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Nanoparticles Enhance Antioxidant System and Improve Yield Quality of Sugar Beet (*Beta vulgaris* L.) Plants Irrigated with Wastewater

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IN DEVELOPED and low-economy countries, water scarcity due to climate change and rapid population growth has led to reuse of untreated wastewater in the agricultural sector resulting in oxidative stress in plants. The oxidative stress tolerance of sugar beet, the second most important sugar producing plant economically, can be improved by applying novel nanotechnology. A field experiment was conducted to investigate the effect of application of different doses (control; 0.0, 50, 100 and 200 mg. L^{-1}) of SiO_2, MgO and a mixture of SiO_2 + MgO NPs in improving the growth and yield quality of sugar beet plants irrigated with wastewater. Low dose (50 mg.L^{-1}) of SiO_2 + MgO NPs resulted in the highest improvement in root length and diameter, but MgO NPs was the best in improving FM and DM by about 187 and 277.2 %, respectively. Application of high level of (200 mg.L^{-1}) SiO_2 and MgO NPs affect root parameters and pigments pools negatively. The results showed that 50 mg.L^{-1} of SiO_2 NPs was the best at enhancing the activity of CAT, POX, PPO and APX in leaves by about 151.7, 139.1, 175.9 and 213.9 % greater than untreated leaves. In long term effect, 100 and 200 mg.L^{-1} of NPs were more effective in improving the morphological characteristics of root and the maximum increase in crop quality was enhanced by 200 mg.L^{-1} of SiO_2 NPs. It was concluded that MgO NPs improved total Chl content and sucrose accumulation in leaves, while SiO_2 NPs treatments were the best in improving antioxidant enzyme activities and ameliorated the oxidative stress and in the long-term effect in improving the crop quality of sugar beet plants and enhancing sucrose accumulation in roots.

Keywords: Nanoparticles, Wastewater, Oxidative stress, Sucrose %, Yield quality.

1. Introduction

The scarcity of freshwater resources is largely affected by growth of population, pollution, urbanization and climate change, where about 1.6 billion people live in region with low water supply as well as 1.2 billion people living in river basins suffer from water shortage (FAO, 2012). Early FAO (2018) explained by withdraws of more than 25% of fresh water resources of a country triggers a water stress but withdrawal of more than 60 % triggers water scarcity and sever water scarcity will persist beyond 75%. Therefore, Egypt, Jordan, Libya, Saudi Arabia, Turkmenistan and Uzbekistan exposed to high water stress while Afghanistan, China, India and South Africa were subjected to very high water stress. Reusing wastewater to irrigate agricultural crops in low-income, rain-deficit countries would meet agricultural needs and could continue to boost economies by monitoring nutrients from water to adjust and apply them to crops through different fertilization methods (Bedbabis et al., 2014). However, irrigation with wastewater leads to the accumulation of potentially toxic elements with organic matter through aging (Abbas and Bassouny, 2018).

High levels of salinity and heavy metals accumulate with long-term irrigation with wastewater, affecting soil fertility and human health (Erel et al., 2019). By 2050, the world will need to double its agricultural output to meet growing population demands (FAO, 2019). Under stress conditions, reactive oxygen species (ROS) accumulate in plants, altering membrane integrity and nutrient balance, causing cell death and reducing growth and productivity. The generation of ROS threatens the cells due to oxidation of macromolecules proteins, nucleic acids, peroxidation of lipids and inhibition of enzymes that leads to programmed cell death (Mozaffari and Fatholahy, 2020). Plants have an enzymatic system including catalase (CAT), superoxide dismutase (SOD), polyphenol oxidase (PPO), and ascorbate peroxidase (APX) and a non-enzymatic system including phenolic, flavonoids, and alkaloids capable of scavenging accumulated ROS (Bienert et al., 2006).

Egypt is one of the top ten countries in sugar beet production (FAO, 2019). Besides sugar production, sugar beet, which is one of the most water-intensive crops, is used as biofuel due to its high potential for ethanol production and shorter growing period (Mall et al., 2021). Recently, Ramazi et al. (2024) have suggested that the interaction

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between genotype and diverse environmental conditions significantly affects root productivity, sugar content, sucrose stability, sucrose extraction coefficient, and levels of impurities including α-amino N, Na^+, and K^+. In addition, sugar beet plants can grow well in soils irrigated with wastewater and high levels of salinity, and can be used as a salt-tolerant crop due to its predecessor, sea beet (Rozema et al., 2015).

A novel nanotechnology approach used in agriculture to prepare and design of materials at nanoscale. Recently, nanotechnology has been applied in agricultural sector with different purposes as increasing resistance of crop plants to abiotic and biotic stresses and productivity of plants (Khaled et al., 2019. The application of NPs is an effective long-term approach to alter the toxic effects, nutritional imbalance resulting from abiotic stress and enhance growth and productivity (Singh et al., 2024b, Raza et al., 2023). Nanoparticles (NPs) characterized by nano-sized with large surface area and high reactivity (Singh et al., 2024a), thus they have the ability to penetrate plant cells easily, have higher infiltration capacity, controlled and slow release, protect plants against stressors, enhances ROS scavenge, reduced oxidation of photosynthetic systems and undergo a complex reactions in soil such as aggregation and redox reactions (Fatima et al., 2021). It was reported that single or multiple NPs foliar application enhanced plant tolerance and resistance against stresses compared to soil amendment *via* stimulating, enhanced up taking and assimilation of fertilizers (Garcia-lopez et al., 2019), and increase yield quality with more shelf life of plant products (Natasha et al., 2020).

Although silicon hasn't any clear physiological role in plants, it is considered beneficial element since its accumulation in different plants improves antioxidant defence system and stress tolerance with metabolism modification. However, silicon nanoparticles (SNPs) exerted a significant ameliorative effect on the physiological functions, growth and productivity of stressed plants by improving water and mineral uptake, nitrogen metabolism, photosynthesis and gas exchange processes (Liang et al., 2023). Sousa et al. (2019) reported that oxidative stress produced by abiotic and biotic stresses can be reduced by silicon NPs priming defence reactions. Magnesium (Mg^{2+}) plays a fundamental role in plant physiology and biochemistry. It is a component of chlorophyll molecule and thus enhances photosynthesis, activates many enzymes and thus plant growth and productivity. Kanjana (2020) reported that application of MgO NPs on cotton plant leaves increased the yield by 42.2% compared to untreated plants. Several studies have shown that SiO_2 NPs, TiO NPs, and gold (Au) NPs positively affected the seed germination of crop plants such as tomato, rice, maize, wheat, barley, soybean, and pearl millet (Khan et al., 2019). It was found that MgO NPs treatment of *Daucu carota* plants grown under lead stress increased activities of CAT and SOD enzymes by 32% and 29%, respectively, compared to untreated control plants (Faiz et al., 2022). Application of nanoparticles can increase stress tolerance by enhancing photosynthesis rate and photoprotection in stressed plants (Khan and Upadhyaya, 2019). However, photosynthetic rate (Pn), photosynthetic efficiency of PSII (Fv/Fm) and maximum photooxidation P700 (Pm) increased by 74.5%, 16.7% and 21.3%, respectively, in sugarcane plants treated with MgO nanoparticles.

Our study aimed to study the reuse of untreated wastewater in irrigation of sugar beet (*Beta vulgaris* L.) crop, and we try to improve oxidative stress tolerance and thus crop productivity by applying various concentrations of different nanoparticles (SiO_2, MgO and a mixture of SiO_2 + MgO). On the other hand, we planned to show and compare the responsive effect of antioxidant systems in leaves and roots to SiO_2 and MgO NPs or their combination to conclude which one is better in improving the crop quality.

2. Materials and Methods

2.1. Materials

Magnesium nitrate (Mg $(NO_3)_2.6H_2O$), sodium silicate, and potassium hydroxide (KOH) trichloroacetic acid (TCA), thiobarbituric acid (TBA), anthrone and quercetin purchased from Sigma Chemical Co (*via* Egyptian center, Cairo, Egypt).

2.2. Synthesis of nanoparticles

2.2.1. Synthesis of magnesium oxide (MgO) nanoparticles

Preparation of MgO NPs by sol-gel method, where 1 g of magnesium nitrate (Mg $(NO_3)_2.6H_2O$) was dissolved in 100 ml DDW and mixed with 0.1 M of potassium hydroxide that added drop by drop with stirring vigorously for 2h until white ppt of magnesium hydroxide formed (pH 12). The white ppt was purified with ethanol and DDW several times and dried at 50 ºC. The white powder was calcined at 450 ºC (El-Shafai et al., 2021).

