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Influence of lead, cadmium, and chromium with titanium dioxide and silica nanoparticles on in vitro growth and morphogenesis of *Chrysanthemum morifolium*

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ABSTRACT

This study investigated the effects of heavy metals – lead (Pb), cadmium (Cd), and chromium (Cr) – and the influence of titanium dioxide (TiO_2) and silica (SiO_2) nanoparticles on the *in vitro* growth and development of *Chrysanthemum morifolium* microshoots cultured on Murashige and Skoog (MS) medium. Microshoots were cultured for five weeks on MS medium supplemented with varying concentrations of Pb, Cd, or Cr, individually and in combination with TiO_2 or SiO_2 nanoparticles. The results demonstrated that supplementation with Pb, Cd, or Cr significantly influenced shoot proliferation, shoot length, leaf number, and biomass accumulation. The addition of TiO_2 and Si nanoparticles further enhanced shoot formation and leaf number compared to control treatments. For Pb treatments, the highest number of shoots (14.2 ± 1.29 per explant) was obtained on MS medium containing 1.0 mg/L Pb and 25 mg/L Ti. In the presence of Cd, shoot multiplication increased notably, with the maximum number of shoots (15.84 ± 1.93) achieved at 6.0 mg/L Cd and 25 mg/L Si nanoparticles. Similarly, Cr treatments enhanced shoot regeneration, with the highest number of shoots (15.6 ± 1.02) observed at 6.0 mg/L Cr and 25 mg/L Si. Although shoot length generally decreased with increasing heavy metal concentrations, the number of leaves and both fresh and dry biomass increased significantly ($p < 0.05$) in the presence of nanoparticles. Metal uptake analysis revealed that Pb and Cd accumulated in the explants proportionally to their concentrations in the medium, while Cr absorption remained negligible. Overall, moderate levels of Pb, Cd, or Cr, particularly when combined with TiO_2 or Si nanoparticles, promoted *in vitro* morphogenesis and biomass production in *C. morifolium*. However, concentrations beyond optimal levels exhibited inhibitory effects on growth parameters.

Keywords: *Chrysanthemum morifolium*, heavy metals, titanium dioxide nanoparticles, silica nanoparticles, micropropagation, *in vitro* culture, morphogenesis.

INTRODUCTION

Chrysanthemum morifolium is a perennial herb that belongs to the Composaetae (Asteraceae) family (Arora, 1990). Chrysanthemum is a cut flower that is economically important worldwide, as there are a great number of cultivars for cut flowers, pot flowers, and garden flowers (Teixeira *et al*., 2004; Shatnawi *et al*., 2011). This plant propagates asexually by shoot cuttings or suckers. Moreover, this method is very slow (Karim *et al.,* 2002), where cuttings are obtained from the progenitor plant. On the other hand, progenitors can be exposed to virus infection. However, as a result thus production costs increased (Teixeira *et al*., 2004; Shatnawi *et al*., 2011). In the future, there is a requirement for easy and quick methods of propagation. The only way is by applying *in vitro* propagation procedures, which can be applied to the clonal propagation of many plants, including Chrysanthemum (Kumar *et al*., 2006).

In vitro propagation can augment the reproduction rates (Alrayes *et al*., 2018; Al Shhab *et al.,* 2021; Osman *et al*., 2021). The culture will be a true-to-mother type of plant. This procedure has previously been used to study high proliferation of *C. morifolium* (Shatnawi *et al*., 2022). Accordingly, there is a need to cultivate an affordable system for high-production chrysanthemum plants that are adapted to stress such as salinity and heavy metals. Heavy metal and salinity are one of the high important abiotic stresses for plant cultivation. Heavy metals and salinity can cause catastrophic damage to land. On the other hand, heavy metals and salinity disturb biological processes in plants, resulting in a decrease in growth and yield. The only way for easy methods of monitoring plant responses to heavy metals and salinity is by using in vitro culture (Shibli *et al*., 2000; 2007). Consequently, it is vital to study the association between *in vitro* and responses to both heavy metals and salinity. Heavy metal can change physiological processes in plants by reducing cellular division and leading to plant death (Ali et al., 1994). This is because, under *in vitro* conditions, growth and physiological responses could be controlled.

Nanoparticles are substances of very small size with unique properties. Recently, the use of nanoparticle synthesis has played an essential role in agriculture and the biological system (Kim *et al*., 2017; Shibli *et al*., 2022; Kasmi *et al*., 2005). These methods can develop new processes for significant needs (Jadczak *et al*., 2019). Using nanotechnology can simplify the development of resources with specific characteristics for positive utilization (Gupta *et al*., 2007; 2014). The addition of nanoparticles to tissue culture medium can increase the propagation rate of many plants. However, AgNPs had a positive influence on date palm callus growth, the rate of stimulus depended on AgNPs concentration and plant genotypes (Rahmawati *et al*., 2022). Many studies have shown that nanoparticles enhance the growth of tissue culture plants by changing medium constituents and altering their physical conditions. Nanoparticles have high accessibility because of the increased specific surface area, making absorption easier (Mousavi and Rezaei, 2011). Saxena *et al*. (2016) reported that nanoparticles have been replacing mineral nutrients to enhance growth. Wang *et al*. (2016) mention that nanoparticles increase plant growth yield, development, secondary metabolites production, and seed germination.

The use of nanoparticles has increased in tissue culture since it can increase their growth processes and biochemical variables (Sharma *et al*., 2012). However, the current study attempted to evaluate the effects of different kinds of nanoparticles on *in vitro C. morifolium* plants grown under heavy metals and salts. *In vitro* culture has a significant impact on the production of ornamental plants to improve their performance under abiotic stress (Shanawi *et al*., 2021). In plant nanotechnology, improved plant characters and crop output by decreasing input losses and ensuring efficient nutrient and water management. However, until now, there has been no published report on the application of nanomaterial for evaluating the influence of nanoparticles on the *in vitro* growth of *C. morifolilim* and abiotic stress. Thus, the current study evaluated the possible influence of nanoparticles on the *in vitro* growth of *C. morifolium* tissue culture plants grown under heavy metals.

NPs play a significant role in improving plant growth and are responsible for the transfer of energy needed for metabolic processes within the plant. Therefore, the current investigation did not consider the possibility of toxic effects of nanoparticles at higher concentrations and their potential long-term impact on regenerated plants, this because *C. morifolium* is a cut flower plant, which is not an edible plant, and it is difficult to enter the food chain.

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MATERIAL AND METHODS

Plant materials

In vitro Chrysanthemum morifolium plantlets obtained from Biotechnology Laboratories/Faculty of Agriculture, Al-Balqa University, Jordan (Shatnawi *et al*., 2010).

