis. NJ acknowledge the receipt of the fellowship under UGC research fellowship program from Dr. Harisingh Gour Vishwavidyalaya, Sagar, Madhya Pradesh, India. AP acknowledge the receipt of the fellowship from DBT under "DBT-BUILDER-Dr Harisingh Gour Central University, Sagar, Madhya Pradesh Interdisciplinary Life Science Program for Advance Research and Education (No. BT/INF/22/SP47619/2022).

**Authors Contributions** Conceptualization and designing of the research work by NJ and CPU; Execution of lab experiments and data collection by NJ and AP; Analysis of data and interpretation by NJ, DCU and AP; Preparation of manuscript by CPU and DCU.

**Funding** The authors acknowledge the funding receipt from DBT under "DBT-BUILDER-Dr Harisingh Gour Central University, Sagar, Madhya Pradesh Interdisciplinary Life Science Program for Advance Research and Education (Level II No. BT/INF/22/SP47619/2022)".

## Declarations

**Conflict of Interest** On behalf of all authors, the corresponding author states that there is no conflict of interest.

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