2.2.2. Synthesis of silicon dioxide (SiO_2) nanoparticles

10 mL of sodium silicate (Na_2SiO_3) was placed in a 250 mL beaker with stirring for 10 min, and 40 mL of hydrochloric acid (1M) was added drop wise until white ppt was formed. The mixture was stirred overnight until ppt formation was complete, ppt was washed with mixed solvent water and ethanol several times and then the drying process was carried out at 50 °C (El-Wakeil et al., 2024).

2.3. Field trial and plant material

A field experiment was conducted on a private farm irrigated with wastewater from Aptor drain in Qaleen (latitude 31° 2' 52" N, longitude 30° 51' 9" E, 7.76 m above sea level), Kafr El-Sheikh, Egypt. The soil preparing and sowing were according to agricultural practices previously recommended by Ministry of Agriculture. 30 kg/acre of calcium superphosphate (15.5% P_2O_5) was added as a phosphorus source to the soil before planting. The growing season period was 6 months from early October to early April 2021/2022 and was characterized by a climate of average humidity; 63.83%, average rainfall; 7.276 mm, average temperature; 23.73/12.63 °C and a photoperiod of 16/8 h light/dark.

Five surface soil samples were collected before planting from a depth of 0-30 cm and subjected to physicochemical analysis as described by Klute and Dirksen (1986), and results are represented in table 1. Soil organic carbon was estimated using dichromate digestion method (Shi et al., 2011) and exchangeable elements were estimated by using flame photometer. In addition, 5 wastewater samples from the Aptor drain were collected in clean plastic bottles in triplicate and transported to the Plant Physiology Laboratory, Department of Botany and Microbiology, Faculty of Science, Kafrelsheikh University, Kafrelsheikh, Egypt for analysis. Physicochemical analysis included pH, EC, The wastewater analysis showed the following results: pH, 7.5; EC, 3.52 $dS.m^{-1}$; TDS, 226.8; K^+, 3.4; Na^+, 12.4; Mg^{++}, 6.9; Ca^{++}, 19.3; Cl^-, 4.3; SO_4^{++}, 22.6; NO^{-3}, 0.53; Fe, 0.14; B, 0.03; Cd^{++}, 0.004; Cr, 0.006^{++}; Cu, 0.005; Mn, 0.022; Pb, 0.027; Zn, 0.01 $mg.L^{-1}$.

Table 1. Physicochemical analysis of experimental field soil (Data are means of 5 samples).

Soil properties	mean
Physical properties	
Coarse sand %	6.24
Fine sand %	27.38
Silt %	14.85
Clay %	51.63
Textural class	clay
Field capacity %	39.31
Permanent wilting point %	19.50
Chemical properties	
pH	7.71
EC ($ds.m^{-1}$)	1.61
O.C %	2.25
$CaCO_3$ %	1.66
Available elements ($mg.kg^{-1}$ soil)	
N	479
K	18.20
P	16.50
Mg	6.61
Na	1.85

2.3.1. Evaluation of the effect of NPs on the growth and productivity of sugar beet plants

Sugar beet (*Beta vulgaris* L.) cv. "karam" seeds were voluntarily provided by the Agricultural Research Center, Sakha, Kafr El-Sheikh, Egypt. The seeds were evenly sterilized with 0.5% sodium hypochlorite solution for 10 min, then washed thoroughly several times with distilled water and dried with a tissue, divided into ten equal groups. Different concentrations of each of SiO_2, MgO and SiO_2 + MgO nanoparticles were applied at different levels: 50, 100 and 200 $mg.L^{-1}$. For the first priming application, each seed group was soaked for 24 h at room temperature in the dark in different NPs solutions and distilled water as a control with continuous aeration (Khan et al., 2017) and in distilled water as a control. An area of 10*20 m was divided into separate rows with a distance of 0.65m (about 30 rows with a length of 10 m), 3 rows for each treatment, then each group of seeds was planted in studied rows. The four-leafed plants were thinned at 30 days of age to ensure one plant/hill followed by supply of urea as nitrogen fertilizer at the rate of 80 kg/acre twice. Irrigation was done with wastewater from Aptor drain. For the second foliar application, 55-day old (about 8 weeks) sugar beet plants were foliar sprayed by the different pre-prepared NPs solutions and distilled water (control). The instructions recommended by Sugar Crops Research Institute were followed during the growing season.

2.3.2. Plant sampling

First sampling: After 10 days of foliar spraying (65-day-old plants), 20 randomly selected homogeneous plants for each treatment were collected and placed in clean, refrigerated plastic bags. They were then transferred to the Plant Physiology laboratory, and washed thoroughly with tap water followed by dist. water and dried with paper towels. Five plants were used to measure root morphological characteristics (root length, diameter, fresh and dry mass). For biochemical analysis, roots were separated from shoots, leaf samples and root slices were taken and stored in a freezer at -20°C until use. Second sampling: Five homogeneous roots were randomly selected at harvest stage after 180 days of sowing for each treatment.

2.4. Biochemical analysis

2.4.1. Extraction and estimation photosynthetic pigments content

Discs (0.5 gm) of freshly harvested NPs treated or untreated sugar beet leaves were homogenized with 5 ml cooled 80% acetone in pre-chilled porcelain mortar with adding $CaCO_3$. Plant homogenate was centrifuged at 8000 xg for 5 min at 4 ^{O}C. the precipitate was re-extracted three times by 80 % and the absorbance of the collected supernatant was read at 470, 648.6 and 664 nm using spectrophotometer ((Jasco V-730 UV-Visible Spectrophotometer). According to Lichtenthaler (1987), total chlorophylls and carotenoids content were determined as mg.g^{-1} FM.

2.4.2. Estimation of glucose and sucrose contents

To estimate the glucose and sucrose contents, 0.5 g of fresh leaves and roots tissues was kept in 10 ml of ethanol (95%) overnight and filtered after homogenization. The residue was washed and filtered three times. The resulting fractions were mixed, adjusted to a known total volume and used for the determination of glucose and sucrose as described by Van Handel (1968). The sucrose content was determined by incubating a mixture of 0.1 ml of the extract and 0.1 ml of 30% KOH in a boiling water bath for 10 min. After cooling to room temperature, a portion of anthrone reagent was added and boiled for 5 min, then cooled and the absorbance at 620 nm was read using a spectrophotometer (Jasco V-730 UV-Visible Spectrophotometer).

2.4.3. Estimation of lipid peroxidation content and H_2O_2 level

The method of Uchiyama and Mihara (1978) was followed to determine the content of malondialdehyde (MDA) in the leaves and roots of sugar beet plants treated and untreated with nanoparticles. 0.5 g of plant tissue was ground in a pre-cooled mortar with 5 ml extraction solution (100 mM phosphate buffer; pH 7.5 containing 0.1 mM EDTA). After centrifugation at 4 °C for 15 min at 13,000 × g, a mixture of equal volumes of the supernatant and a solution of 0.8% TBA and 20% TCA was heated in a boiling water bath for 15 min, cooled on ice, and centrifuged at 12,000 × g for 5 min. The absorbance at 532 nm was read using a spectrophotometer (Jasco V-730 UV-Visible Spectrophotometer). As described by Loreto and Velikova (2001), H_2O_2 content was determined using the standard curve and expressed as µmol.g^{-1}.

2.4.4. Extraction of tested antioxidant enzymes

As described by Slabbert and Krüger (2014), 0.5 gm of freeze-dried leaves and roots were ground in a pre-chilled mortar and pestle with 10 ml of 50 mM phosphate buffer (pH, 7.8), followed by centrifugation at 15000xg at 4^{O}C for 20 min. Enzyme extract was stored at refrigerator for further assaying the activity of catalase (CAT; EC 1.11.1.6), polyphenol oxidase (PPO; EC 1.14.18.1), Guaiacol peroxidase (POX; EC 1.11.1.7) and ascorbate peroxidase (APX; EC 1.11.1.11).

Catalase activity was assayed according to Velikova et al. (2000), the decrease in H_2O_2 concentration at 240 nm was recorded using a spectrophotometer after incubation of 40 µl of the extract with 2.6 ml of 50 mM phosphate buffer (pH 7.0) and 400 µl 15 mM H_2O_2. The activity was expressed in µmol H_2O_2 .g^{-1} FM.min^{-1}. PPO activity was estimated after incubation of 0.5 ml of enzyme extract, 1 ml of 100 mM phosphate buffer (pH 6.0) and 1 ml of 100 mM catechol for 5 min at 25°C, reaction is stopped by adding 1 ml of 2.5 N H_2SO_4 (Velikova et al., 2000). The absorbance of the developed color was measured at 495 nm and PPO activity was expressed as µmol .g^{-1} FM.min^{-1}. POX activity was determined according to the modified method of Angelini et al. (1990). Absorbance was measured at 470 nm using spectrophotometer and activity is calculated as µmol tetraguaiacol.g^{-1} FM.min^{-1} using the extinction coefficient of tetraguaiacol. APX activity was assayed by measuring the decrease in absorbance at 290 nm and the activity was expressed µmol ascorbate.g^{-1} FM.min^{-1} (Nakano and Asada, 1981).