Culture media

The plant tissue culture media used were MS medium (Murashige and Skoog, 1962) – all – all the constituents of the MS medium dissolved in water. Growth regulators were melted in water with added drops of 1.0 N NaOH. Sucrose (30 g/L) was supplementary as a source of carbon. Eight grams per liter of agar was used as a solidifying agent, and 75 ml of medium was dispensed into a 250 ml glass flask. The media pH was adjusted to 5.8±2 before autoclaving. For gas exchange, a cotton wool closer was inserted into each flask. Then the medium was sterilized in an autoclave at 121 °C for 15 minutes. Repeated subculture on MS medium to increase *C. morifolium* micoshoots. Subculturing was achieved every four to five weeks to a fresh medium for propagation and to evade the appearance of brown color. Microshoots were cultivated on the growth root under cool white fluorescent lights at 24 ± 2 °C with a 16-hour photoperiod and a photosynthetic photon flux density (PPFD) of 40–50 µmol m^2s^1.

Effect of nanoparticles on plant tissue culture

This study examined how different concentrations of silicon dioxide (SiO_2) nanoparticles (Natsheh et al., 2023) and titanium dioxide (TiO_2) (Kandiel et al., 2012) affected the growth of *C. morifoloium* microshoots. The microshoots, which had an apical meristem and measured 10–12 mm, were placed in an MS (Murashige and Skoog) basal medium. Different concentrations of the nanoparticles (0.0, 0.25, 0.5, 1.0, and 5.0 mg/L) and 0.5 mg/L of the plant growth regulator benzyl adenine (BA) were added to this medium. Before being added to the medium, the nanoparticles were sonicated for 20 minutes and then stirred for another 20 minutes to guarantee adequate dispersal. Its pH was adjusted to 5.8. Five replicates of each concentration were tested in 250 mL flasks, each holding 75 mL of medium and five microshoots.

To measure the developmental response to the nanoparticle treatments, the researchers gathered data following a five-week growth period. Maximum shoot height, total number of shoots, number of leaves, and fresh and dry weight (biomass) were among the parameters measured. Before being weighed, the microshoots were harvested and dried at 85 °C for 24 hours in order to calculate their dry weight. An evaluation of the effects of each nanoparticle concentration on the overall growth and vigor of microshoots was made possible by recording the final biomass as the mean weight in grams.

Effect of different concentrations of heavy metals

C. morifoloium microshoots with apical meristem 10–12 mm in length were subculture on MS containing different concentrations of titanium dioxide and silica (0.0, 0.25, and 25 mg/L) nanopartices with the addition to different concentrations of heavy metals such as chromium (Cr), lead (Pb), or cadmium (Cd) at a concentration of 0, 1, 3, or 6 mg/. The pH of the medium was adjusted to 5.8. Each replicate containing 75 mL of the medium aliquot in a 250 mL flask. The medium was solidified with 8.0 g/L agar. The medium was solidified with 8.0 g/L agar. Every treatment had five microshoots, and each treatment contained five replicates. The culture condition is as stated above. After five weeks of growth periods, data were collected on maximum shoot height, number of shoots, number of leaves, fresh and dry weight.

Experimental design and data analysis

The experimental design of the current investigation was operated using a completely randomized design (CRD) with five replications in each treatment which were repeated twice. The data were collected after five weeks. Analysis of variance (ANOVA) was used to analyze the results. The results were analyzed using the SPSS program (version 19). Standard errors were calculated for each mean and the mean was separated according to the Tukeys HSD test at a 0.05 level of probability (SPSS, 2017).

RESULTS

C. morifolium microshoots were cultured for five weeks on MS medium augmented with various heavy metal concentrations of lead, cadmium, or chromium, with the addition of titanium or silica nanoparticles. The increase in lead, cadmium, and chromium was found to increase shoot number, shoot length, number of leaves, and biomass (Figures 1, 2, and 3). The addition of titanium and silica in the medium influences shoot formation. The supplementation of lead, cadmium, or chromium decreased shoot length significantly (Figures 1, 2, and 3). However, media containing lead, cadmium, or chromium with or without titanium and silica did not show any noticeable signs of phenotypic change. The growth of the shoot biomass (dry weight) was not influenced by the addition of lead at 0.5 mg/L in the medium, comparable to the control. Moreover, a high concentration of lead in the medium led to an increase in biomass growth. However, the highest number of new shots (14.2) was noticed in the MS medium supplemented with 1.0 mg/L lead and 25 mg/L titanium in the medium (Figure 1).

Several shoots formed under different concentrations of lead with the supplementation of titanium or silica, ranging from 8.84±1.38 to 14.2±1.29 per explant. Notably, in the existence of different concentrations of titanium, silica, with cadmium, shoot multiplication was higher than the control (Figure 1). The highest number of leaves was observed in the medium comprising 6 mg/L of lead with 25 mg/L silica, reaching 108.64±8.26. Conversely, lower doses of lead (1.0 mg/L) with lower doses of silica (0.25 mg/L) resulted in the maximum number of shoots (13.36±1.66) and with a shoot length of 3.81±0.25. Furthermore, the titanium and silica nanoparticles use concentration has significant positive effects on the number of shoots, leaf number, and biomass (fresh and dry) weight.

Titanium and silica nanoparticles in the presence of cadmium showed an increase in the number of shoots, a decrease in the length of shoots, increase in the number of leaves at increasing concentrations of titanium and silica nanoparticles. In the presence of cadmium, fresh and dry

