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The Effect of Manganese Oxide Nanoparticles and Zinc Oxide Nanoparticles on Seed Germination of Medicinal Chicory Plant *Cichorium intybus* L.

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Abstract

The chicory plant, scientifically known as *Cichorium intybus*, has been recognized for its medicinal and nutritional properties since ancient times. Due to its practical importance, this plant is extensively cultivated across various region of the world. Therefore, enhancing both the quality and quantity of chicory production is paramount importance. The role of Nanotechnology is crucial, particularly in the production of nano-sized particles that exhibit diverse properties. Following the disinfection, the seeds, they are soaked in a solution containing zinc and manganese oxide nanoparticles at concentrations of 0.1, 0.05, and 0.01 g/l for a duration of two hours. Subsequently, the seeds are transferred to a petri dish lined with moist filter paper. Each Petri dish receives a daily application of a corresponding solution at a volume of 0.5 cc. Measurement of the root length and seed germination percentage are then conducted and evaluated. The results indicate a significant enhancement in growth with the increased application of nanoparticles at the two lower concentration. However, at the highest concentration, decreasing decline in effectiveness was noted. The optimal concentration for both types of nanoparticles was determined to be 0.05 g/l ($p< 0.001$). This decreasing trend at a concentration of 0.1 g/l was more pronounced for manganese oxide nanoparticles in comparison to zinc oxide nanoparticles. The findings indicated beneficial effects of both nanoparticles at the two lower concentration, while the highest concentration exhibited toxic effects. Zinc and manganese are vital elements for plant growth and development; thus, the concentration is critical as it can lead to detrimental effects on plants due to either deficiency or toxicity of these essential elements. Based on the outcomes of this study, it is recommended to utilize two nanoparticles of zinc oxide and manganese oxide at a concentration of 0.05 g/l are suggested to be used as fertilizers.

Keywords: Chicory, Nanoparticles, Zinc oxide, Manganese oxide, Germination

Introduction

The chicory plant, scientifically referred to as *Cichorium intybus* L., is a perennial herbaceous plant species that can grow up to half a meter in height and features blue flowers. It grows in relatively moist and shaded environments. The radicles are brown and sometimes reach a length of one meter, with a white interior that contain a milky sap. The leaves exhibit deep grooves that

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culminate in a triangular shape at their trips (Janda et al., 2021). Chicory is recognized for its numerous health benefits, including liver detoxification, alleviation of urinary issues, and fever reduction. This plant has received approval from the FDA of the United States Food and Drug Administration (Street et al., 2013). Chicory is rich in inulin, which possesses various probiotic properties and contributes to the improvement of heart conditions, joint pain and constipation, immune system enhancement, sedation, and cancer prevention. This plant possesses the capacity to absorb bodily fluids and is beneficial in improving inflammation and rheumatism. It contains a variety of compounds such as water, carbohydrates, especially cellulose and starch, and a variety of terinoids, particularly sesquiterpenes and lactones. Other significant constituents include lactocin, which has a bitter taste, tannin, unsaturated sterols, tartaric acid, chicory acid. The leaves are rich in apigenin, quercetin, cichorin and luteolin. Additionally, it features various types of flavonoids and essential minerals such as phosphorus, calcium, potassium and iron, which are abundantly found in its flowers (Qadir et al., 2022).

Nanotechnology has emerged as a significant and appealing field of research in contemporary science, primarily due to the creation of nano-sized particles that exhibit unique features in shape, distribution, chemical composition and properties. This field is experiencing rapid growth and expansion, with an increasing increasingly production and utilization of nanomaterials globally (McNeil, 2005). Nanoparticles are defined as compounds measuring between5 to 555 nm, and the characteristics of these materials in this scale are differ markedly from those of the same chemical compounds in larger dimensions. Additionally, metal oxide nanoparticles possess a variety of crystal structures and exhibit unique electronic and magnetic properties. For this reason, these elements are utilized across various sectors including environmental, chemical, catalyst and medical industries (Kubik et al., 2005). The essential mineral elements present in the soil possess unique physiological properties and are categorized into high-use and low-use elements based on the relative concentrations required in plant tissues. Low-usage elements such as zinc, copper, and cobalt can lead to plant toxicity when their concentration exceeds a certain threshold (Mukhopadhyay, 2014). Zinc plays a crucial role in the formation of diastases and its deficiency causes disruption in ribonucleic acid formation, consequently affecting the synthesis of plant proteins. Zinc serves multiple functions in plant enzyme activities, including activation, catalysis, structural support. The transport of zinc within the plant is complex and its distribution in the phloem is notably restricted in the (Sabir et al., 2014). This micronutrient is essential for the normal metabolic processes of the plant, influencing the metabolism of various compounds such as carbohydrates, proteins, auxin. A deficiency in zinc can significantly impair reproductive processes and overall plant growth. Zinc deficiency leads to a reduction in photosynthesis and chlorophyll content, structural damage to chlorophyll, a decrease in the number of vascular sheath chloroplasts, a reduction in the permeation proteins of the living membrane, and an increase in inorganic phosphorus levels (Sharma et al., 2012). Manganese, an essential yet low-use element for plants, is crucial due to its ability to transition between oxidation states, thereby facilitating oxidation and reduction reactions. Manganese plays a role in nitrogen metabolism and its toxic effects are reduced through the reduction of nitrate. Additionally, Manganese serves as a cofactor for the superoxide dismutase enzyme, which is vital for the antioxidant mechanisms in plants. A deficiency in manganese compromises the plant 's resilience to environmental stress (Rashed et al., 2019).