2.4.5. Estimation of non-enzymatic antioxidant compounds

Ethanolic extract was prepared by grinding 0.5 g of freeze-dried plant tissue in aliquot of ethanol (80%, v/v in water) followed by ultra-sonication at 35 °C for 60 min. Then, the homogenate is filtered and stored at 4 °C for further analysis. For estimation of phenolic content (TPC), 0.2 ml of supernatant was mixed with 2.5 ml of diluted Folin-Ciocalteau reagent and 0.2 ml of Na_2CO_3 (7.5 %). After 30 min incubation at 40 °C, absorbance was read at 760 nm and concentration was determined using standard curve as µg Gallic acid equivalent.gm^{-1} FM (Maurya and Singh, 2010). Flavonoids content was estimated as described by Zhishen et al. (1999) and expressed as µg quercetin equivalent.gm^{-1} FM. Alkaloids content was estimated following the method of Selvakumar et al. (2019).

2.4.6. Evaluation of the qualitative characteristics of the roots harvested at 180 days of age

The roots were washed well under tap water, then with distilled water and dried with a paper towel. After measuring the length, diameter and weight of the roots, they were transferred to the Delta Sugar Company laboratory in El-Hamoul, Kafr El-Sheikh Governorate to evaluate the qualitative characteristics of the roots. Polarization % (sugar %): A known weight of root slices was ground using an electric mixer, mixed with distilled water, filtered and adjusted to a known volume, and the sugar % was recorded using a saccharometer. The Brix % was recorded by transferring one drop of the ground root slices juice to the screen of a manual refractometer.

The purity ratio was determined using the following equation:

$$\text{Purity \%} = \text{Sugar \%} / \text{Brix \%} \times 100$$

Quality characteristics of yield including sucrose %, sodium, potassium and alpha amino nitrogen contents (Milliequivalent /100 g) were determined using the Analyzer-HG according to the AOAC method. Recoverable sugar percentage (corrected sugar %) = sugar % -0.029-0.343 (sodium + potassium) -0.094 (α- amino nitrogen)

2.5. Analysis of data

Data were expressed as mean ± standard error (SE) in figures and tables with 3 replicates except for root characteristics at 65 and 180 days of age where there were 5 replicates. One-way analysis of variance, least significant difference (LSD) was performed using IBM SPSS statistical software version 22 to compare the means of different treatments at $P \leq 0.05$.

3. Results

3.1. Characterization of nanoparticles

3.1.1. FT-IR spectra

Fig. 1a shows that the bands of SiO_2 NPs that appeared at 480 cm^{-1} are related to Si-O, while that at 3419 cm^{-1} is due to the effect of the atmosphere. The MgO NPs have a distinct peak at 448 cm^{-1}.

3.1.2. XRD analysis

Fig. 1b shows that the X-ray diffractogram (XRD) of MgO NPs has strong characteristic XRD patterns at 42.8° and 62°, also their characteristic patterns at 18.3°, 36.6°, 37.7°, 50.7°, 58.57°, 74.3°, and 78.6° (El-Shafai et al., 2021). SiO_2 NPs has characteristic patterns at 22°C (El-Wakeil et al., 2024). The grain size was calculated by the Scherrer equation, where the average diameter of SiO_2 NPs is 45 nm, and the diameter of MgO NPs is 33 nm

3.1.3. Zeta potential/particle size

The surface charge of synthesized nanoparticles was displayed *via* the zeta device at room temperature, the samples were dispersed in double-distilled water. Zeta potential values were related to the stability of the particles in the solution and related to the application fields utilized for nanomaterials (El-Shafai et al., 2023). Generally, zeta potential values have a range value related to the dispersion of the particles, where the moderate value of NPs is around ± 30 mV which refers to electrostatic stability and is a guideline to evaluate the stability of NPs, while the large values between ± 40 to ± 60 mV reflected good stability (El-Shafai et al., 2022). The samples were prepared freshly, where 5 mg was dispersed in double-distilled water, and five runs average was performed. The zeta values were recorded at -34 mV for SiO_2 NPs and + 14 mV for MgO NPs (Fig. 1c), the results referred to the stability of fabricated nanoparticles

3.1.4. Field emission scanning electron microscopy (FE-SEM)

The FE-SEM has been illustrated the micrograph shape of the fabricated SiO_2 and MgO NPs (Fig. 2), The shape of the fabricated SiO_2 and MgO NPs appeared a spherical shape with the pore surface on the SiO_2 NPs.

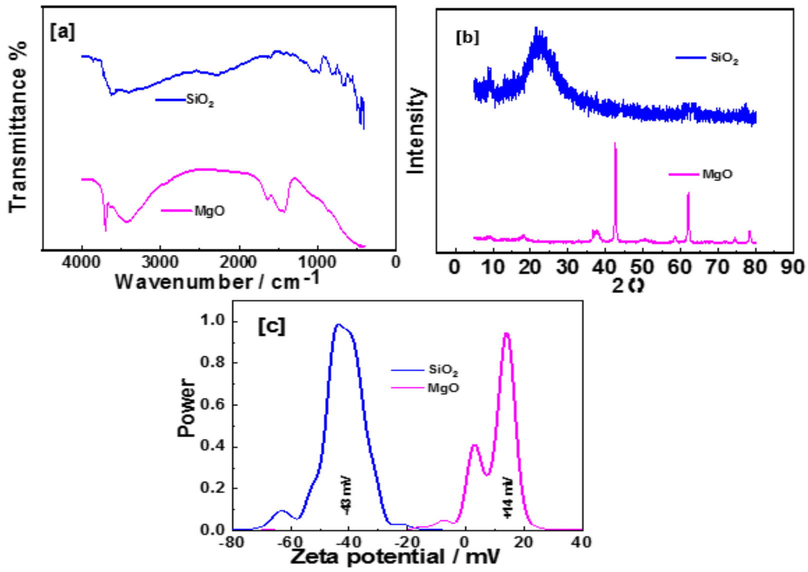

Fig. 1. FT-IR spectra (a), XRD patterns (b), and Zeta potential diagram of synthesized nanoparticles (c).

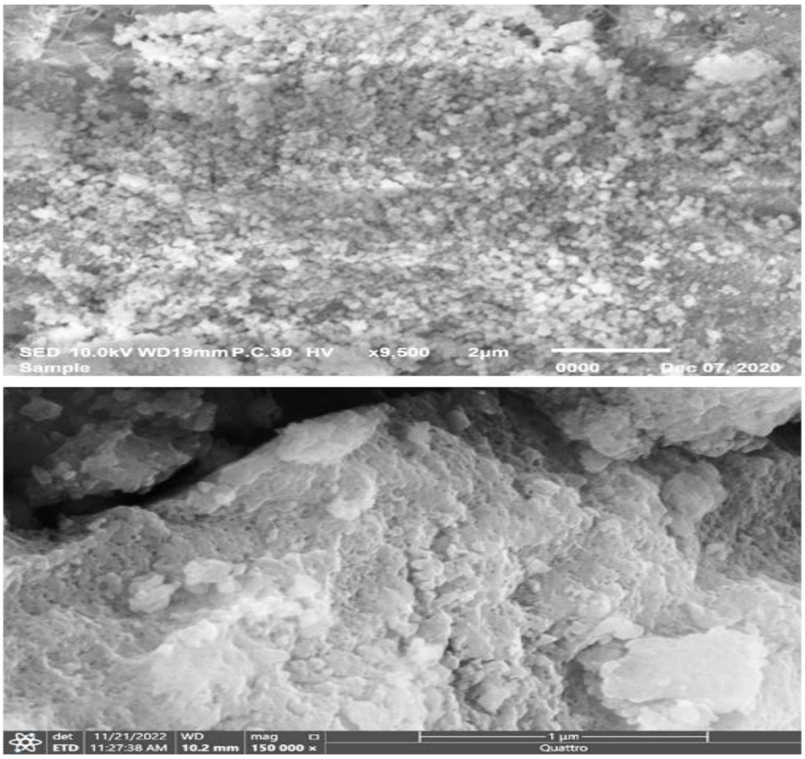

Fig. 2. FE-SEM micrograph of the synthesized nanoparticles: MgO NPs (Upper) and SiO_2 NPs (down).