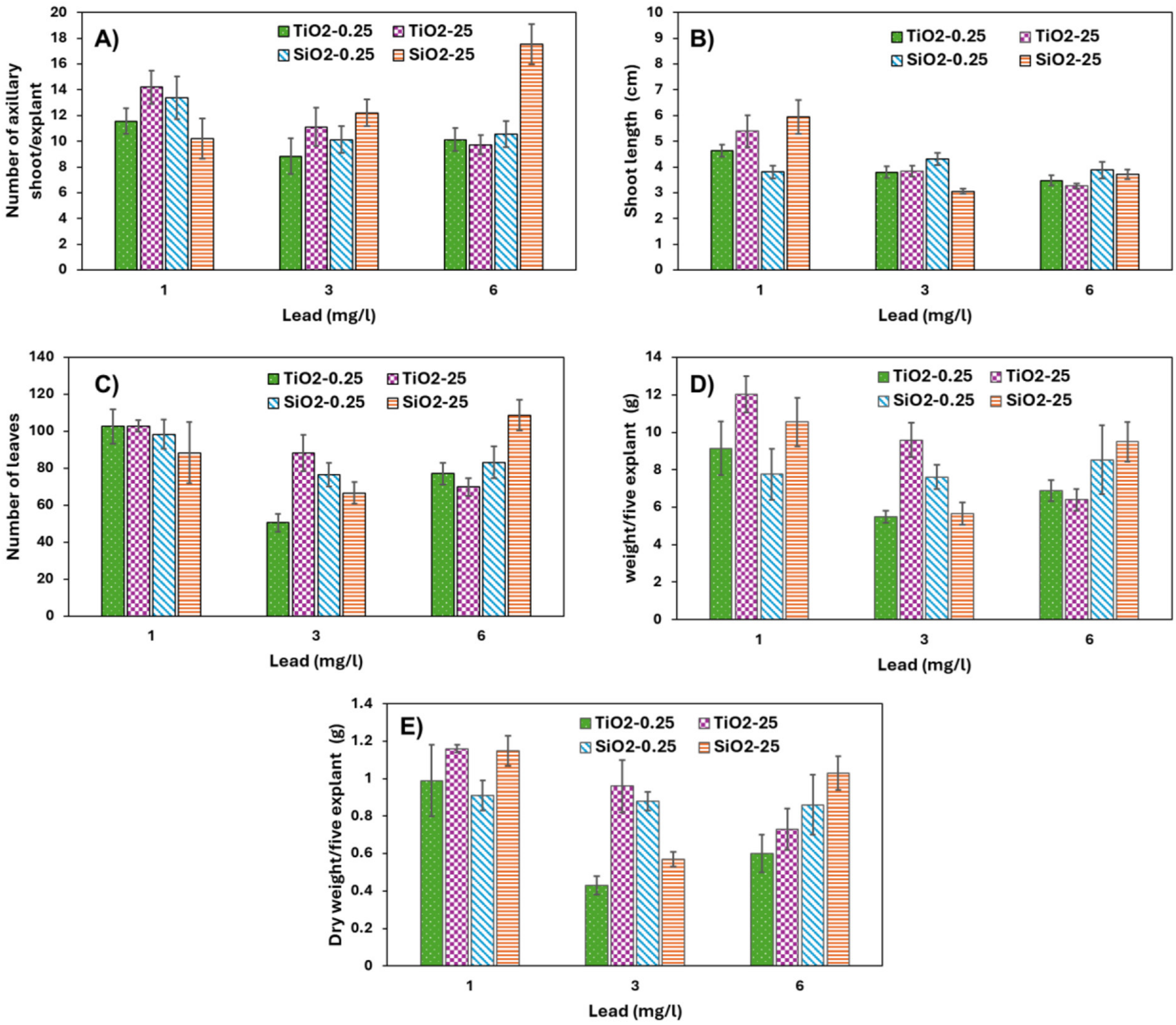

Figure 1. Influence of various concentrations of titanium (TiO_2) and silica nanoparticles on the *in vitro* growth of *Chrysanthemum morifolium* on MS medium containing various concentrations of Lead after five-week growth period

weight increased in the presence of nanoparticles (titanium and silica) (Figure 2). The number of shoots per explant ranged from 3.52±0.43 to 15.84±1.93 when MS medium was supplemented with cadmium nanoparticle (titanium or silica). In the current study *C. morifolium* microshoots were statistically significant with Cd and nanoparticle (titanium and silica) applications. In culture media containing Cd and nanoparticles (titanium and silica), the maximum number of shoots per explant (15.84±1.93) was recorded on MS medium supplemented with 6.0 mg/L cadmium and 25 mg/L silica nanoparticles.

The lowest shoot numbers were detected on the control medium. Maximum shoot lengths (6.29 ±0.26) were detected using MS medium augmented with 6.0 mg/L cadmium and 25 mg/L silica nanoparticles. The lowest shoot lengths in Cd and nanoparticle (titanium and silica), applications were obtained on MS medium containing 1.0 mg/L cadmium and 25 mg/L silica (Table 2). The highest leaf number (128.12±7.11) was achieved from the explants in MS medium supplemented with 3.0 mg/L cadmium and 25 mg/L titanium. While the lowest was recorded with the control treatment (33.24±3.82). Moreover, maximum fresh weight (15.64±1.07) was recorded on MS medium supplemented with 3.0 mg/L cadmium and 25 mg/L titanium. As the cadmium concentrations increased in the presence of nanoparticles, the fresh weight of the plants increased. There were statistically significant decreases in fresh and dry weight with the application of nanoparticles compared to the control ($p < 0.05$). The lowest dry weight (0.67±0.1) was detected control treatment.

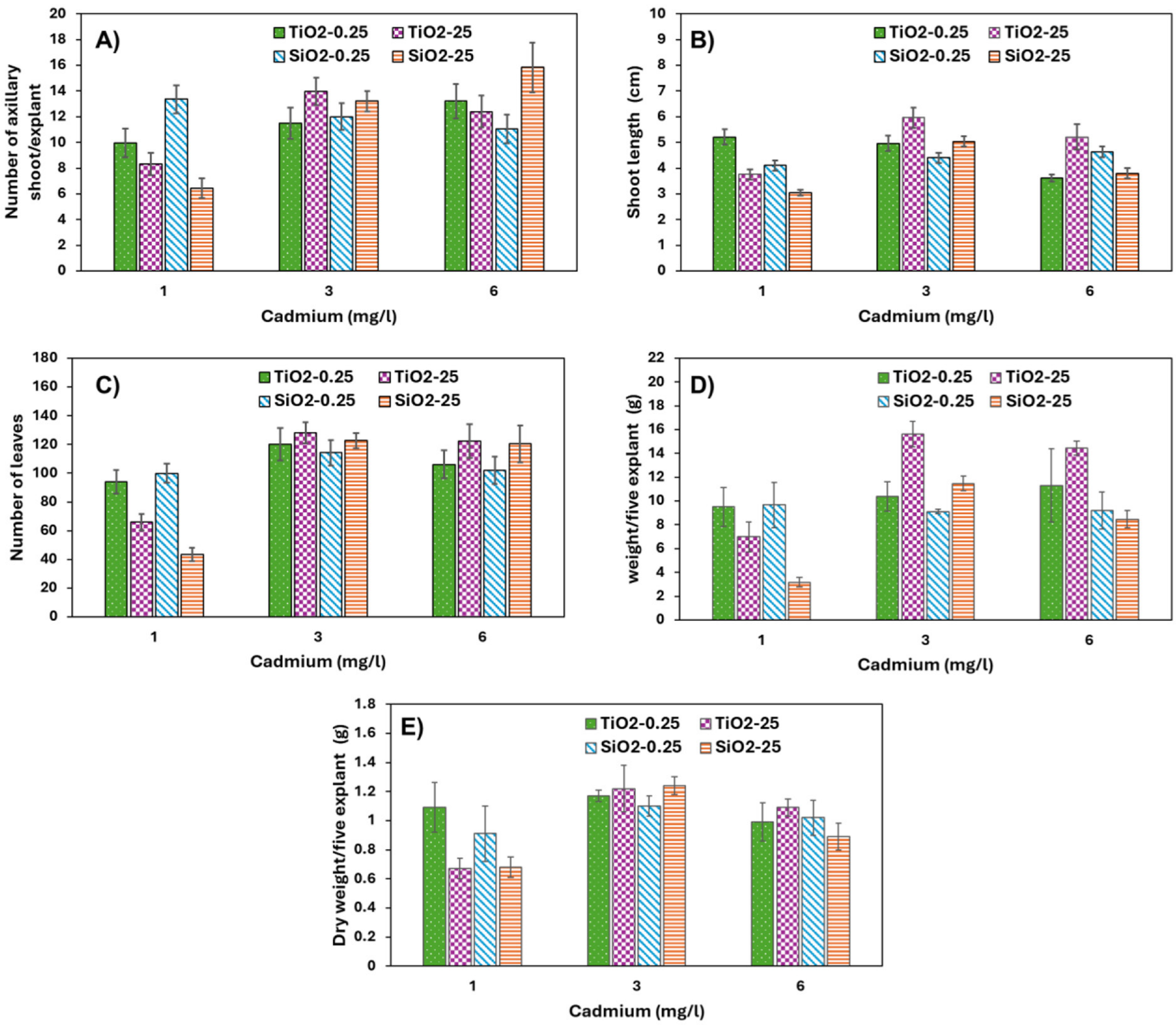

Figure 2. Influence of various concentrations of titanium and silica nanoparticles on the *in vitro* growth of *Chrysanthemum moriflium* on MS medium containing various concentrations of Cadmium after five weeks of growth period