The seeds of the chicory plant exhibit physiological dormancy. Given this plant's industrial and medicinal cultivation, enhancing its germination efficiency and growth is of great importance. Zinc and manganese play significant roles in many physiological mechanisms and plant enzymes, suggesting their potential influence on the germination chicory (Qadir et al., 2022). Considering the limited findings about the effects of zinc oxide and manganese oxide nanoparticles on seed germination in chicory in Iran, it is essential to conduct further studies in this area. The present study was undertaken in light of these considerations and their importance.

Material and methods

Nanoparticle preparation

Distilled water was used to prepare the nanoparticle solution. Zinc oxide and manganese oxide nanoparticles, sourced from US Nano Research, were utilized. Following the heating of distilled water to a temperature of 30°C, 100 milliliters of stock solution were prepared for each nanoparticle. Treatment solutions were then prepared in three concentrations: 0.1, 0.05, and 0.01 g/l (Alavi et al., 2022).

Seeds disinfection

Chicory plant seeds were obtained from Pakan Seed Company located in Isfahan. To ensure the seeds are disinfected, they were immersed in a 2% sodium hypochlorite solution. This was followed by three rinses with distilled water. The treatments were done in three replicates, resulting in a total of seven groups including a control group. The experimental design is completely randomized and performed in three replications (Tajadod and Majd, 2007).

Seed germination measurements

Following the disinfecting of the seeds, they are immersed in a solution containing zinc and manganese oxide nanoparticles at 0.01, 0.05 and 0.1 g/l concentrations for a duration of two hours while being agitated on a shaker. Subsequently, the seeds are washed again with distilled water. The seeds were then transferred to a petri dish lined with wet filter paper. The seeds are stored under conditions storage conditions of the seeds were 25 ºC with a light cycle of 16 hours of illumination followed by 8 hours of darkness. Initially, the seeds are undergoing disinfection in a sodium hypochlorite solution for 10 minutes, after which they are thoroughly washed multiple times with distilled water. The seeds are then arranged on cotton or filter paper within a Petri dish, with 10 seeds allocated to each Petri dish. A solution corresponding to the respective doses is applied daily at a volume of 0.5 ml for each Petri dishes. The dished are sealed with parafilm and aluminum paper and placed in an incubator set at 25 °C. After 3 days, the germination rate and radicle length of the are measured. Healthy germinated seeds are used to measure radicle length and germination percentage. The percentage of seed germination is calculated using the following formula (1).

%G=n/N.100 (1)

where G represents the final germination percentage or seed vigor, n denotes the final number of germinated seeds, and N indicates the number of planted seeds (Esatu et al., 2022).

Statistical analysis

All results obtained from this research were statistically measured and analyzed using SPSS software version 21. Following the calculation of the mean and standard deviation of the data, normality is assessed. Subsequently, a one-way ANOVA and Tukey's post hoc test were conducted under the condition of $P < 0.05$ to determine the significant differences between the sample groups and the control group was calculated. Finally, the graphs representing the obtained data were created using Excel software.

Results

Healthy seeds were utilized to measure radicle length and germination percentage. A Petri dish culture was employed to calculate these quantities, the culture used. For the formulation of nanoparticle solutions, zinc oxide and manganese oxide nanoparticles, sourced from US Nano Research, along with distilled water, were utilized. According to the manufacturer's reliable statements, the nanoparticles measured between 10-30 nanometers in size. The treatment solutions were then used in 0.01, 0.05, and 0.1 g/l concentrations.