3.2. Effect of NPs on growth and yield of sugar beet plants irrigated with wastewater

3.2.1. Effect of NPs on root characteristics

The variable significant or non-significant ($P \leq 0.05$) responses of root morphological characteristics of 65-day old sugar beet grown under wastewater stress to different concentrations of SiO_2, MgO and a mixture of SiO_2 + MgO NPs were showed in Fig. 3. The highest significant increases in root length and diameter were recorded in response to 50 $mg.L^{-1}$ SiO_2 + MgO NPs treatment, about 120 and 135.5 % greater than untreated plants, respectively. Conversely, high level 200 $mg.L^{-1}$ SiO_2 or MgO NPs significantly reduced root length by about 21.6 and 18.3 % and diameter by about 33.3 and 10.87 % less than control, respectively. The largest significant reduction in root FM from 488.3 to 248.5 $g.plant^{-1}$ was observed in 200 $mg.L^{-1}$ SiO_2 NPs treated plants. Regarding root DM, 50 $mg.L^{-1}$ MgO NPs treatment induced the largest increase of 277.2 % more than control. But 200 $mg.L^{-1}$ SiO_2 NPs resulted in a loss of 21.3 % than the control.

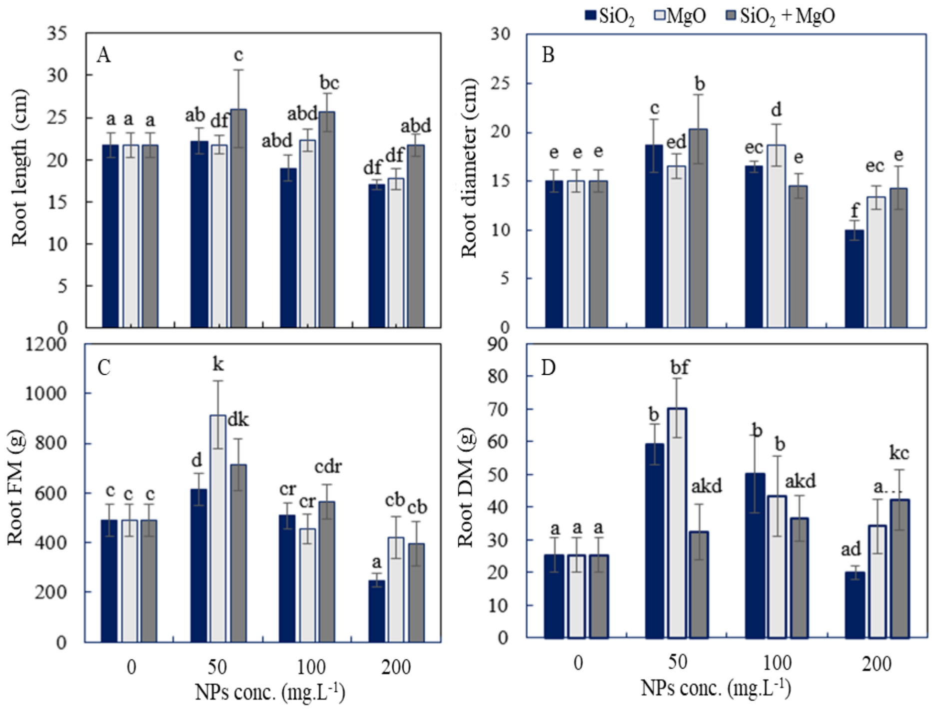

Fig. 3. Effect of various NPs (SiO_2, MgO and SiO_2 + MgO) at different doses on root growth parameters (length, A; diameter, B; FM, C and dry matter, D) of 65-day old sugar beet (*Beta vulgaris* L.) plants irrigated with wastewater. Values are the mean from five replicates and vertical bars represent standard errors. Different letters indicate the presence of significant differences (LSD test $p \leq 0.05$).

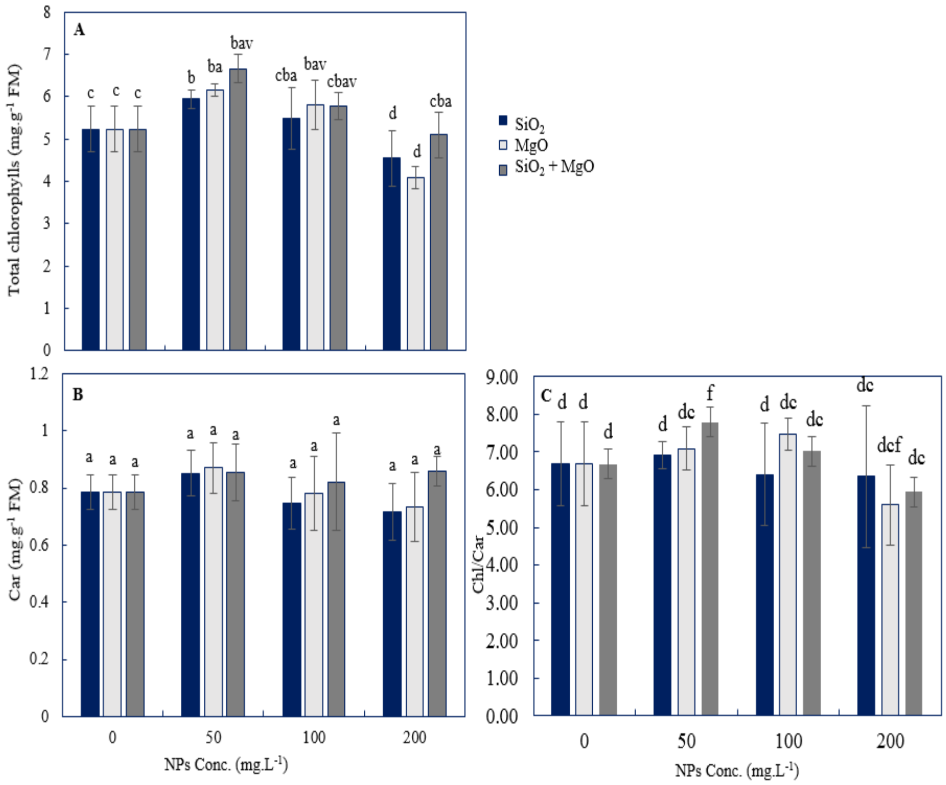

Fig. 4. Effect of various NPs (SiO_2, MgO and SiO_2 + MgO) at different doses on photosynthetic pigment pools (Total Chl, A; Car content, B and Chl/Car ratio) of 65-day old sugar beet (*Beta vulgaris* L) plants irrigated with wastewater. Values are the mean from three replicates and vertical bars represent standard errors. Different letters indicate the presence of significant differences (LSD test $p \leq 0.05$).

3.2.2. Effect of NPs on photosynthetic pools

The data displayed in Fig. 4 showed the variable significantly or insignificantly changes in contents of total chlorophylls (A), carotenoids (B) and the Chl/Car ratio (C) in response to different concentrations of NPs treatments in 65-day old sugar beet plants irrigated with wastewater. Low doses 50 $mg.L^{-1}$ of each of SiO_2, MgO and SiO_2 + MgO NPs stimulated significant increases in total Chl to about 113.6, 117.6 and 127.1% greater than control, receptively. High dose 200 $mg.L^{-1}$ SiO_2 and MgO NPs triggered significant decreases by about 13.2 and 21.8 % less than control, respectively. Treatment of sugar beet plants with 100 $mg.L^{-1}$ NPs increased total Chl non-significantly. Non-significant variable changes in leaf Car content were observed in response to different NPs treatments. The highest increases from 0.78 to 0.87 and 0.86 $mg.g^{-1}$ FM were observed in response to 50 $mg.L^{-1}$ MgO and SiO_2 + MgO NPs treatments. The Chl/Car ratio decreased or increased non-significantly in response to most applied NPs doses. It increased significantly from 6.68 to 7.79 in 50 $mg.L^{-1}$ SiO_2 + MgO NPs treated leaves. It showed the same trend of response of the total Chl and Car contents to 200 $mg.L^{-1}$ SiO_2 and MgO NPs treatments where it decreased non-significantly to 6.35 and 5.59, respectively.

3.2.3. Effect of NPs on glucose and sucrose contents

A significant or non-significant ($P \leq 0.05$) increases in glucose and sucrose levels in both leaves and roots of 65-day old sugar beet plants grown under wastewater stress were observed in response to different applied NPs (Table 2). It should be noted that although 200 $mg.L^{-1}$ SiO_2 + MgO NPs led to the highest significant increase in glucose content in leaves (6.85 $mg.g^{-1}$FM) compared to control (2.62 $mg.g^{-1}$FM), it led to lowest non-significant increase from 17.12 to 17.64 $mg.g^{-1}$ FM in roots. It was observed that different SiO_2 NPs doses significantly enhanced glucose accumulation in leaves and roots and the highest glucose levels in leaves and roots (4.44 and 25.30 $mg.g^{-1}$ FM, respectively) were recorded in response to 50 $mg.L^{-1}$ SiO_2 NPs treatment compared to control (2.6 and 17.1 $mg.g^{-1}$ FM). In addition, all doses of MgO NPs induced insignificant increases in glucose level in leaves and significant increases in root compared to control and in relative to each other. Sucrose is the main transport sugar and has a vital regulatory role in carbon partitioning and thus plant growth and productivity. There was a significant increase in the level of sucrose in leaves treated with 100 $mg.L^{-1}$ SiO_2 + MgO NPs, amounting to about 164 % greater than untreated plants. The best improvement sucrose accumulation was in the roots of plants treated with 50 $mg.L^{-1}$ SiO_2 NPs, as it increased to 35.0 $mg.g^{-1}$ FM compared to 27.2 $mg.g^{-1}$ FM in the control.