The amount of chromium supplemented to MS with titanium or silica nanoparticles was shown to promote growth. The number of new shoots was found to increase with the increase of chromium in the presence of titanium or silica nanoparticles. The highest number of new shoots (15.6±1.02) was found on MS medium cultured on MS medium supplemented with 6.0 mg/L chromium and 25 mg/L silica nanoparticles (Figure 3). The maximum length of shoot 6.29±0.26 cm was observed in the control, and the shortest shoot (4.08±0.21) length was obtained on MS medium augmented with 3.0 mg/L chromium and 0.25 titanium. Moreover, shoot length decreased significantly with the addition of chromium in the presence of titanium and silica nanoparticles.

The number of leaves increased with the presence of chromium with and without titanium or silica nanoparticles. The highest number of leaves was detected on MS medium supplemented with 6.0 mg/L chromium and 25 mg/ L silica nanoparticles. There were significant compared with the control treatment. There were significant variations in the number of leaves affected by chromium supplementation. However, changes were only significantly high in the MS medium supplemented with chromium with the addition of titanium or silica nanoparticles. There were statistically significant increases in fresh and dry weight with the application of chromium and nanoparticles compared to the control ($p < 0.05$). Thus, maximum fresh weight was recorded on MS medium added with 1.0 mg/L chromium and 0.25 mg/L silica. The lowest dry weight (0.67±0.1) was detected on the controlled treatment. There were highly significant changes in the average

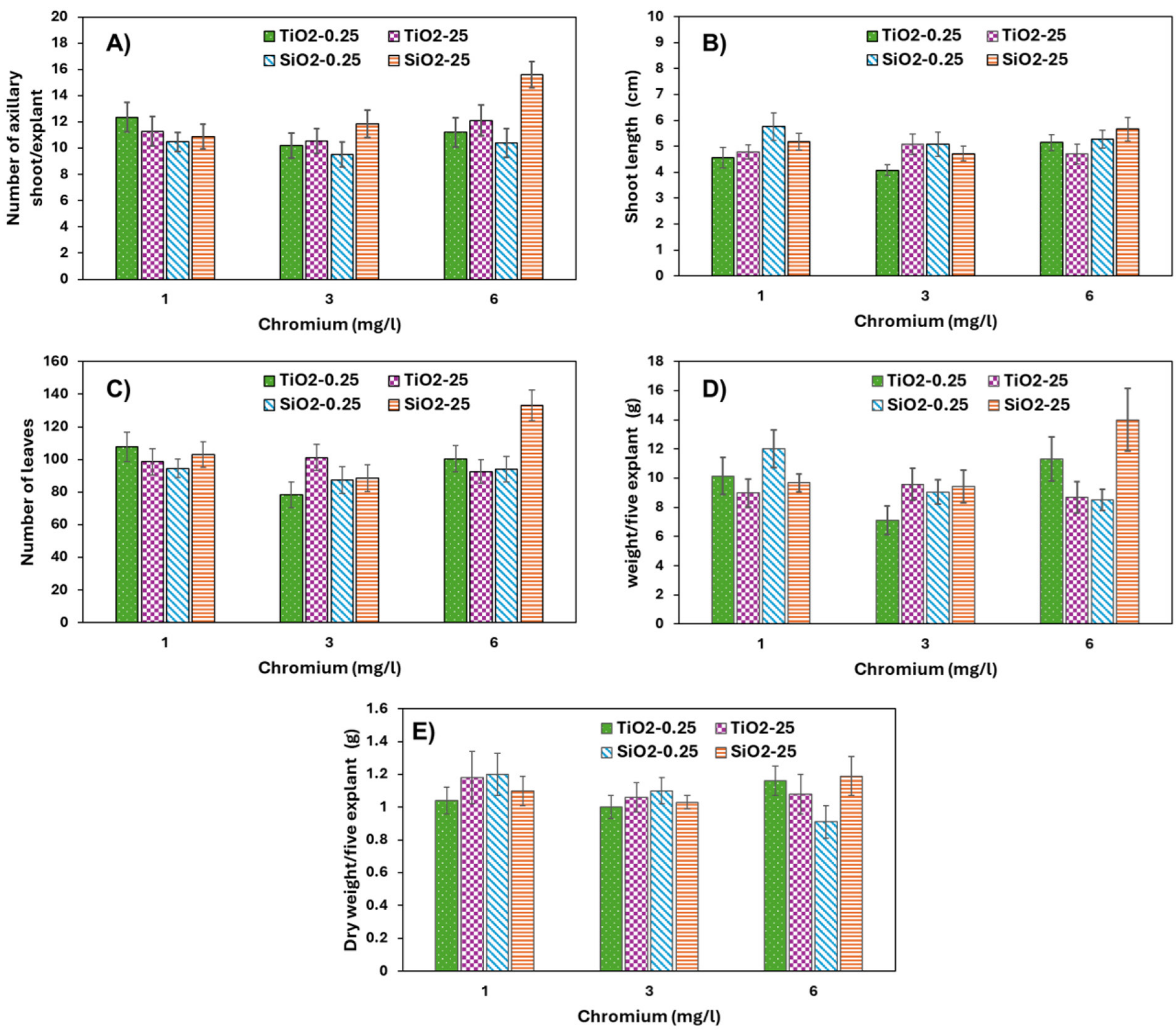

Figure 3. Influence of various concentrations of titanium and silica nanoparticles on the *in vitro* growth of *Chrysanthemum morifolium* on the MS medium containing various concentrations of Chromium after five weeks of growth period

fresh and dry weight were detected when chromium used with silica and titanium nanoparticles.

MS medium supplemented with leads, cadmium or chrome showed high differentiation in *in vitro* growth of *C. morifolium*. The increase in the leads, cadmium, or chrome metals concentration over the optimal concentration showed a detrimental growth effect on the explants. MS medium supplemented with lead, cadmium, or chrome and nanoparticles showed a high regrowth (Table 1). The increase in the leads, cadmium, or chrome concentration showed a detrimental effect on the explants. As the leads increased from 1.0 to 3.0 mg in the medium, there were no leads in *C. morifolium* ex-plants. Furthermore, an increase in leads up to 6 mg/L showed an increased lead in the explants (21.165). Moreover, increased Cr concentration in the medium did not increase Cr in the explant. On the other hand, increased lead in the medium (6 mg) caused an increase in leads in the explants (21.165). Meanwhile, cadmium absorption increased with increasing cadmium concentration in the media to reach a maximum value of (210.35) in shoot pre-exposed explants. Meanwhile, no influence on growth was obtained by the end of the experiment, most explants had normal growth after five weeks of growth periods, and no Cr was absorbed by the explant.

