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STUDY ON REPLACING SOME MICRONUTRIENT METALLIC SALTS WITH METALLIC NANOPARTICLES IN THE MICROPROPAGATION OF ORNAMENTAL PLANTS (CHRYSANTHEMUM, GERBERA, AND AFRICAN VIOLET)

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INTRODUCTION

Rationale for Selecting the Topic

The 21st century has witnessed an explosion of nanotechnology across all aspects of life, from cosmetics, textiles, household goods, and food to the environment and healthcare. Life sciences in general, and biology and biotechnology in particular, including biotechnology in plant breeding, are also leveraging and adopting these advances to promote development.

Current research on the application of nanoparticles (NPs) in *in vitro* culture mainly focuses on adding NPs to culture media to enhance morphogenesis, growth, improve crop yield, improve gene transfer efficiency, and protect plants from environmental impacts. However, there remains a gap in the application of NPs as a nutrient source to replace metal salts in *in vitro* culture media.

Therefore, the topic "study on replacing some micronutrient metallic salts with metallic nanoparticles in the micropropagation of ornamental plants (chrysanthemum, gerbera, and african violet)" was chosen to deeply investigate the potential of NPs to replace metal salts in Mourashige et Skoog (1962) (MS) medium [1]. The topic focuses on investigating the effectiveness of iron nanoparticles (FeNPs), molybdenum nanoparticles (MoO_3NPs), and cobalt nanoparticles (CoNPs) in replacing the corresponding trace metal salts in MS medium in the process of asexual propagation and subsequent growth in the nursery stage of flowering plants such as chrysanthemums, marigolds, and lily of the valley. The research results will open up a new and groundbreaking direction in *in vitro* culture.

Objectives of the study

General objective

the research "study on replacing some micronutrient metallic salts with metallic nanoparticles in the micropropagation of ornamental plants (chrysanthemum, gerbera, and african violet)" was conducted with the aim of evaluating the effectiveness of replacing trace metal salts ($FeSO_4.7H_2O$, $Na_2MoO_4.2H_2O$, $CoCl_2.6H_2O$) in MS culture medium with corresponding NPs (FeNPs, MoO_3NPs, and CoNPs) in three plant species: chrysanthemum, gerbera, and lily of the valley. From this, the goal is to use

these NPs to replace trace metal salts in MS culture medium – optimizing the growth, development, and quality of seedlings.

Specific objectives

Micronutrients are indispensable components throughout the life cycle of plants. This thesis investigates the roles of MoO_3 NPs (in callus induction, shoot regeneration, rapid shoot multiplication, growth, and rooting of chrysanthemums), CoNPs (in shoot regeneration, rapid shoot multiplication, growth, and rooting of petunias and marigolds; and rooting of chrysanthemums), and FeNPs (in rooting of chrysanthemums, marigolds, and petunias). Furthermore, experiments were conducted to evaluate the influence of NPs on photosynthesis, antioxidant enzyme activity, hydrolytic enzyme activity, and the uptake of macronutrients and micronutrients during plant culture.

Abnormalities in in vitro culture, such as ethylene accumulation, vitreous humor issues, browning of explants, and oxidative stress, reduced the efficiency of tissue culture by decreasing seedling quality or causing explant death. Besides using NPs as a nutrient source, the study also aimed to evaluate the impact of CoNPs in reducing abnormalities in in vitro culture (money plant and lily of the valley).

Residues of metal salts absorbed by plants during in vitro culture can have positive or negative effects on seedling growth and development. This study also generally assessed the effects of NP residues (MoO_3NPs, CoNPs, FeNPs) on the studied plant groups during rooting and development in the nursery. The aim was to use NPs as an effective alternative nutrient source in MS culture media.

New contributions of the dissertation

This thesis systematically demonstrated the ability to replace traditional trace metal salts in MS medium with metal nanoparticles (FeNPs, MoO_3NPs, CoNPs) in three flowering plant species (chrysanthemum, marigold, and lily of the valley), thereby opening a new approach to improving in vitro culture media.

- Determined the optimal concentration of each type of metal nanoparticle for each developmental stage (callus induction, shoot regeneration, rapid multiplication, and rooting) in each plant species.

• Provided evidence of the physiological and biochemical mechanisms of metal nanoparticles, through increased activity of antioxidant enzymes (SOD, CAT, APX), decreased activity of hydrolytic enzymes (cellulase, pectinase), and limited ethylene accumulation, thus reducing physiological disturbances in in vitro culture. • Clarify the role of metal nanoparticles in enhancing the absorption of mineral nutrients (macro and micronutrients), contributing to improved growth, development, and seedling quality.

• Demonstrate that seedlings obtained from treatments using metal nanoparticles have better adaptability, stable growth, and improved flower quality under nursery conditions, confirming the practical application potential of this research direction.

CHAPTER 1. LITERATURE REVIEW

The dissertation has reviewed and summarized six main topics related to: (1) MS culture medium; (2) Some limitations of plant tissue, cell, and organ culture methods; (3) Metallic nanoparticles; (4) Metallic nanoparticles in plant clonal propagation; (5) Iron, cobalt, and molybdenum nanoparticles in clonal propagation; (6) Overview of the study subjects.

CHAPTER 2: SUBJECTS, CONTENT, AND RESEARCH METHODS

2.1. Subjects

2.1.1. Plant Material

The plant materials used in the experiments were 30-day-old *in vitro* culture samples, including leaves, stem sections, and shoot tips of Chrysanthemums (*Chrysanthemum morifolium* Ramat cv. "Jimba"), Gerbera (*Gerbera jamesonii* "Revolution Yellow"), and African violet (*Saintpaulia ionantha* Wendl.). The plant samples were uniform in size and were propagated at the Plant Molecular Biology and Plant Breeding - VAST

2.1.2. Metallic Nanoparticle Solutions

The metallic nanoparticle solutions (FeNPs, CoNPs, and MoO_3NPs) were provided by the Institute of Environmental Technology (VAST, Hanoi, Vietnam).