3.2.4. Effect of NPs on H_2O_2 and MDA levels

Membrane oxidative damage assessment revealed variable changes in both H_2O_2 stress index and MDA levels in response to various NPs applied (Table 3). The highest significant decrease in H_2O_2 level was recorded in leaves and roots of 65-day old sugar beet plants irrigated with wastewater and treated with 100 and 200 $mg.L^{-1}$ SiO_2 + MgO NPs to about 52.9 and 47.6 % less than in leaves and root of control plants, respectively. Nanoparticles treatments can be applied to mitigate harmful changes in morphology, biochemical and physiological activities, accumulation of ROS, membrane damage, and nutritional imbalance in stressed plants. Although 200 $mg.L^{-1}$ SiO_2 resulted in the highest increase in H_2O_2 levels in leaves and roots by about 112% and 150% greater than untreated plants, it caused a decline in MDA content by about 37 and 37 % lesser than control, respectively. It was noted that MDA increased non-significantly only in root of plants treated with 200 and 50 $mg.L^{-1}$ of MgO NPs and SiO_2 + MgO NPs from 1.02 to 1.32 and 1.06 $\mu mol.g^{-1}$ FM, respectively.

3.2.5. Effect of various NPs on non-enzymatic antioxidants

The results in table 4 showed the response of antioxidant activities (phenolic, flavonoids and alkaloids) to different NPs applied to the leaves and roots of 65-day old sugar beet plants irrigated with wastewater. The Results showed that the treatments of 50 $mg.L^{-1}$ of MgO and of SiO_2 NPs increased the content of phenolic compounds in leaves by about 244 and 242 % more than the control, respectively. Insignificant decrease in the phenolic content from 3.4 to 3.1 µg GAE gm^{-1} FM was also recorded in leaves treated with 200 $mg.L^{-1}$ SiO_2 + MgO NPs. All NPs applied at different concentrations led to a significant increase in phenolic content in roots and the flavonoids content in leaves and roots. However, the highest significant increase in phenolic was found in roots treated with 50 $mg.L^{-1}$ SiO_2 NPs by about 206 % greater than control. A gradual decrease in flavonoids content in leaves and roots was observed with increasing NPs concentrations, but the highest increases were triggered by SiO_2 NPs application. Flavonoids increased by about 217 % in leaves treated with 100 $mg.L^{-1}$ and by about 207 % in roots treated with 50 $mg.L^{-1}$ than untreated, respectively. It was observed that SiO_2 NPs treatments resulted in greater improvement in flavonoids more than SiO_2 + MgO NPs treatments than MgO NPs treatments. Treatment of 65-day sugar plants with different concentrations of SiO_2 or MgO NPs led to non-significant decreases in the leaf alkaloid content but the highest decreases from 0.12 to 0.08 and 0.09 µg $AE.g^{-1}$ FM were recorded in plants treated at 50 and 100 $mg.L^{-1}$, respectively. On the other hand combination between SiO_2 + MgO NPs at 100 and 200 $mg.L^{-1}$ resulted in non-significant increase about 118 and 102 % compared with control, respectively. Except for 200 $mg.L^{-1}$ SiO_2 and SiO_2 + MgO NPs treatments, all other treatments resulted in a decrease in roots alkaloid content in roots. The greatest decrease by about 31.8 % compared to control was found in plants treated by 50 $mg.L^{-1}$ MgO NPs and the highest increase was in 200 $mg.L^{-1}$ SiO_2 and SiO_2 + MgO NPs treated roots by about 188 % and 106 %, respectively compared to untreated. That might be reflect an antagonized effect between SiO_2 and MgO NPs.

Table 2. Effect of various NPs (SiO_2, MgO and SiO_2 + MgO) at different doses on sucrose and glucose levels in leaves and roots of 65-day old sugar beet (*Beta vulgaris* L) plants irrigated with wastewater. Values are the mean ± SE (n = 3).

NPs Conc. ($mg.L^{-1}$)		Sucrose content($mg. g^{-1}$ FM)		Glucose content ($mg. g^{-1}$ FM)	
		Leaves	Root	Leaves	Root
Control	0.0	10.4±0.9^{d}	27.2±2.4^{c}	2.62±0.3^{f}	17.1±0.7^{a}
SiO_2	50	11.2±0.7^{dc}	35.0±4.9^{b}	4.44±0.7^{a}	25.3±1.4^{b}
	100	11.2±0.9^{dc}	29.8±3.7^{cb}	3.80±0.4^{ae}	23.8±1.6^{bc}
	200	12.3±0.7^{dc}	26.3±1.6^{ce}	3.92±0.4^{ae}	23.2±1.1^{bce}
MgO	50	10.5±0.8^{dcr}	26.2±1.9^{ce}	3.85±0.3^{ae}	22.3±0.9^{ce}
	100	13.8±1.1^{cj}	27.4±2.1^{ce}	3.64±0.2^{aef}	21.3±0.6^{ced}
	200	16.1±1.0^{e}	26.2±1.75^{ce}	3.47±0.4^{aef}	20.2±0.9^{edf}
SiO_2 + MgO	50	16.9±0.8^{e}	28.2±3.1^{ce}	3.05±0.2^{fed}	19.2±0.7^{adfk}
	100	17.0±2.2^{e}	29.5±2.3^{cbe}	2.94±0.2^{fed}	18.1±1.1^{afk}
	200	14.0±0.8^{cje}	28.8±1.9^{cbe}	6.85±0.4^{b}	17.6±0.9^{afk}

Different letters in the same column indicate significant difference at $p \leq 0.05$ LSD

Table 3. Effect of various NPs (SiO_2, MgO and SiO_2 + MgO) at different doses on H_2O_2 and MDA levels in leaves and roots of 65-day old sugar beet (*Beta vulgaris* L) plants irrigated with wastewater. Values are the mean ± SE (n = 3).

NPs Conc. ($mg.L^{-1}$)		H_2O_2 level (µmol. g^{-1} FM)		MDA content (µmol. g^{-1} FM)	
		Leaves	Root	Leaves	Root
Control	0.0	4.52±0.39^{a}	2.88±0.28^{x}	0.89±0.09^{b}	1.02±0.12^{a}
SiO_2	50	2.99±0.30^{b}	2.74±0.29^{xa}	0.64±0.02^{b}	0.73±0.04^{ab}
	100	2.97±0.25^{b}	3.76±0.66^{k}	0.61±0.02^{b}	0.72±0.01^{ab}
	200	5.05±0.35^{a}	4.33±0.36^{k}	0.56±0.04^{b}	0.64±0.02^{b}
MgO	50	2.44±0.27^{be}	2.72±0.25^{xa}	0.76±0.003^{b}	0.87±0.04^{abc}
	100	3.11±0.23^{bej}	2.78±0.20^{xa}	0.57±0.03^{b}	0.85±0.06^{abc}
	200	3.90±0.35^{a}	2.63±0.20^{xa}	0.78±0.06^{b}	1.32±0.13^{a}
SiO_2 + MgO	50	4.08±0.08^{a}	1.87±0.17^{ae}	0.88±0.18^{b}	1.06±0.16^{abc}
	100	2.13±0.25^{en}	1.65±0.14^{eh}	0.84±0.29^{b}	1.00±0.18^{abc}
	200	2.47±0.25^{bejn}	1.51±0.12^{eh}	0.82±0.23^{b}	0.98±0.17^{abc}

Different letters in the same column indicate significant difference at $p \leq 0.05$ LSD

Table 4. Effect of various NPs (SiO_2, MgO and SiO_2 + MgO) at different doses on the antioxidants (phenolic, A; flavonoids, B and alkaloids, C) contents in leaves and roots of 65-day old sugar beet (*Beta vulgaris* L) plants irrigated with wastewater. Values are the mean ± SE (n = 3).