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Meanwhile, cadmium absorption increased with increasing cadmium concentration in the media to reach a maximum value of 210.35 mm in shoot pre-exposed explants. Meanwhile, no influence on growth was obtained by the end of the experiment; most explants had normal growth after five weeks of growth periods, and no Cr was absorbed by the explant (Table 2).

The results obtained in the current study can be transferred to greenhouse or field conditions only to a limited and conditional extent. The degree of transferability depends on the plant's biological system that is being evaluated, and how closely the *in vitro* environment is similar to the real growing conditions. The *in vitro* studies are effective for introductory selection of treatments, allowing the investigator to narrow down candidates. Moreover, the straight prediction of plant growth, yield, or long-term stress tolerance in greenhouse or field conditions is often unreliable. In addition, *in vitro* conditions are highly controlled, while greenhouse and field environments include changes in temperature, light, wind, soil structure, and biotic interactions.

DISCUSSION

C. morifolium is an important plant and shows good heavy metal accumulation. Moreover, there is no complete and extensive study of *C. morifolium* responses to nanoparticle stress in tissue culture conditions (Figures 1, 2 or 3). Therefore, this study has been conducted to study the influence of heavy metals (Cd, Cr, or Pb) and nanomaterials (titanium or silica) on the *in vitro* growth of *C. morifolium*. In the current studies, the growth responses of the three types of heavy metals showed differences based on the concentration and

Table 1. Influence of various lead and different titanium (TiO_2) nanoparticles on lead concentration in the explants after five weeks of growth periods

Titanium (TiO_2) concentration (mg/L)	Lead concentration (mg/L)	Lead concentration per explant (µg)
0.0	0.0	Nil
0.5	1.0	Nil
50	3.0	Nil
50	6.0	21.165 ±0.2

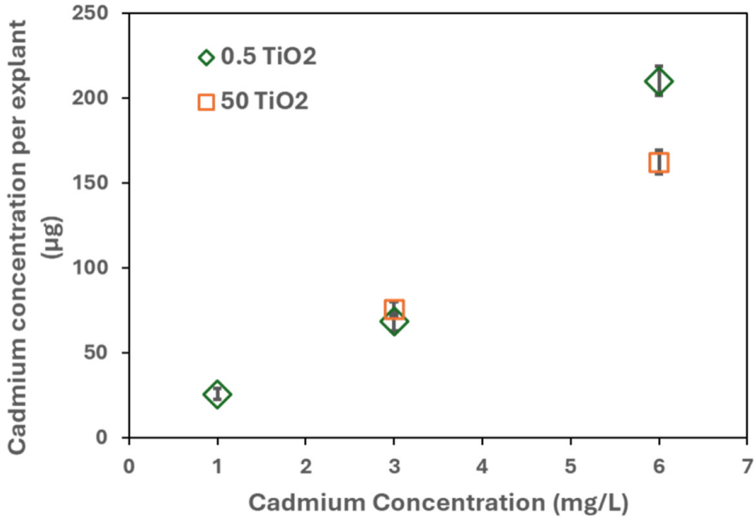

Figure 4. Influence of various cadmium and different titanium (TiO_2) dioxide on cadmium concentration in the explants after five weeks of growth periods

Table 2. Influence of various chromium and different titanium (TiO_2) dioxide on chrome concentration on microshoots of *Chrysanthemum morifolium* after a five-week growth period

Titanium (TiO_2) concentration (mg/L)	Chromium concentration (mg/L)	Chromium concentration per explant (µg)
0.0	0.0	Nil
50	1.0	Nil
50	3.0	Nil
0.5	3.0	Nil
0.5	6.0	Nil
50	6.0	Nil

combinations of nanomaterials used. Using heavy metals, the environmental systems were transferred from biotic to abiotic (Misra *et al*., 2009).

The difference in shoot formation between different types of heavy metals and nanoparticles used could be due to increased or decreased growth of the *in vitro* plant. Moreover, Prażak and Molas (2015) reported that an increase in copper (CuO) concentrations in MS increases the micropropagation rate. In addition, AL-Mayahi (2014) reported that 2.0 µM copper sulphate and 2.0 µM cobalt chloride enhanced regeneration frequency and root induction in *Phoenix dactylifera* plant. This is similar to our results, where Cd, Cr, or Pb enhanced shoot formation. The application of 150 µM $CdCl_2$ on *Momordica cymbalaria* affected growth and reduced the regeneration capability of the *in vitro* plant (Chaitanya *et al*., 2022). This may be due to the effectivity of the heavy metals on cells' totipotency, enzyme activity, and photosynthesis activity (Chen *et al*., 2015; Amari *et al*., 2017).

The application of nanoparticles increased the growth of *in vitro* plants (Figures 1, 2, and 3). This observation is similar to previous findings on *Indica rice* using 40–50 mg/L TiO_2 nanoparticles (Chutiatifit and Sutjaitorrakul, 2018). TiO_2 nanoparticles can encourage in vitro growth, improve cell division, enhance plant growth, and production of secondary metabolites (Mahmoodzadeh *et al*., 2013). Moreover, TiO_2 has confirmed its effectiveness in inducing photosynthetic gene expression (Mandeh *et al*., 2012). In addition, nanoparticles could emerge as new chemical elicitors acting as signaling agents affecting plant metabolism (Gonçalves *et al*., 2021). In addition, Gonçalves *et al*. (2021) indicated that the addition of NPs into the culture medium depended on the type of NP tested and the plant species.

The addition of ZnO NPs was found to reduce the growth of *Solanum melongena* seedlings (Thunugunta *et al*., 2018). Moreover, this is similar to a previous finding by Baskar *et al*. (2020) on *Abelmoschus esculentus*. In addition, CuO NPs at high concentrations reduced shoot and root growth in *Oryza sativa* plants (Da Costa *et al*., 2016). Chang *et al*. (2012) reported that CuO-NPs could cause genotoxicity in plant tissues if the CuO-NP amounts exceed the physiological tolerance range because Cu is indispensable to maintaining homeostasis in living systems. In this study, nanoparticles were employed to improve the *in vitro* growth of *C. morifolium* under an MS medium containing different concentrations of heavy metals. This is because nanoparticles can inhibit ethylene biosynthesis or may activate growth regulators in plants (Sarmast *et al*., 2011; Aghdaei *et al*., 2012). Kim *et al*. (2017) indicated that nanoparticles appeared as elicitors and contributed as signaling agents, prompting plant metabolism. However, various concentrations of nanoparticles have a positive effect on the induction of secondary metabolites (Fatima *et al*., 2020).

