

Biochemical attributes *Portulaca oleracea* as affected by manganese oxide and magnesium oxide nanoparticles

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Abstract

Due to the limitation of freshwater resources and quality loss of soils, the use of halophyte plants is inevitable. Halophyte species such as purslane (*Portulaca oleracea*) are one of the important and rich sources of different metabolites, however, very few studies have been done in this regard. The application of metal nanoparticles in improving plant growth characteristics and also knowing the threshold of using these nanoparticles can lead to plant growth and development. Therefore, the present study is aimed at investigating the effect of manganese oxide (Mn₂O₃) and magnesium oxide (MgO) nanoparticles (NPs) on biochemical attributes and plant growth of purslane in a controlled condition based on a balanced completely random design. The treatments included Mg and Mn nanoparticles in three concentrations: 10, 100, and 500 mg L⁻¹, bulk metal Mg and Mn in three concentrations of 10, 100, and 500 mg L⁻¹, and a control treatment. The results showed that nano treatments had a significant effect on the concentration of phenol, flavonoid, anthocyanin, quercetin, half-maximal inhibitory concentration (IC₅₀), total fresh weight, and total dry weight. Except for quercetin, the results indicated a positive effect of Mg and Mn, especially in the form of nanoparticles, on the quality and quantity of purslane plants. Increasing the concentration of Mg and Mn metals in nanoparticle form did not increase IC₅₀, however, it was associated with an increase in this index in bulk form, which indicates a decrease in the negative effect of metals in nanoparticle form. Considering the salinization of water and soil resources and the importance of preventing environmental pollution, the use of Mg and Mn nanoparticles on halophyte species is very important; which requires more research.

Keywords: anthocyanin; flavonoid; halophytes; nanoparticles; phenol

Introduction

Purslane (*Portulaca oleracea*) is an important plant with multiple benefits that is a rich source of various phytochemicals, including omega-3 fatty acids, which are not commonly found in terrestrial plants (Kumar *et al.*, 2022). Purslane has been traditionally used as a nutritious and ethnomedicinal food across the globe, which

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has been found to have anti-inflammatory, antidiabetic, antioxidant, and neuroprotective properties, among others (Aini *et al.*, 2022). Purslane is also known for its tolerance against multiple stresses and ability to grow in diverse environmental conditions. It could be used as a stress-tolerant food and a biosaline crop. Furthermore, purslane has been investigated for its potential role in the prevention and treatment of metabolic syndrome, including conditions such as obesity and type 2 diabetes mellitus. It is also considered a superfood for its high nutrient content and medicinal benefits. Purslane shows promise for future use in the food industry, dietary supplements, drugs, cosmetics, and products for body care (Popescu *et al.*, 2018).

Purslane tissue contains phenolic acids, flavonoids, and anthocyanins. The flower fraction of purslane has the highest total phenolic acid content and O₂-scavenging activities, while the leaf fraction has the highest amount of total flavonoids and ascorbic acid (Siriamornpun and Suttajit, 2010). The stem fraction has a significantly lower β -carotene content than the leaf and flower fractions (Popescu *et al.*, 2018). Nanoparticles, microscopic particles ranging from 1 to 100 nanometers, can have both positive and negative effects on plants. They can be used to deliver nutrients, pesticides, and herbicides more efficiently, promoting plant growth and reducing chemical usage. Some nanoparticles, like nanoscale silicon dioxide or zinc oxide, have been shown to enhance photosynthesis, nutrient absorption, and enzymatic activity, leading to improved crop yields (Hussain *et al.*, 2019; Ammulu *et al.*, 2021). However, certain nanoparticles may also be toxic to plants, causing stress, reduced growth, or plant death. The environmental impact of nanoparticle accumulation in soil, water, and plants is a concern that requires further study. Responsible and sustainable use of nanoparticles in agriculture is being researched and regulated.