NPs Conc. (mg.L-1)		Phenolic (µg GAE gm^{-1} FM)		Flavonoids (µg QE. gm^{-1} FM)		Alkaloids (µg AE.g^{-1} FM)	
		leaves	Root	Leaves	Root	Leaves	Root
Control	0.0	$3.4±0.6^{a}$	$7.8±0.5^{w}$	$1.0±0.03^{z}$	$1.3±0.2^{g}$	$0.12±0.010^{b}$	$0.11±0.004^{a}$
SiO_2	50	$8.3±0.9^{m}$	$15.7±1.1^{k}$	$2.1±0.2^{k}$	$2.7±0.4^{h}$	$0.08±0.011^{b}$	$0.08±0.009^{c}$
	100	$3.7±0.2^{a}$	$13.3±2.0^{k}$	$2.1±0.3^{k}$	$2.3±0.2^{ha}$	$0.09±0.003^{b}$	$0.08±0.008^{c}$
	200	$3.7±0.5^{a}$	$9.7±0.4^{m}$	$2.1±0.2^{k}$	$2.2±0.2^{ha}$	$0.10±0.002^{b}$	$0.20±0.115^{b}$
MgO	50	$8.4±0.8^{m}$	$9.3±0.9^{m}$	$1.5±0.1^{p}$	$1.8±0.1^{ab}$	$0.10±0.006^{b}$	$0.07±0.011^{c}$
	100	$6.5±0.6^{p}$	$11.3±0.6^{m}$	$1.3±0.1^{zpj}$	$1.6±0.2^{gb}$	$0.11±0.004^{b}$	$0.08±0.009^{c}$
	200	$3.7±0.5^{a}$	$9.23±0.5^{m}$	$1.2±0.03^{zpjs}$	$1.43±0.1^{gb}$	$0.10±0.011^{b}$	$0.10±0.002^{acf}$
SiO_2 + MgO	50	$3.7±0.3^{a}$	$10.1±1.1^{m}$	$1.9±0.2^{kpr}$	$2.6±0.2^{ha}$	$0.11±0.005^{b}$	$0.09±0.011^{acf}$
	100	$3.7±0.4^{a}$	$12.7±0.5^{n}$	$1.7±0.1^{kpjr}$	$2.1±0.1^{hab}$	$0.14±0.019^{b}$	$0.07±0.031^{c}$
	200	$3.1±0.3^{a}$	$9.9±0.2^{m}$	$1.5±0.1^{pjsr}$	$2.1±0.1^{ab}$	$0.12±0.008^{b}$	$0.11±0.018^{af}$

Different letters in the same column indicate significant difference at $p \leq 0.05$ LSD

3.2.6. Effect of NPs on activity of antioxidant enzymes (CAT, POX, PPO and APX)

The data in table 5 showed that all various doses of different NPs applied enhanced the activity of the antioxidant enzymes under study compared to untreated plants. It was observes that low doses of NPs achieved the highest antioxidant enzyme activity compared to high doses of NPs- treated plants on the one hand and with untreated plants on the other hand. It was noticed that highest antioxidant enzymes activity reached in 50 and 100 mg.L^{-1} of SiO_2 NPs treated plants compared to the same levels of MgO or mixture of SiO_2 + MgO NPs.

The results showed that 50 mg.L^{-1} of SiO_2 NPs was the best in enhancement the CAT, POX, PPO and APX activities in leaves by about 151.7, 139.1, 175.9 and 213.9 % greater than untreated leaves. The same trend in roots was found, where CAT, POX, PPO and APX activity significantly increased in response to 50 mg.L^{-1} SiO_2 NPs treatment by about 203, 164, 134 and 190 % more than untreated plants. The activity of enzymes under study was noticed to decrease with increasing the NPs concentration but still greater than control except for 200 mg.L^{-1} SiO_2 + MgO NPs which insignificantly reduced the PPO activity in leaves from 10.32 in untreated to 10.07 µmol .g^{-1} FM.min^{-1}. However, 50 mg.L^{-1} MgO NPs stimulated the activity of CAT, POX, PPO and APX activity in leaves by 134.0, 124.7, 157.6 and 166. % and in roots by 175.8, 143.1, 121.9 and 145.0 % more than control group. The least effective treatment in enhancing the activity of enzymes under study was 200 mg.L^{-1} SiO_2 + MgO NPs, which increased the activity of CAT, POX and APX in leaves by 111.7, 100.4, 114.6 % and increased the activity of CAT, POX, PPO and APX in roots by116, 104.8, 101.7 and 124.8 % more than the control, respectively.

3.2.7. Effect of NPs on root traits of 180-day old sugar beet plants

Figure 5 showed that various doses of NPs significantly or non-significantly improved root quality of 180-day old sugar beet plants grown under wastewater conditions. Regarding SiO_2 NPs treatments, 100 mg.L^{-1} of SiO_2 NPs caused the highest increase in root length, diameter and weight to 28.7 cm, 26.3 cm and 1410 g compared to 25.3 cm, 20 cm and 1013 g in the control group, respectively. Regarding MgO NPs treatments, the highest increase in length (35.3 cm) was in response to 200 mg. L^{-1}, in diameter (28.7 cm) was in response to 100 mg. L^{-1} and in weight (2136 g) was in response to 50 mg. L^{-1} that matches the highest increase in total chl and Car. It was observed that the 200 mg. L^{-1} SiO_2 + MgO NPs treatment caused the best improvement in root length and weight (40.8 cm and 2267 g) for both SiO_2, MgO NPs and control treatments.

Table 5. Effect of various NPs (SiO_2, MgO and SiO_2 + MgO) at different doses on the activity of the antioxidant enzymes (CAT, POX, PPO and APX) in leaves and roots of 65-day old sugar beet (*Beta vulgaris L*) plants irrigated with wastewater. Values are the mean ± SE (n = 3).

NPs Conc. (mg.L-1)		Enzyme activity (µmol .g^{-1} FM.min^{-1})			
		CAT	POX	PPO	APX
Leaves					
Control	0.0	13.3±1.2^{b}	7.9±0.4^{f}	10.32±1.00^{f}	1.51±0.3^{h}
SiO_2	50	20.2±1.5^{d}	11.0±0.9^{d}	18.15±1.0^{a}	3.23±0.1^{b}
	100	19.1±1.4^{de}	10.6±0.5^{de}	17.91±1.0^{ae}	2.81±0.3^{bc}
	200	14.5±0.1^{c}	9.6±0.4^{eb}	17.16±0.8^{aec}	2.25±0.2^{cf}
MgO	50	17.8±1.1^{deca}	9.9±0.4^{debc}	16.26±1.3^{ecb}	2.51±0.2^{cf}
	100	16.3±0.6^{caf}	9.4±0.3^{ebc}	15.04±0.6^{cbd}	2.45±0.2^{cf}
	200	14.2±0.8^{bcf}	8.8±0.4^{fbc}	14.18±0.7^{bdg}	2.17±0.2^{fk}
SiO_2 + MgO	50	17.3±1.1^{decaf}	8.7±0.4^{fbcj}	14.26±0.5^{bdg}	1.77±0.2^{hkn}
	100	18.4±0.9^{decaf}	8.6±0.3^{fbji}	13.24±0.5^{gm}	1.67±0.1^{hkn}
	200	14.8±0.4^{bcf}	8.0±1.0^{fji}	10.07±0.6^{f}	1.73±0.2^{hkn}
Roots					
Control	0.0	11.33±0.7^{j}	5.68±0.4^{u}	8.77±0.7^{u}	1.29±0.18^{c}
SiO_2	50	23.03±1.0^{b}	9.3±0.5^{w}	11.79±0.6^{b}	2.45±0.4^{a}
	100	22.2±1.4^{bd}	8.8±0.4w	11.24±0.9^{bc}	2.13±0.2^{d}
	200	13.0±0.9^{da}	8.4±0.5^{wv}	10.67±0.6^{bce}	1.68±0.2^{de}
MgO	50	19.9±1.2^{ae}	8.1±0.5^{wz}	10.69±0.8^{bced}	1.87±0.1^{de}
	100	19.2±1.4^{ae}	8.6±0.6^{70wz}	10.22±0.7^{cedj}	1.84±0.1^{deb}
	200	18.4±1.4^{aec}	7.6±0.3^{vzs}	9.61±0.8^{uedj}	1.67±0.2^{eb}
SiO_2 +MgO	50	17.6±0.4^{ecw}	7.1±0.5^{vzsq}	9.64±0.5^{uedjn}	1.51±0.2^{ceb}
	100	16.3±0.4^{cwv}	6.2±0.3^{uq}	9.28±0.7^{uejn}	1.53±0.1^{ceb}
	200	13.1±0.5^{v}	6.0±0.2^{uq}	8.92±0.8^{ujn}	1.61±0.2^{ceb}

Different letters in the same column indicate significant difference at $p \leq 0.05$ LSD

3.2.8. Effect of NPs on yield root quality of 180-day old sugar beet plants

Data in table 6 revealed a variable significant or non-significant responses of the yield quality of 180-day old sugar beet plants irrigated with wastewater to long-term effect of different NPs. All NPs treatments improved the percentage of sucrose, TSS, and juice purity. The highly significant increases in percentage of sucrose, TSS, and juice purity were recorded in SiO_2 NPs treated sugar beet plants followed by MgO NPs and SiO_2 + MgO NPS treated plants compared to untreated plants, respectively. The maximum increases were boosted by 200 mg.L^{-1} SiO_2 NPs where percentage of sucrose increased to about 150, TSS to about 127 and juice purity to about 109 of the respective values of control plants. However, the highly significant increases in SR and Brix percentage were 14.1 and 20.1 % in 50 mg.L^{-1} of SiO_2 + MgO treated plants compared to 10.3 and 16.7 % in case of control plants.