Heavy stress can affect plant growth performance. *In vitro* plants need heavy metals in small quantities during plant growth (Sarkar *et al*., 2010; Radovanovic *et al*., 2015). However, *C. morifolium* shows different tolerance to the presence of lead, cadmium, or chromium in its shoots (Table 1 or 2 and Figure 4). This is similar to a previous finding by Hatamian *et al*. (2020) on hackberry seedlings. The presence of titanium particles did not increase lead concentration in the ex-plants. Meanwhile, using cadmium and titanium showed increased cadmium concentration in the explants (Table 2). Correspondingly, the presence of cadmium and titanium increased the ability of the ex-plant to absorb heavy metals. Moreover, cadmium detected in the explant increased with increasing cadmium concentration in the media.

Increasing chromium concentration in the medium did not show an increase in chromium in the plants. It has been proposed that TiO_2 and SiO_2 interact with enzymes and inactivate them; thus, increasing chromium concentrations was not observed in the plant. Although TiO_2 and SiO_2 may stabilize chromium in the medium, making it difficult to absorb. As reported by Chavan et al. (2020), titanium also makes plants more resistant to environmental stress conditions. Titanium promotes micronutrient absorption and can replace it is deficient in the plant (Ghoto *et al*., 2020).

The physiological mechanism responsible for the stimulating effect of TiO_2 and SiO_2 nanoparticles on *Chrysanthemum morifolium* growth under heavy metal stress showed a positive effect on *in vitro* growth of *C. morifolium*, This because nanoparticles may reduce heavy metal uptake, by holding together metal ions, reducing metal bioavailability, and decreasing heavy metal uptake into the plants, changing cell wall characteristics through increasing cell rigidity. Moreover, TiO_2 and SiO_2 nanoparticles can enhance the antioxidant defense plant system by generating excess reactive oxygen species (OS), or increased superoxide dismutase enzyme (Misra *et al*., 2013; Moghadam *et al*., 2015). In addition, TiO_2 and SiO_2 nanoparticles may enhance photosynthetic activity in the plants and then increase the biomass of the plants. In addition, TiO_2 and SiO_2 may decrease oxidative stress, then protect chlorophyll and photosynthetic pigments from degradation. Furthermore, TiO_2 and SiO_2 nanoparticles may affect phytohormone levels such as abscise acid, salicylic acid, auxin, or cytokinin, which may enhance the expression of metal detoxifying enzymes.

TiO_2 and SiO_2 may result in low availability of heavy metals, so the plant may dedicated uptake system. NPS can protect plants from environmental stresses, such as heavy metals. This is because NPs can eliminate free radicals and protect plant cells from oxidative damage. In addition, NPS can withstand the impact of terpenes, ketones, aldehydes, alkaloids, and phenols (Bacilieri *et al*., 2017). Although the absence of microbe, under *in vitro* conditions, showed a change in morphology and acclimation to stress, which can change plant responses. Chavan *et al*. (2020) indicated that TiO_2 and SiO_2 make plants more resistant to environmental stress conditions. NPs influence the accumulation of salicylic acid within the plant, which increases plant biological stresses. TiO_2 and SiO_2 reduce the bioavailability of chromium, improving plant growth, biomass, yield, and carbon metabolism through enhanced chlorophyll content and reducing oxidative stress (Almohammedi *et al*., 2023). Wu *et al*. (2016) NPs decreased the activity of the enzymes superoxide dismutase, ascorbate peroxidase, and catalase. Consequently, NPs can enhance bioremediation under in vitro conditions, thus enhancing plant metabolic processes (Srivastav *et al*., 2018). Thus, using TiO_2 and SiO_2 nanoparticles at different concentrations could serve to remediate heavy metals. Furthermore, the presence TiO_2 and SiO_2 the phytohormones in the medium that are involved in the *in vitro* grwoth and development and plant defense responses to stress.

CONCLUSIONS

In this study, *Chrysanthemum morifolium* was tested under in vitro conditions for its response to different concentrations of Pb, Cd, and Cr. Moderate levels of these metals supported plant growth, but higher concentrations inhibited development. The addition of TiO_2 nanoparticles mitigated cadmium toxicity in plant cultures by modulating photosynthetic and antioxidative systems, thereby improving physiological responses under heavy metal stress. Nanoparticle-supplemented *in vitro* systems may be helpful for stress screening and increasing biomass production in the presence of heavy metals. Because *C. morifolium* can tolerate and accumulate certain heavy metals, it could be considered for phytoremediation research. It is essential to adjust nanoparticle and metal doses to prevent toxic effects, and more research is needed to understand the underlying physiological mechanisms and to test these findings in conditions outside the laboratory.