The effect of manganese oxide (MnO) and magnesium oxide (MgO) nanoparticles on phenol, flavonoid, anthocyanin, and quercetin content, as well as half-maximal inhibitory concentration (IC₅₀), has been investigated in various studies. It has been found that foliar application of MgO nanoparticles had a significant effect on flavonoids and anthocyanin content in Roselle plants (Kiapour *et al.*, 2020). Another research. formulated ginger flower extract into nanoparticles using chitosan and NaTPP, which showed good absorption efficiencies of phenol, flavonoid, and anthocyanin (Lestari *et al.*, 2018). Hussain *et al.* (2019) demonstrated that the combination of thidiazuron (TDZ) growth regulator and MgO nanoparticles increased phenolic and flavonoid contents in radish plants, as well as lead phytoaccumulation. It also synthesized MgO nanoparticles using Pterocarpus marsupium extract, which exhibited good antioxidant activity and anti-inflammatory activity (Ammulu *et al.*, 2021). Zwijnenburg (2021) studied the properties of MgO nanoparticles and found that the optical gap remained fixed with particle size, while the highest occupied and lowest unoccupied quasiparticle states showed significant delocalization.

Although good studies have been done on the effect of manganese oxide and magnesium oxide nanoparticles and comparing their effect with their bulk metal on different plants, there is little information about their effect on biochemical and IC₅₀ especially in purslane. Considering that purslane is a halophyte plant with a high tolerance to salinity and heavy metal pollution, this issue becomes more important. So, this paper aims to investigate the effects of manganese oxide and magnesium oxide nanoparticles on the biochemical attributes of *Portulaca oleracea*.

Materials and Methods

Experimental design and treatments

This experiment was carried out at Islamic Azad University, North Tehran Branch (35° 44' 47" N, 51° 34' 26" E, 1504 m above sea level) in controlled conditions based on a balanced completely random design (CRD) with three replications and three samples in each replication. The treatments included magnesium (Mg) and manganese (Mn) nanoparticles (NPs) in three concentrations: 10, 100, and 500 mg L⁻¹, bulk metal

Mg and Mn in three concentrations of 10, 100, and 500 mg L⁻¹, and a control treatment. The nanoparticles used in this project included magnesium oxide (MgO) and manganese oxide (Mn₂O₃).

Experimental procedure

In each pot filled with sand and perlite in a ratio of 1:1, 10 sterilized seeds of purslane (supplied by Agricultural Research, Education & Extension Organization, Tehran) were planted at a depth of 1 cm on November 28, 2021. The pots were placed in a greenhouse with a temperature of 25±1 °C with a photoperiod of 16 hours of light and 8 hours of darkness (Figure 1). All the pots were irrigated three times a week with distilled water up to the field capacity (FC) and were nutrient by Hoagland nutrient solution every 10 days. Treatments either bulk or nanoparticles of MgO and Mn₂O₃ were applied on December 5, 2021, to the plants twice a week.

60-day-old purslane plants were harvested to measure total fresh weight and total dry weight. Their initial weight was considered as fresh weight and the weight of the samples after keeping them in an oven at 60 °C for 72 hours was considered as dry weight.



Figure 1. Images of purslane plants under different treatments of nanoparticles or bulk metal magnesium and manganese in the growth chamber

Sampling and measurements

At the end of the experiment, all the plants of each pot unit were harvested and transported to the laboratory to measure phenolic, flavonoid, anthocyanin, and quercetin content and half-maximal inhibitory concentration (IC₅₀). The leaf discs prepared from the plant weighing 0.5 g were ground completely in a mortar and some acidic methanol at a volume ratio of 99:1, and the extracts were placed in the dark at a temperature of 25 °C for 24 h to measure the amount of flavonoids and anthocyanin. The amount of flavonoids in purslane leaves was measured by reading the absorbance at a wavelength of 300 nm using the spectrophotometer (Krizek *et al.*, 1998). To measure the amount of anthocyanin, leaf discs prepared from the plant weighing 0.5 g and absorbing at a wavelength of 550 nm using the spectrophotometer were used according to the method of Wagner (1979). The amount of phenolic compounds from 0.1 g of fresh leaf tissue was obtained based on the absorbance at 725 nm wavelength in a spectrophotometer (Philler scientific su-6100) and according to the gallic acid standard curve (Seever and Daly, 1970).