High levels of amino N, Na^+ and K^+ in roots result in their accumulation in molasses and reduce sucrose crystallization that affect the efficiency of sucrose extraction and quality. The different NPs treatments at different doses triggered a decrease in K, Na and α-amino N impurities in roots of sugar beet plants grown under wastewater stress condition except Na content of 200 mg.L^{-1} SiO_2 NPs treated plants increased. These decreases might be improved the sugar quality production. The highest decrease in K and Na levels from about 72.6 and 22.9 in untreated root pasta to about 68.3 and 17.1 mmol.kg^{-1} root pasta of 50 mg.L^{-1} SiO_2 + MgO NPs treated sugar beet plants and α-amino N decreased significantly ($p \leq 0.05$) from about 19.4 in control untreated plants to about 12.3 mmol.kg^{-1} root pasta of 200 mg.L^{-1} SiO_2 NPs treated plants.

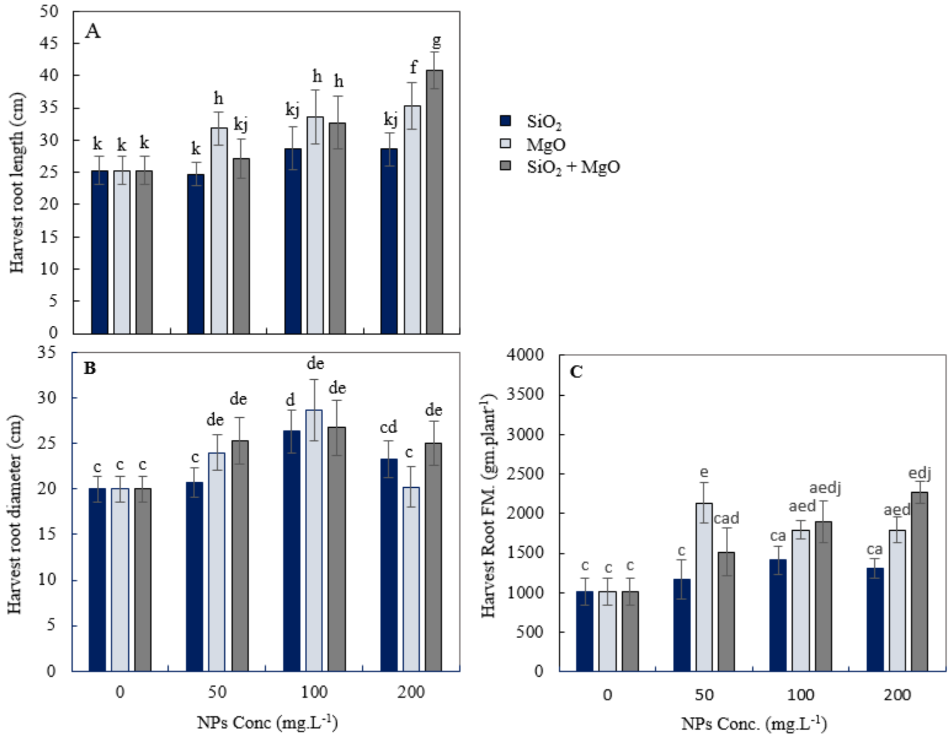

Fig. 5. Effect of various NPs (SiO_2, MgO and SiO_2 + MgO) at different doses on root characteristics (length, A; diameter, B and FM, C) of 180-day old sugar beet (*Beta vulgaris* L.) plants irrigated with wastewater. Values are the mean from five replicates and vertical bars represent standard errors. Different letters indicate the presence of significant differences (LSD test p ≤ 0.05).

Table 6. Effect of various NPs (SiO_2, MgO and SiO_2 + MgO) at different doses on yield quality traits of 180-day old sugar beet (*Beta vulgaris* L.) plants irrigated with wastewater. Values are the mean ± SE (n = 5).

NPs Conc. ($mg.L^{-1}$)		Sucrose %	TSS %	K content	Na content	α-amino	Juice Purity%	SR %	Brix %
Control	0.0	15.8^{f}	18.3^{e}	72.6^{c}	22.9^{a}	19.4^{b}	$79.3.^{8c}$	10.3^{b}	16.7^{c}
SiO_2	50	22.1^{a}	22.5^{f}	71.4^{cd}	21.9^{a}	16.2^{a}	84.9^{a}	10.1^{b}	16.3^{c}
	100	23.1^{a}	22.8^{f}	71.7^{cd}	22.5^{ba}	15.5^{a}	85.3^{a}	9.2^{b}	15.2^{c}
	200	23.7^{a}	23.2^{f}	72.4^{cd}	23.4^{ba}	12.3^{e}	86.2^{a}	9.2^{b}	14.8^{c}
	Mean	22.9	22.8	71.5	22.9	14.67	85.47	9.5	15.4
MgO	50	19.9^{b}	20.2^{d}	70.3^{cd}	$19.7.5^{c}$	17.3^{a}	83.7^{a}	12.1^{ab}	18.8^{d}
	100	20.5^{b}	21.0^{d}	70.8^{cd}	20.3^{ac}	17.2^{a}	84.3^{a}	11.6^{b}	18.5^{dc}
	200	21.5^{a}	21.3^{d}	71.2^{cd}	21.3^{ac}	16.3^{ac}	84.8^{a}	10.5^{b}	17.2^{dc}
	Mean	20.6	20.8	70.8	20.4	20.3	84.3	11.4	18.2
SiO_2 + MgO	50	17.2^{c}	18.6^{e}	68.3^{d}	17.1^{d}	19.2^{b}	81.4^{c}	14.1^{a}	20.1^{d}
	100	18.6^{b}	19.3^{de}	69.4^{d}	$18. 5^{cd}$	18.5^{b}	82.6^{c}	13.2^{a}	19.2^{d}
	200	19.2^{b}	19.9^{de}	69.7^{d}	19.1^{cd}	18.1^{b}	83.0^{c}	12.8^{a}	19.0^{d}
	Mean	18.3	19.3	69.1	18.2	18.6	82.3	13.4	19.4

K, Na and α-amino N are expressed as $mmol.Kg^{-1}$ root pasta
Different letters in the same column indicate significant difference at $p \leq 0.05$ LSD

4. Discussion

Plants use strategies such as structural defense and antioxidants including phenolics, flavonoids, and alkaloids (Sachdev et al., 2021). Enhancing the activity of antioxidant enzymes through the application of nanoparticles counterbalances the excess accumulation of ROS, thereby improving the growth and productivity of stressed plants. We applied SiO_2 and MgO NPs to enhance enzymatic (CAT, APX, POX, and PPO) and non-enzymatic (phenolic compounds and flavonoids) antioxidants with the aim of reducing ROS accumulation and minimizing membrane damage in sugar beet plants grown under wastewater stress. The highest significant increase in root length, diameter and total Chl of 65-day old sugar beet plants were in response to 50 $mg.L^{-1}$ SiO_2 + MgO NPs treatment, about 120 and 135.5 and 127.1 % greater than untreated plants, respectively. But 200 $mg.L^{-1}$ SiO_2 NPs significantly reduced root length by about 21.6 and diameter by about 33.3 and total Chl by about 13.2 % less than control that led to loss in root FM by about 49.1 %. Recently, Hao et al. (2023) showed that low concentrations of SiO_2 NPs enhanced the RWC of maize seedlings, while high concentrations impaired it. In the same regard, Khan et al. (2016) observed that the germination and growth parameters of *Silybus marianum* were enhanced by Ag NPs at 30 mg/L but inhibited at higher levels.