REFERENCES

1. Aghdaei M, Salehi H, Sarmas MK. (2012). Effects of silver nanoparticles on *Tecomella undulata* (Roxb.) Seem. Micropropagation. *Advances in Horticultural Science. 26*(1): 21–24. https://doi.org/10.13128/ahs-12748
2. Al Shhab M., Shatnawi M., Abu Romman, S., Majdalawi M and Odat N. (2021). Micropropagation and *in vitro* conservation of *Ruta graveolens. Research on Crops Journal. 22*(2): 398–409. https://doi.org/10.31830/2348-7542.2021.085
3. AL-Mayahi AMW. (2014). Effect of copper sulfate and cobalt chloride on the growth of the *in vitro* culture tissues for date palm (*Phoenix dactylifera*) cv Ashgar. *American Journal of Agricultural and Biological Sciences. 9*(1): 6–18. https://doi.org/10.3844/ajabssp.2014.6.18
4. Almohammedi O., Sekhi Y and Ismail M. (2023). A review of nano fertilization and its role on growth, yield and quality characteristics of fruit trees. *Tikrit J. Agric. Sci. 23*(1): 158–167.
5. Alrayes LMH., Shatnawi MA and Al Khateeb WM. (2018). *In vitro* studies on callus induction of *Moringa peregrina* (Forsk) Fiori and antifungal activity of plant extract. *Jordan Journal of Agricultural Science. 14*(2): 146–156.
6. Amari T., Ghnaya T. and Abdelly C. (2017). Nickel, cadmium, and lead phytotoxicity and potential of halophytic plants in heavy metal extraction. *South Africa Journal of Botany 111*: 99–110. https://doi.org/10.1016/j.sajb.2017.03.011
7. Arora JS. (1990). *Introductory Ornamental Horticulture*. Kalyani Publishers, New Delhi. 1990; 8.
8. Bacilieri FS., Vasconcelos ACP., de-Lana RMQ., Mageste JG., Torres JLR. (2017). Titanium (Ti) in plant nutrition – A review. *Aust. J. Crop Sci. 11*(4): 382–386.
9. Baskar V., Safia N., Preethy K and Dhivya S., Thiruvengadam M and Sathishkumar R. (2020). A comparative study of phytotoxic effects of metal oxide (CuO, ZnO, and NiO) nanoparticles on *in vitro* grown *Abelmoschus esculentus*. *Plant Biosystems. 155*: 374–383. https://doi.org/10.1080/11263504.2020.1753843
10. Baskaran P. and Jayabalan N. (2005). Role of basal media, carbon sources, and growth regulators in micropropagation of *Eclipta alba* – a valuable medicinal herb. *Science Journal. 5*(2): 469482.
11. Chaitanya G., Pavani C and Shasthree T. (2022). Effect of heavy metals on *in vitro* growth and development of the *Momordica cymbalaria* Fenz. *International Journal of Environmental Science and Technology*. https://doi.org/10.1007/s13762-022-04437-9
12. Chang YN., Zhang M., Lin Xia L., Zhang J. and Xing G. (2012). The toxic effects and mechanisms of CuO and ZnO nanoparticles. *Materials 5*(12): 2850–2871. https://doi.org/10.3390/ma5122850
13. Chavan S., Sarangdhar V. and Nadanathangam V. (2020). Toxicological effects of TiO_2 nanoparticles on plant growth promoting soil bacteria. *Emer. Cont. 6*: 87–92
14. Chen M., Zhang LL., Li J., He XJ and Cai JC. (2015). Bioaccumulation and tolerance characteristics of a submerged plant (*Ceratophyllum demersum* L.) exposed to toxic metal lead. *Ecotoxicology and mental Safety. 122*: 313–321. https://doi.org/10.1016/j.ecoenv.2015.08.007
15. Da Costa MVJ. and. Sharma PK. (2016). Effect of copper oxide nanoparticles on growth, morphology, photosynthesis, and antioxidant response in *Oryza sativa*. *Photosynthetica. 54*(1): 110–119. https://doi.org/10.1007/s11099-015-0167-5
16. Fatimaa K., Abbas S. R., Ziaa M., Sabirc S. M., Khand R. T., Khane A. A., Hassane Z and Zamanf R. (2020). Induction of secondary metabolites on nanoparticles stress in callus culture of *Artemisia annua* L. *Brazilian Journal of Biology. 81*(2): 474–483. https://doi.org/10.1590/1519-6984.23293.
17. Ghoto K., Simon M., Shen ZJ., Gao GF., Li PF., Li H. and Zheng HL. (2020). Physiological and root exudation response of maize seedlings to TiO_2 and SiO_2 nanoparticles exposure. *Bio. Nano. Sci. 10*(2): 473–481.

18. Gonçalves S., Mansinhos I., Rodríguez-Solana R. Pereira-Caro G., Moreno-Rojas JM., Romano, A. (2021). Impact of metallic nanoparticles on *in vitro* culture, phenolic profile and biological activity of two Mediterranean *Lamiaceae* Species: *Lavandula viridis* L'Hér and *Thymus lotocephalus* G. López and R. Morales. *Molecules. 26*: 6427. https:// doi.org/10.3390/molecules26216427
19. Gupta AK. and Sinha S. (2007). Phytoextraction capacity of the *Chenopodium album* L. grown on soil amended with tannery sludge. *Bioresource Technology. 98*(2): 442–446. https://doi.org/10.1016/j.biortech.2006.01.015. Epub 2006 Mar 15.
20. Gupta B and Huang B. (2014). Mechanism of salinity tolerance in plants: Physiological, biochemical, and molecular characterization. *International Journal of Genome. 18*. Article ID 701596. https://doi.org/10.1155/2014/701596
21. Hahn EJ, JH Bae, Lee YB. (1998). Growth and leaf surface characteristics of *Chrysanthemum plantlets* in micropropagation and microponic system. *J. Kor. Soc. Hort. Sci. 39*(6): 838–842.
22. Hatamian M., Nejad AR., Kafi M., Souri MK and Shahbaz K. (2020). Nitrate improves hackberry seedling growth under cadmium application. *Heliyon 6*(1): e03247. https://doi.org/10.1016/j.heliyon.2020.e03247
23. Jadczak P., Kulpa D., Bihun M. and Przewodowski W. (2019). Positive effect of Ag NPs and Au NPs in *in vitro* cultures of *Lavandula angustifolia* Mill. *Plant Cell, Tissue, and Organ Culture. 139*: 191–197. https://doi.org/10.1007/s11240-019-01656-w
24. Kandiel, TA., Dillert, R. and Bahnemann, D. (2012). Titanium dioxide nanoparticles and nanostructures. *Curr. Inorg. Chem*. *2*: 94–114. https://doi.org/10.2174/1877944111202020094
25. Karim MZ., Amin M., Asaduzzaman N., Islam S., Hossin F. and Alam R. (2002). Rapid multiplication of *Chrysanthemum morifolium* through *in vitro* Culture. *Pakistan Journal of Biological Sciences. 5*: 1170–1172.
26. Kim DH., Gopal J. and Sivanesan, I. (2017). Nanomaterials in plant tissue culture: The disclosed and undisclosed. *RSC Advances. 7*: 36492–36505. https://doi.org/1039/C7RA07025J
27. Kumar I and Sharma K. (2018). Production of secondary metabolites in plants under abiotic stress: An overview. *Significances of Bioengineering and Biosciences. 2*(4): 196–200. http://dx.doi.org/10.31031/sbb.2018.02.000545
28. Mahmoodzadeh H., Nabavi M. and Kashefi H. (2013). Effect of nanoscale titanium dioxide particles on the germination and growth of canola (*Brassica napus*). *Journal of Ornamental and Horticultural Plants. 3*(1): 25–32.
29. Mandeh M., Omidi M. and Rahaie M. (2012). *In vitro* influences of TiO_2 nanoparticles on barley (*Hordeum vulgare* L.) tissue culture. *Biological Trace Element Research. 150*: 376–380. https://doi.org/10.1007/s12011-012-9480-z
30. Misra AN., Misra M and Singh R. (2013). Nanotechnology in agriculture and food industry. *Int. J. Pure Appl. Sci. Technol. 16*: 1–9.
31. Misra V., Tiwari A., Shukla B and Seth CS. (2009). Effects of soil amendments on the bioavailability of heavy metals from zinc mine tailings. *Environmental Monitoring and. Assessment. 155*(4): 467–475. https://doi.org/10.1007/s10661-008-0449-5
32. Moghadam M. M., Motalleb G. and Fakheri BA. (2015). Nanoparticles and plant Biotechnology. *Research in Biotechnology, 6*(5): 1–15. www.researchinbiotechnology.com
33. Mousavi SR. and Rezaei M. (2011). Nanotechnology in agriculture and food production. *Journal of Applied Environmental and Biological Sciences. 1*: 414–419.
34. Murashige T. and Skoog F. (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Plant Physiology. 15*: 473–497. https://doi.org/10.1111/j.1399-3054.1962.tb08052.x
35. Natsheh, IY., Elkhader, MT., Al-Bakheit, AA., Alsaleh, MM., El-Eswed, BI., Hosein, NF., Duaa, K. and AlbadawiI, DK. (2023). Inhibition of acinetobacter baumannii biofilm formation using dif ferent treatments of silica nanoparticles. *Antibiotics*. *12*: 1–18. https://doi.org/10.3390/ antibiotics1209136
36. Osman AE, Shatnawi M., Shibli R., Majdalawi M., Al Tawaha AR and Qudah T. (2021). Salts induced salinity and *in vitro* multiplication of *Paronychia argentea. Ecological Engineering and Environmental Technology.22* (5): 55–64. https://doi.org/10.12912/27197050/139408
37. Prasad TN., Sudhakar P., Sreenivasulu Y., Latha P., Munaswamy V., Reddy KR and Pradeep T. (2012). Effect of nanoscale zinc oxide particles on the germination, growth and yield of peanut. *Journal of Plant Nutrition. 35*(6): 905–927. https://doi.org/10.1080/01904167.2012.663443
38. Pratibha Misra, Datta SK. (2007). Standardization of in vitro protocol in *Chrysanthemum* cv. Madam E Roger for development of quality planting material and to induce genetic variability using γ –radiation. *Indian Journal of Biotechnology. 6*: 121–124.
39. Prażak R. and Molas J. (2015). Effect of copper concentration on micropropagation and accumulation of some metals in the *Dendrobium Kingianum* bidwill Orchid. *Journal of Elementology. 20*(3): 693–703. https://doi.org/10.5601/jelem.2014.19.4.748
40. Radovanovic V., Djekic I and Zarkovic B. (2015). Characteristics of cadmium and lead accumulation and transfer by *Chenopodium Quinoa* Will, *Sustainability. 12*(9): 3789–3798. https://doi.org/10.3390/su12093789