HPLC device was used to measure quercetin in 0.1 g of dry powder of four-month-old leaves (Dmitrienko *et al.*, 2012; Jaimand *et al.*, 2013). To determine the IC_{50} , different volumes (25, 50, 100, 150 μ l) of dry leaf powder extract were prepared in methanol solvent, and after 30 minutes of keeping the tubes in the dark, the optical absorption of the samples was read using the spectrophotometer at a wavelength of 517 nm. In this experiment, ascorbic acid was used as a positive control to compare the IC_{50} of the extracts with a pure antioxidant, and 3 ml of absolute methanol with 1 ml of 0.004% DPPH was used as a negative control.

Statistical analysis

The data were subjected to analysis of variance (ANOVA) after the homogeneity of variances test. Means were compared based on the least significant difference (LSD) at the 1% probability level.

Results

There was a significant difference between the concentrations of phenol and flavonoids in the studied treatments (Figure 2). The highest concentration of phenol was equal to 970.6 mg g^{-1} , which was obtained in BulkMn₂O₃ treatment with a concentration of 10 mg L^{-1} . After that, other concentrations of BulkMn₂O₃ and all concentrations of Mn₂O₃NPs had higher phenol concentrations (Figure 2a). The phenol concentration in all manganese treatments (bulk or nanoparticles) was higher than in the control (747.6 mg g^{-1}). The difference between treatments was greater in flavonoid than phenol concentration. The lowest concentration of flavonoid was obtained in the control treatment, which was less than all the treatments using manganese and magnesium, either in bulk or in nanoparticle form (Figure 2b). The highest flavonoid concentration was obtained in the treatments of MgONPs at 100 mg L^{-1} concentration equal to 1185.8 mg g^{-1} and Mn₂O₃NPs at 10 mg L^{-1} concentration equal to 1169.5 mg g^{-1} .

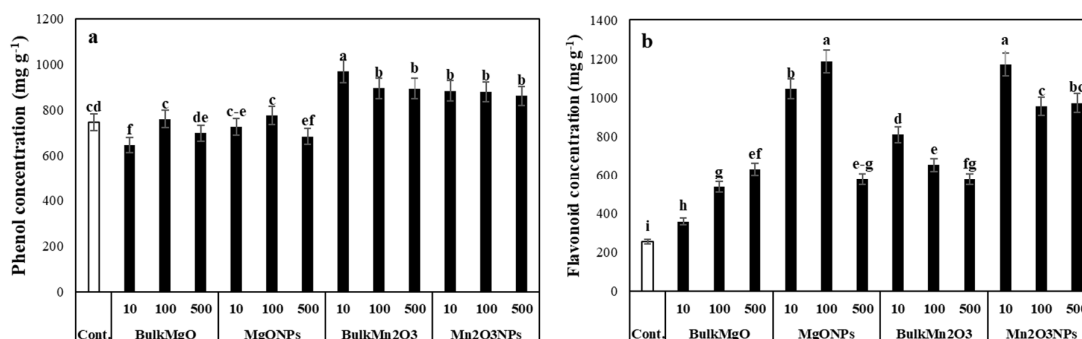


Figure 2. Concentrations of phenol and flavonoids as affected by varied concentrations of manganese and magnesium (mg L^{-1}) in bulk or nanoparticle forms. Averages with at least one common letter do not have a significant difference based on the LSD at the 1% probability level.

Although the concentration of anthocyanin was affected by the experimental treatments (Figure 3a), this effect was slightly different from the effect on phenol (Figure 2a) and flavonoids (Figure 2b). The highest concentration of anthocyanin was obtained in BulkMgO and MgONPs treatments at 500 mg L^{-1} concentrations equal to 240 and 239 mg g^{-1} , respectively; which was 82.0% and 81.3% more than the control, respectively. All the magnesium application treatments, both bulk and nanoparticle, had significantly higher anthocyanins than the control, while the anthocyanin in the control plants was significantly higher than all manganese application treatments (Figure 3a).