Similarly, Jayarambu et al. (2016) reported that 50, 100 and 150 mg. L^{-1} of MgO NPs significantly increased the chlorophyll content in 20-day-old maize plants but higher concentrations caused a gradual decrease in chlorophyll. In this study, the Chl/Car increased significantly from 6.68 to 7.79 in 50 $mg.L^{-1}$ SiO_2 + MgO NPs treated leaves and decreased to 6.35 and 5.59 in response to 200 $mg.L^{-1}$ SiO_2 and MgO NPs respectively. In agreement, Kashyap and Siddiqui (2022) found that low rate (100 mg/L) of SiO_2 NPs increased the growth parameters including length, fresh mass, dry mass and number of nodules with enhanced chlorophyll, carotenoids and proline contents in pea (*Pisum sativum* L.) plants. In addition, Iqbal et al. (2019) showed that treatment of heat-stressed wheat plants with silver nanoparticles improved the length and mass of fresh and dry roots and shoots, which could be attributed to the enhancement of photosynthetic machinery. Also, El-Sonbaty (2025) found that application of nano potassium silicate led to a highest increase in photosynthetic pigments and nutrients maize leaves compared to composite treatment.

The highest decreases in H_2O_2 level were induced by SiO_2 + MgO NPs in leaves at 100 $mg.L^{-1}$ and in roots at 200 $mg.L^{-1}$ by about 52.87 % and 47.57 % less than control. However, nanoparticle treatments can be applied to alleviate the detrimental changes in morphology, biochemical and physiological activities, accumulation of reactive oxygen species, membrane damage and nutritional imbalance in stressed plants (Ashkavand et al., 2015). We found that 200 $mg.L^{-1}$ SiO_2 caused the caused a decrease in MDA content in leaves and roots by about 37.08% and 37.25% less than the control group, respectively. In this regard, Garcia-Lopez et al. (2019) reported that foliar application of single or multiple nanoparticles enhanced plant tolerance and stress resistance compared to soil amendment by stimulating and improving fertilizer uptake and assimilation. Lu et al. (2020) previously suggested that foliar application of NPs at high levels interferes with DNA, RNA, phosphorylation, membrane integrity and ATP synthesis, and inhibits photosynthesis due to damage to the chloroplast membrane and inhibition of gas exchange capacity.

However, in roots 50 $mg.L^{-1}$ SiO_2 NPs produce the best enhanced level of phenolic by 201.5 and flavonoids by 206.9 % and the best improvement in CAT, POX, PPO and APX activities by about 203.3, 164.1, 134.4 and 189.9 % and in leaves by about 151.7, 139.1, 175.9 and 213.9 % greater than that in control, respectively. Similar findings, Si NPs enhanced the antioxidant defense system in maize plants by increasing the activities of SOD, APO, DHAR and GR in arsenic-stressed leaves (Tripathi et al., 2016) and increasing the accumulation level of phenolics and organic acids in aluminum-stressed roots (Sousa et al., 2019). Our study found that SiO_2 NPs treatments resulted in greater improvement in flavonoids more than SiO_2 + MgO NPs more than MgO NPs treatments. Namjoyan et al. (2020) demonstrated that low concentration of Si NPs enhanced photosynthetic activity, non-enzymatic antioxidants namely, quercetin, rutin and glycine and enzymatic antioxidants (SOD, GPX and CAT) beside decreased level each of H_2O_2 and MDA. Except 200 $mg.L^{-1}$ SiO_2 and SiO_2 + MgO NPs treatments, all other treatments resulted in a decrease in alkaloid content in roots. The 200 $mg.L^{-1}$ SiO_2 and SiO_2 + MgO NPs treatments increased alkaloid content by 187.9 % and 105.6 %, respectively compared to untreated. That might be revealed an antagonized effect between SiO_2 and MgO NPs. Foliar application of Ag NPs resulted in an increase in alkaloid contents in *Borago officinalis* (Seifsahandi and Sorooshzadeh, 2013).

In agreement, treatment of *Daucu carota* plants grown under lead stress with MgO NPs resulted in increasing the activities of CAT and SOD enzymes by 32% and 29%, respectively, compared to untreated control plants (Khan et al., 2019). However, Wang *et al.* (2016) showed that application of different concentrations of ZnO NPs treated ROS toxicity in *Gossypium hirsutum* with increased POX and SOD activities accompanied by decreased MDA content. In this study, the least effective treatment in enhancing the activity of enzymes under study was 200 $mg.L^{-1}$ SiO_2 + MgO NPs. The use of nanofertilizers has been promised for their improving effect on seed germination, photosynthesis and antioxidant system activity (Faizan et al., 2023).

This study found that 50 $mg.L^{-1}$ SiO_2 NPs induced the highest glucose level in roots by about 148 %, 200 $mg.L^{-1}$ SiO_2 + MgO NPs induced the highest increase in glucose accumulation in leaves about 261 %, 100 $mg.L^{-1}$ SiO_2

+ MgO NPs induced the highest accumulation of sucrose in leaves amounting to about 164 % and 200 mg.L^{-1} SiO_2 NPs induced the highest content of sucrose in roots by about 128.6 % compared to 65-day old untreated sugar beet plants (Table 2). It was reported that the increased level of accumulation of sucrose and reducing sugars in various organs of sugar beet gradually affects the sugar metabolism process and enzymes activities (Ali, 2018). In addition, Zhu et al. (2018) recorded a decrease in sucrose degrading enzymes activity at maturation matched with a reduction in their transcript of their encoding genes beside a decrease in activity of sucrose phosphate synthase although of its high transcript. These and our results could be attributed the increase levels of sucrose and glucose accumulation in NPs treated leaves and roots of sugar beet plants to the enhancement of chlorophyll content and thus the improvement of photosynthetic efficiency and a decline in sucrose degradation.

Root characteristics of 180-day old sugar beet plants grown under wastewater conditions were improved significantly or non-significantly by applying SiO_2 and MgO NPs (Fig. 5). Applying of 200 mg. L^{-1} SiO_2 + MgO NPs treatment caused the best improvement in root length and weight (40.8 cm and 2267 g). Also, Gautam et al. (2023) reported an increase in photosynthetic pigment content of mustard plants exposed to MgO NPs resulting in increased biomass and carbohydrates along with increased total grain yield. Analysis of root yield quality revealed that application of 200 mg.L^{-1} SiO_2 NPs induced the best percentage of sucrose (150 %), TSS (127 %), and juice purity (109 %) of the respective values of control plants (Table 6). However, 50 mg.L^{-1} of SiO_2 + MgO NPs induced highly significant increases in SR and Brix percentage from 14.1 and 20.1 % to 10.3 and 16.7 % in control plants. In agreement, Jakiene et al. (2015) have shown that the application of nanoparticles improved photosynthetic productivity, root yield, sucrose content, total soluble solids and juice purity in sugar beet plants.

High levels of amino N, Na^+ and K^+ in roots result in their accumulation in molasses and reduce sucrose crystallization that affect the efficiency of sucrose extraction and quality. In this study, different NPs at different doses treatments triggered a decrease in K, Na and α-amino N impurities in roots of sugar beet plants grown under wastewater stress condition except Na content of 200 mg.L^{-1} SiO_2 NPs treated plants increased. However, Artyszak et al. (2021) reported that the increase in the pure sugar yield after silicon foliar application under water deficit stress is due to increase in their function efficiency not is a result of chemical composition changes.

5. Conclusion

In summary, wastewater stress led to accumulation of ROS followed by high MDA content which decreased photosynthetic pigments, root characteristics and activities of antioxidant systems and hence yield and quality. Application of nanoparticles (SiO_2, MgO and SiO_2 + MgO) at lower rate (50 mg.L^{-1}) in short term effect was best in reducing wastewater induced oxidative stress by increasing in non-enzymatic and enzymatic antioxidants activities. MgO NPs were best in improving Chl content, FM and DM. But SiO_2 NPs was the most effective in improving antioxidant defense and ameliorated the oxidative stress. In long term effect, SiO_2 and SiO_2 + MgO NPs improved yield quality of 180-day old sugar beet plants which may be attributed to the improvement of photosynthesis efficiency by MgO NPs, the reduction of oxidative stress by SiO_2 NPs, and the increase in sucrose transport from source leaves to roots. It was observed that there were different responses of leaves and roots to different NPs and also antagonized effect between SiO_2 + MgO NPs in some effects and synergistic effect in other. Further study is still needed to understand the interaction between SiO_2 and MgO NPs in combined form as well as their interference with wastewater stress. The Increase of root yield and sugar yield caused by foliar applications by nanoparticles could be attributed to the stimulating effect on photosynthesis process in plant such as translocation of sucrose from the top to root.

List of abbreviations:

FM: fresh matter

DM: dry matter

Chl: chlorophylls

Car: carotenoids

Declarations

Ethics approval and consent to participate

Consent for publication: The article contains no such material that may be unlawful, defamatory, or which would, if published, in any way whatsoever, violate the terms and conditions as laid down in the agreement.

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