Radicle length measurement

In the results concerning to radicle length measurement, a notable enhancement in radicle growth was observed at the two lower concentration of 0.01 and 0.05 g/l for both magnesium oxide and zinc oxide nanoparticles. This enhancement was statistically significant at the levels of $p< 0.05$ and $p< 0.001$, respectively. However, when the concentration of nanoparticle solution was increased to 0.1 g/l, toxic effects were evident. In this group, a decline in radicle growth was observed for both types of nanoparticles, with the reduction being more pronounced for magnesium oxide nanoparticles (Fig.1).

Germination percentage measurement

In the analysis of germination percentage, an enhancement was observed at the concentration of 0.01 g/l for both zinc oxide and manganese oxide nanoparticle solutions. While the increase of zinc oxide did not reach statistical significance, the rise of manganese oxide was significant at $p < 0.05$. When the concentration was raised to 0.05 g/l, the germination percentage for both types of nanoparticles showed a significant increase with $p< 0.001$. However, as the concentration of nanoparticle solutions continued to rise, a decline in germination percentage was observed, with manganese oxide nanoparticles exhibiting a more pronounced decrease. In the group treated with manganese oxide nanoparticles at a concentration of 0.1 g/l, no statistically significant difference was found compared to the control group (Fig. 2).

Discussion

Nanoparticles exhibit elevated levels due to their small size, which has led to their propensity to aggregate. This aggregation may contribute to toxicity and adverse effects on plants (Mahootforoshha et al., 2023). The variation in metal and metal oxide concentrations in their nano form across to different plant species is attributed to the permeability characteristics of the pod and seed coat to various compounds. The seed pod plays a crucial role in protecting the plant. As mentioned, this feature is also applicable for nanoparticles. Whitin

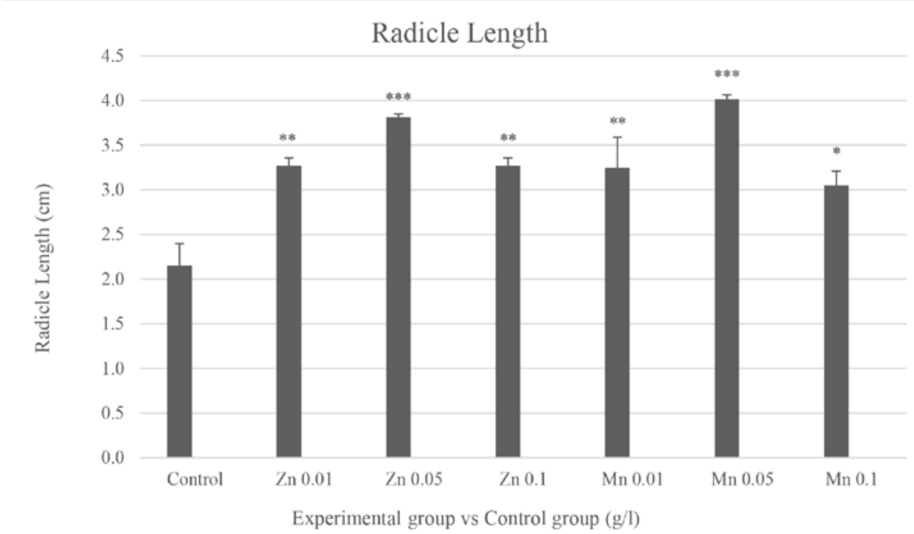

Fig. 1. The radicle length measurement

*: significant difference with the control group (p<0.05), **: significant difference with the control group (p< 0.01), ***: significant difference with the control group (p < 0.001)

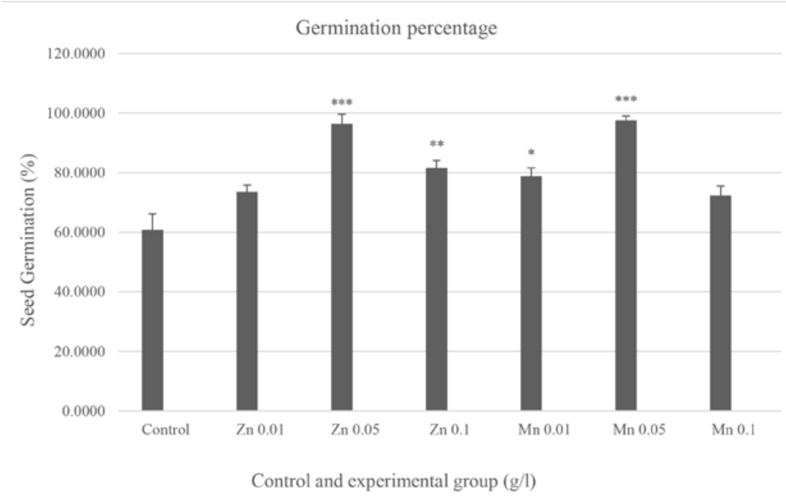

Fig. 2. The seed germination percentage

*: significant difference with the control group (p< 0.05), **: significant difference with the control group (p< 0.01), ***: significant difference with the control group (p< 0.001)