41. Rahmawati M., Mahfud C., Risuleo G. and Jadid N. (2022). Nanotechnology in plant metabolite improvement and in animal welfare. *Applied Sciences. 12*(2): 838. https://doi.org/10.3390/app12020838
42. Sarkar T., Vijay Anand KG and Reddy MP. (2010). Effect of nickel on regeneration in *Jatropha curcas* L. and assessment of genotoxicity using RAPD markers. *Biomaterials. 23*: 1149–1158. https://doi.org/10.1007/s10534-010-9364-7
43. Sarmast MK., Niazi A., Salehi H. and Abolimoghadam A. (S2015). Silver nanoparticles affect ACS expression in *Tecomella undulata in vitro* culture. *Plant Cell Tissue, and Organ Culture. 121*: 227–236, https://doi.org/10.1007/s11240-014-0697-8
44. Saxena R., Tomar RS. and Kumar, M. (2016). Exploring nano-biotechnology to mitigate abiotic stress in crop plants. *Journal of Pharmaceutical Sciences and Research. 8*(9): 974–980.
45. Shatnawi MA. (2013). Multiplication and cryopreservation of Yarrow (*Achillea millefolium* L., Astraceae). *Journal of Agriculture Science and Technology. 15*: 163–173. http://jast.modares.ac.ir/article-23-769-en.html
46. Shatnawi MA., Shibli RA., Abu-Romman SM., Al-Mazra'awi MS., Al Ajlouni ZI., Shatanawi, WA. and Odeh WH. (2011). Clonal propagation and cryogenic storage of the medicinal plant *Stevia rebaudiana*. *Spanish Journal of Agriculture Research. 9*(1): 213–220. https://doi.org/10.5424/SJAR/20110901-021-10
47. Shatnawi M. A., Shibli R. A., Qrunfleh I., Bataeineh K. and Obeidat M. (2007). *In vitro* propagation and cryopreservation of *Prunus avium* using vitrification and encapsulation dehydration methods. *Journal of Food, Agriculture and Environment. 5*(2): 204–208. EID: 2-s2.0-34249802311
48. Shatnawi MA., Shibli RA., Shahrour WG., Al-Qudah TS. and Abu-Zahra T. (2019). Micropropagation and conservation of Fig (*Ficus carica* L.). *Journal of Advances in Agriculture. 10*: 1669–1679. https://doi.org/10.24297/jaa.v10i0.8160
49. Shatnawi M., Abubaker S., Odat, N., Al-Tawaha AR. and Majdalawi, M. (2021). Antimicrobial activity and micropropagation of selected Jordanian medicinal plant. *Journal of Ecological Engineering. 22*(6): 151–158. https://doi.org/10.12911/22998993/137679
50. Shatnawi M., Al-Fauri A., Megdadi H., Al-Shatnawi M. K., Shibli R.A., Abu-Romman S and Al-Ghzawi A. (2010). *In vitro* multiplication of *Chrysanthemum morifolium* Ramat and its responses to NaCl induced salinity. *Jordan Journal of Biological Sciences. 3*(3): 101–110.
51. Shatnawi M., Faour, A., Al-Mazraawi M., Shibli R. and Makhadmeh I. (2009). Tissue culture and salt stress in *Chrysanthemum morifolium*. *Acta Horticulturae. 829*: 189–196. https://doi.org/10.17660/ActaHortic.2009.829.27
52. Shatnawi M., Majdawi M., Shahrour W., R. Abu-Zahra T. and Al-Tawaha, A. (2022). Germination and *in vitro* propagation of *Gundelia tournefontii* as an important medicinal plant. *Ecological Engineering and Environmental Technology. 23*(1): 57–64. https://doi.org/10.12912/27197050/143006
53. Shatnawi M., Osman N. A., Shibli R., Odat N., Al-Tawaha AR. Qudah T. and Majdalawi M. (2021). Effect of heavy metal on the *in vitro* growth of *Paronchia argentea* and its antimicrobial activity. *Ecological Engineering and Environmental Technology. 22*(3): 142–151. https://doi.org/10.12912/27197050/135655
54. Shibli R., Mohusaien R., Abu-Zurayk R., Tamara Qudah T. and Tahtamouni R. (2022). Nanoparticles (Ag NPs) boost the mitigation powers of *Chenopodium Quinoa* (Q6 Line) grown under *in vitro* salt-stressing conditions. *Water. 14*(19): 1–13. https://doi.org/10.3390/w14193099
55. SPSS. (2017). Complex samples, SPSS INC., Chicago ILL: USA.
56. Teixeira da Silva JA., Fukai S. (2003). Chrysanthemum organogenesis through thin cell layer technology and plant growth regulator control. *Asian J. Plant Sci. 2*(6): 505–514.
57. Thunugunta T., Reddy AC., Seetharamaiah SK., Hunashikatti LR., Chandrappa SG., Kalathil NC and Reddy, LRDC. (2018). Impact of Zinc oxide nanoparticles on eggplant (*Solanum melongena*): Studies on growth and the accumulation of nanoparticles. *IET Nanobiotechnology. 12*: 706–713. https://doi.org/10.1049/iet-nbt.2017.0237
58. Wu J., Xie Y., Fang Z., Cheng W. and Tsang PE. (2016). Effects of Ni/Fe bimetallic nanoparticles on phytotoxicity and polybrominated translocation diphenylethers of in contaminated soil. *Chemosphere 162*: 235–242.