The response of quercetin to Mn and Mg treatments (Figure 3b) was slightly different from other biochemical characteristics (Figures 2a, 2b, and 3a). The highest concentration of quercetin was obtained in

the treatments of BulkMgO at 500 mg L⁻¹ and BulkMn₂O₃ at 500 mg L⁻¹ concentration, which were not significantly different from the control treatment, however, were significantly higher than other treatments (Figure 3b). The concentrations of quercetin were 14.03, 13.73, and 13.50 mg L⁻¹ in these treatments, respectively. The lowest quercetin concentration was observed in BulkMn₂O₃ at 10 mg L⁻¹ by 9.81 mg L⁻¹.

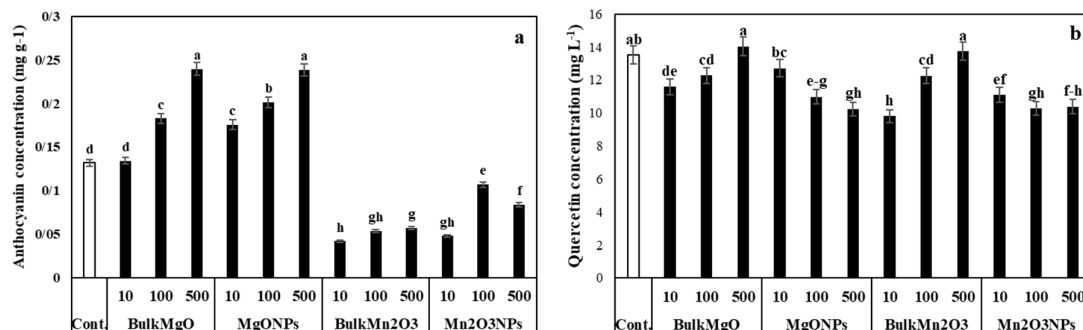


Figure 3. Concentrations of anthocyanin and quercetin as affected by varied concentrations of manganese and magnesium (mg L⁻¹) in bulk or nanoparticle forms
Averages with at least one common letter do not have a significant difference based on the LSD at the 1% probability level.

In the bulk form, increasing the concentration of manganese and magnesium increased the half-maximal inhibitory concentration (IC₅₀) as was shown in Figure 4, so that the highest IC₅₀ was obtained at the concentration of 500 mg L⁻¹ of both metals. However, in the form of nanoparticles, increasing the metal concentration did not increase the IC₅₀. The highest IC₅₀ in the treatments of bulk metals, that is, BulkMgO at 500 mg L⁻¹ and BulkMn₂O₃ at 500 mg L⁻¹ concentration, were obtained as 3.59 and 3.53, respectively; which were higher than the control treatment (Figure 4).

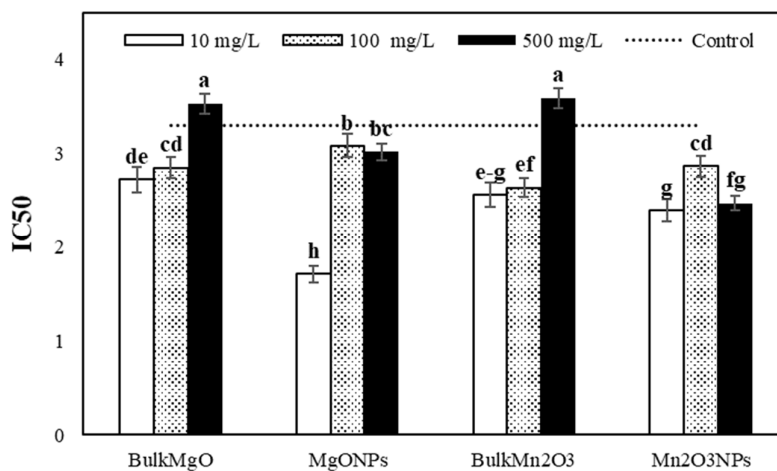


Figure 4. Half-maximal inhibitory concentration (IC₅₀) as affected by varied concentrations of manganese and magnesium in bulk or nanoparticle forms
Averages with at least one common letter do not have a significant difference based on the LSD at the 1% probability level.