the parenchyma of the seed coat, there exist intracellular spaces measuring less than 10 micrometers, which, when filled with an aqueous solution, facilitate the penetration of nano-soluble compounds through various mechanisms (Jiang et al., 2021). Even research has shown that nanoparticles possess the ability to penetrate through the cell wall and access the DNA within the plant cells. Findings have proven that nanoparticles can penetrate the plasma membrane and enter the nucleus (Liu et al., 2016). This research highlights that the rate at which nanoparticles infiltrate to the seed pod varies, and this variation can impact on the germination process. This phenomenon may elucidate the differing effects of zinc and manganese oxide nanoparticles at various concentrations on plant germination and growth. The results further substantiate the capacity of nanoparticles to penetrate both the plasma membrane and the nucleus (Zareei et al., 2023).

Considering that the radicles serve as the initial part of the plant that interact with nanoparticles from the surrounding environment. Their reaction rate significantly influences the potential positive or negative effects of these nanoparticles, making them an effective indicator for the evaluation of nanoparticle effects, particularly regarding the occurrence of toxic effects at varying concentrations. Elevated levels concentrations of these two nanoparticles have been shown to hinder r the water absorption capabilities of the plant and prevention the longitudinal growth of its organs (De Souza et al., 2010). The detrimental effect of high concentrations of zinc and manganese nanoparticles on radicle growth may contribute to the toxic effects observed at elevated doses. The presence of mucilage secreted from the radicle tip, along with pectic substances and hydrated polysaccharides, enhance the absorption of nanoparticles in these regions, thereby increasing the likelihood of adverse effects (Jiang et al., 2009). Nanoparticles typically traverse the cortex and epidermis of the radicle through the apoplastic pathways; however, to access more central structures such as blood vessels, they must penetrate the protoplast of endodermal cells. Examination of tissue sections revealed that the concentration of nanoparticles increased, there was notable damage and destruction in various region of the radicle and stem, leading to a decrease in radicle growth, germination and numerous metabolic processes within the plant. These toxic effects were not observed at lower concentrations. The detrimental impact associated with high concentrations of these two nanoparticles are linked to the destruction of cell parenchyma destruction and the occurrence of vacuolization. Generally, zinc oxide and manganese nanoparticles enhance permeability by forming openings in the cell wall and move, subsequently facilitating movement through plasmodesmata once inside the cell (Lin and Xing., 2008).

Evaluating characteristics such as radicle growth or youth percentage, the positive or negative characteristics of compounds allows for a swift and straightforward assessment in the. Basically, germination is a complex biological process that initiates with the absorption of water by seeds and culminates in the emergence of radicles. The seed coat is one of the factors that can influence the permeability to various substances, including nanoparticles. Consequently, nanoparticles typically do not exhibit a noticeable impact on radicle growth and germination percentages at very low concentrations; however, at significantly high levels, they may aggregate, leading to toxic effects. This accumulation may even occur within cell organelles such as vesicles (Corredor et al., 2009).

Finding of the current research, consistent with the majority of existing research, indicate that low concentrations of zinc and manganese oxide nanoparticles promote both radicle length and germination percentage. Conversely, it is suggested that higher concentration may lead to a reduction in radicle length, as explained by the previously discussed mechanisms.

Conclusion

In this experimental study, the effects of two types of nanoparticles, zinc oxide and manganese oxide on chicory plants were investigated. For this purpose, solutions of nanoparticles of zinc oxide and manganese oxide nanoparticles were prepared at the concentration of 0.1, 0.05 and 0.01 g/l. A total of seven groups were stablished, including a control group. The experiments were designed using a completely randomized approach and were performed in triplicate. Following preparation and disinfection, the seeds were cultivated in Petri dishes and pots. Subsequently, two experiments were carried out to measure the speed and percentage of germination. The finding indicated that the two nanoparticles at the two nanoparticles at the lower concentrations of 0.05 and 0.01 g/l significantly enhanced the growth parameters measured in this research, with the most favorable results observed at the 0.05 g/l concentration for both types of nanoparticles. By increasing the dosage of two nanoparticles to 0.1 g/l resulted in a decline in the biological factors associated with the chicory plant. This decline is attributed to the excessive accumulation of nanoparticles, which disrupts water absorption and transfer, as well as elevates generation of free radicals, leading to toxic stress. It is recommended to apply low concentrations of these nanoparticles in a thoroughly controlled manner to improve both the qualitative and quantitative growth of plants, especially chicory. Further research is required to explore this issue comprehensively.

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