The total fresh weight and the total dry weight of purslane were affected by the experimental treatments (Figure 5). The highest total fresh and dry weights were obtained in the 10 mg L⁻¹ BulkMgO treatment and

100 mg L⁻¹ BulkMgO by 246.67 mg and 50.67 mg, respectively., which was significantly higher than other treatments. The purslane plants treated with 500 mg L⁻¹ Mn₂O₃NPs had the lowest total fresh and dry weights by 31.00 and 3.67 mg, respectively.

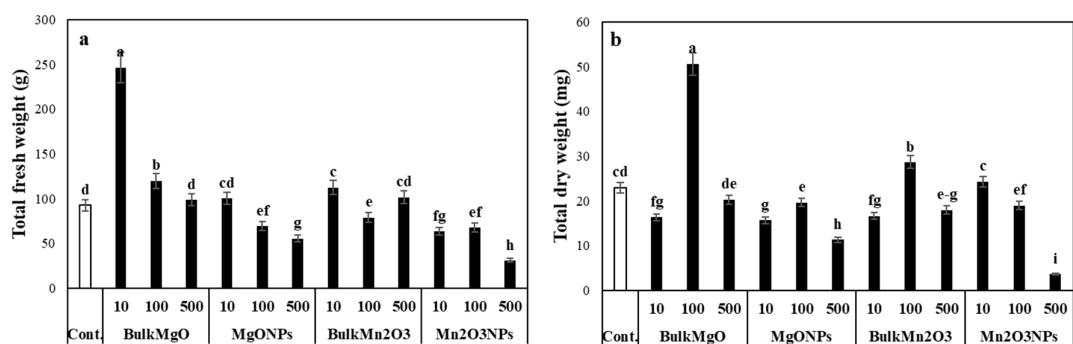


Figure 5. Total fresh and dry weight as affected by varied concentrations of manganese and magnesium (mg L⁻¹) in bulk or nanoparticle forms
Averages with at least one common letter do not have a significant difference based on the LSD at the 1% probability level

Discussion

The concentration of phenol and flavonoids in purslane as a halophyte plant was different under the influence of different treatments. Halophytes the plants with the ability to thrive in high saline environments are known for their ability to biosynthesize phenolic and flavonoids compounds in significant concentrations, which contributes to their remarkable antioxidant and antimicrobial activities (Lopes *et al.*, 2023). This makes halophytes ideal sources of bioactive molecules that can be employed in various sectors, including food and pharmaceutical applications. Phenolic compounds in halophytes play a crucial role in their resistance to harsh environmental conditions, such as excess salts in their habitats (Pungin *et al.*, 2022).

The average concentration of phenol in the purslane plant in this experiment was 803 mg g⁻¹. The total amount of polyphenols in halophytes is twice as high as in glycophytes as the plants that are not adapted to salt stress, and the extracts from halophytes have a high ability to remove free radicals as a high antioxidant capacity (Pungin *et al.*, 2022). Phenol and its derivatives play a significant role in the salinity tolerance of plants through antioxidant properties (helping to mitigate oxidative stress scavenging free radicals and reducing damage to cellular components), osmotic regulation (allowing plants to maintain turgor pressure), signal molecules (triggering stress response pathways and activate genes associated with stress resistance), cell wall structure (the synthesis of lignin), nutrient uptake (influencing root architecture and function), secondary metabolite production (help in the detoxification of harmful ions) and some other processes (Xuan *et al.*, 2022; Pirasteh-Anosheh *et al.*, 2023).

The effect of Mn on phenol concentration was greater than that of Mg; despite the non-significant difference between the two forms of Mn, the effect of the Mg nanoparticle form was greater than the bulk form. Both Mn and Mg as essential nutrients play significant roles in various metabolic processes, including the biosynthesis of phenolic compounds. A study on *Artemisia annua* plants showed that Mn treatments affected the total phenolic content, specifically, Mn foliar application stimulated the synthesis of phenolic compounds mainly in MnSO₄ treatments (Salehi *et al.*, 2022). Another study on snap beans showed that the application of magnesium salts (MgSO₄ and MgCl₂) increased the concentration of total phenols, anthocyanins, flavonoids, and antioxidant capacity (Ciscomani-Larios *et al.*, 2021). In the case of Mn, it is an essential cofactor for the

oxygen-evolving complex (OEC) of the photosynthetic machinery, catalyzing the water-splitting reaction in photosystem II (PSII) (Alejandro *et al.*, 2022).

Both Mn and Mg in both bulk or nanoparticle forms were associated with an increase in the concentration of flavonoids. Meanwhile, the effect of Mn was greater than that of Mg, and the effect of nanoparticles was greater than the bulk form of the metal. The availability and interaction of Mg and Mn can influence the biosynthesis of flavonoids, which are crucial for the plant's defense mechanisms and antioxidant activities. However, the specific effects can vary depending on the plant species and environmental conditions. Mn was found to participate in various soybean plant metabolic processes, such as oxygenic photosynthesis, respiratory action, synthesis of proteins and fatty acids, and activation of enzymes. The study also found that Mn toxicity affected flavonoid biosynthesis (Wang *et al.*, 2023). Research on Arabidopsis, a model plant species, showed that Mn is involved in the flavonoid biosynthetic pathway. The presence of Mn was found to affect the interactions among enzymes involved in this pathway (Burbulis *et al.*, 1999).

Our results showed that magnesium had a positive effect, whereas manganese harmed the anthocyanin concentration of purslane. Magnesium, in the form of magnesium oxide nanoparticles (MgO-NPs), has been found to have a significant impact on the concentration of anthocyanins in plants. However, it's important to note that the specific effects of MgO-NPs on anthocyanin concentration can vary depending on the plant species and environmental conditions. The increase in anthocyanin concentration as a result of MgO-NPs suggested that is not due to the induction of biosynthetic genes but rather due to the inhibition of anthocyanin degradation that typically occurs in the cells (Sinilal *et al.*, 2011). The exact process by which magnesium treatment affects anthocyanin accumulation is still unclear, but the results suggest that at least part of its effect stems from the inhibition of the pigments' catabolism (Sinilal *et al.*, 2011; Gautam *et al.*, 2023). In a study, it was shown that MgO-NPs can improve vegetative growth and enhance productivity, biochemical potency, and storage stability in mustard seeds (Sinilal *et al.*, 2011; Gautam *et al.*, 2023). Another research reported that magnesium treatment led to a 2.5- to 4.5-fold increase in anthocyanin concentration in *Vitis vinifera*. More research is needed to fully understand the mechanisms by which MgO-NPs influence anthocyanin biosynthesis and accumulation in different plant species.

The reaction of quercetin concentration to Mn and Mg treatments did not have a clear-cut trend. These components have a great role in herbal medicines, for example, quercetin inhibits tumors, eliminates free radicals, and reduces blood pressure (Xu *et al.*, 2020). Halophytes are known to accumulate phenolics and flavonoids, including quercetin, at higher levels than glycophytes (Mohammed *et al.*, 2023). The presence of quercetin in halophytes depends on the strength of the salinity in their growing environment and has been reported to have various beneficial properties, including antioxidant activity (Giordano *et al.*, 2021; Mohammed *et al.*, 2023). The average of quercetin in purslane plants in the present study was 11.8 mg ml⁻¹. Pungin *et al.* (2022) reported that two halophytic species consisting of *Spergularia marina* and *Glaux maritima* had a relatively high content of phenolic compounds, including individual flavonoids like quercetin. In another study, Stanković and Jakovljević (2021) highlighted that quercetin, flavonoids, and myricetin are the main active constituents in halophytes. The cultivation of *Apocynum venetum* in saline soils has proven to be feasible and enhances its medicinal quality, particularly through the accumulation of quercetin and kaempferol under salt stress (Xu *et al.*, 2020). So, it can be understood that in addition to the medicinal plant's quality, quercetin contributes to the physiological and biochemical adaptations that enable plants to withstand saline stress, making it a valuable compound in the study of plant resilience.

The interesting result is that increasing the metal concentration in the bulk form increased IC₅₀, while no such effect was observed in higher concentrations of Mg and Mn nanoparticles. This shows that even if Mg and Mn nanoparticles do not have a positive effect, their high concentrations have a less negative effect on plants. However, our study showed that Mn and Mg nanoparticles had a positive effect on the biochemical properties of purslane. The half-maximal inhibitory concentration (IC₅₀) is a measure of the effectiveness of a substance in inhibiting a specific function by 50%, which could refer to the concentration of a substance that

inhibits a particular physiological process or biochemical pathway by 50% (Cai *et al.*, 2018). Mg and Mn play crucial roles in various metabolic processes, however, their specific effects on IC₅₀ values in plants may vary depending on the specific biological process or pathway being inhibited. Manganese is involved in various plant metabolic processes, such as oxygenic photosynthesis, respiratory action, synthesis of proteins and fatty acids, and activation of enzymes. Manganese toxicity can affect plant metabolic processes and lead to oxidative stress, which may impact the IC₅₀ values of certain biochemical pathways (Wang *et al.*, 2023). Magnesium, on the other hand, is a central component of the chlorophyll molecule and is essential for photosynthesis. It also plays a role in activating many enzymes involved in plant growth and development. However, the direct effect of magnesium on IC₅₀ values in plants is not explicitly documented in the provided search results (Alejandro *et al.*, 2020).

The potential environmental implications of this research are significant. By demonstrating that Mg and Mn nanoparticles can enhance the growth and quality of purslane plants without increasing their toxicity (Bhardwaj *et al.*, 2022), this study suggests a viable strategy for improving crop resilience and productivity in saline soils, which are increasingly affected by salinization due to climate change and agricultural practices. Utilizing nanoparticles could also mitigate the negative environmental impact of traditional bulk metal treatments, reducing the risk of toxicity in soil and water ecosystems. Furthermore, the application of these nanoparticles in halophyte species presents a promising avenue for restoring degraded lands and promoting environmental sustainability. As nutrient leaching and pollution from conventional fertilizers pose serious risks to water quality, the targeted use of Mg and Mn nanoparticles could help decrease these concerns while enhancing the efficiency of nutrient uptake by plants. Overall, these findings underline the potential for innovative nanoecological approaches to address ongoing environmental challenges while supporting sustainable agricultural practices.

Conclusions

In conclusion, our study demonstrates that nano treatments of Mg and Mn significantly enhance the concentration of key phytochemicals such as phenols, flavonoids, anthocyanins, and quercetin, along with promoting total fresh and dry weights in purslane plants. Notably, the positive effects of these nanoparticles on plant quality and quantity were observed, except for quercetin. Our findings suggest that while increasing the concentrations of Mg and Mn in bulk form negatively affects the IC₅₀, the nanoparticle form mitigates this effect, indicating a reduced toxicity of these metals when applied in nanoparticle form. Given the ongoing salinization of water and soil resources, the application of Mg and Mn nanoparticles presents a promising strategy for improving halophyte species and mitigating environmental pollution. Further research is essential to explore the full potential and mechanisms of action of these nanoparticles in various agricultural contexts.

Future research should focus on exploring the mechanisms behind the effects of Mg and Mn nanoparticles on halophyte species, conducting field trials to assess their practical applications, and investigating their potential synergistic effects with other nutrients. Additionally, studies on the long-term environmental safety of these nanoparticles, their role in enhancing salinity tolerance, and the optimization of their formulation and delivery methods are essential for advancing sustainable agricultural practices.

Authors' Contributions

Conceptualization: MM, SA and SJM; Methodology: SA, AM and GT; Writing original draft: MM and SA; Reviewing and editing: AM, GT and SJM. All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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