2.1.3. Equipment, instruments, and chemicals

2.1.4. Culture conditions

2.1.5. Culture medium

2.2. RESEARCH CONTENT

Content 1: Study of replacing $Na_2MoO_4.2H_2O$ with MoO_3NPs in the clonal propagation of Chrysanthemums.

Content 2: Study of replacing $CoCl_2.6H_2O$ with CoNPs in the clonal propagation of African violet, Gerbera, and Chrysanthemums.

Content 3: Study of replacing $FeSO_4.7H_2O$ with FeNPs in the clonal propagation of Chrysanthemums, Gerbera, and African violet.

2.3. RESEARCH METHODS

2.3.1. Experimental Layout

2.3.1.1. Study of replacing $Na_2MoO_4.2H_2O$ with MoO_3NPs in the micropropagation of Chrysanthemums

2.3.1.2. Study of replacing $CoCl_2.6H_2O$ with CoNPs in the micropropagation of Gerbera and African violet.

2.3.1.3. Study of replacing $FeSO_4.7H_2O$ with FeNPs in the micropropagation of Chrysanthemums, Gerbera, and African violet.

2.3.2. Some Research Methods

2.3.2.1. Determination of antioxidant enzyme activity

2.3.2.2. Metal absorption

2.3.2.3. Ethylene in culture vessels

2.3.2.4. Hydrolytic enzyme activity

2.3.2.5. Plant morphological anatomy

2.3.2.6. Statistical analysis

2.3.2.7. Method for converting salt concentration to nano-metal concentration

2.4. RESEARCH LOCATION AND DURATION

2.4.1. Research location

2.4.2. Research duration

CHAPTER 3. RESULTS AND DISCUSSION

3.1. Study of replacing $Na_2MoO_4.2H_2O$ with MoO_3NPs in the clonal propagation of Chrysanthemums

3.1.1. Callus induction and shoot regeneration through leaf explant culture

The results observed indicate that 100% of the leaf explants formed callus when cultured on CS0 (+) medium or MoO_3NPs treatments. In contrast, leaf explants cultured on CS0 medium without $Na_2MoO_4.2H_2O$ (-) did not show any callus induction (Table 3.1; Figure 3.1).

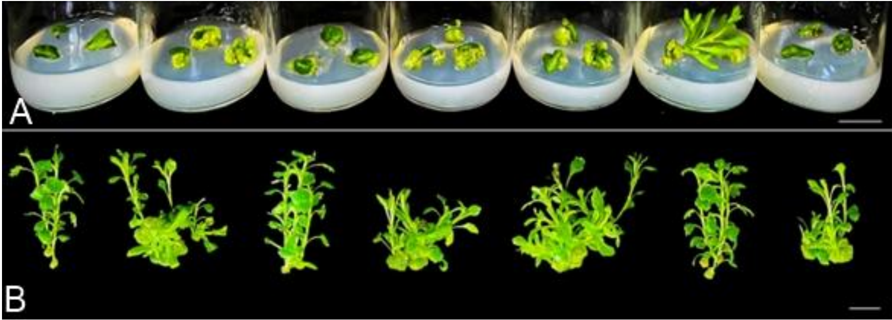

Figure 3.1. Effect of MoO_3NPs on shoot regeneration from leaf explants and shoot multiplication from stem segments of Chrysanthemum after 30 days of culture. (-); (+); 37.25; 74.5; 149; 223.5; 298 µg/L; left to right. Scale bar: 2 cm. **A:** Shoot regeneration from leaf explants; **B:** Shoot multiplication from stem segments.

Table 3.1. Effect of MoO_3NPs on callus induction and shoot regeneration from leaf explants of Chrysanthemum after 30 days of culture.

<table>
<tr><th>MoO_3NPs (µg/L)</th><th>Callus induction rate (%)^x</th><th>Shoot regeneration rate (%)^y</th><th>Number of shoots /sample</th><th>Shoot height (cm)</th><th>Fresh weight (mg)</th></tr>
<tr><td>(-)</td><td>0,00 ± 0,00b*</td><td rowspan="5">0,00 ± 0,00b</td><td rowspan="5">0,00 ± 0,00b</td><td rowspan="5">0,00 ± 0,00b</td><td>163 ± 2,08e</td></tr>
<tr><td>(+)</td><td rowspan="6">100 ± 0,00a</td><td>183 ± 1,15e</td></tr>
<tr><td>37,25</td><td>770 ± 2,89d</td></tr>
<tr><td>74,50</td><td>800 ± 5,68d</td></tr>
<tr><td>149,00</td><td>960 ± 6,56b</td></tr>
<tr><td>223,50</td><td>33,33 ± 0,00a</td><td>1,00 ± 0,00a</td><td>1,14 ± 0,06a</td><td>1210 ± 3,22a</td></tr>
<tr><td>298,00</td><td>0,00 ± 0,00</td><td>0,00 ± 0,00b</td><td>0,00 ± 0,00b</td><td>890 ± 2,65c</td></tr>
</table>

*(-) Negative control: Medium without $Na_2MoO_4.2H_2O$; (+) Positive control: Medium with $Na_2MoO_4.2H_2O$; *The average values ± SD (Standard Deviation) and values with different letters indicate a significant difference between treatments; ^{x}: 15 days; ^{y}: 30 days.*

Table 3.2. Effect of MoO_3NPs on shoot multiplication of stem segment samples of Chrysanthemum after 30 days of culture.

MoO_3NPs (µg/L)	Shoot regeneration rate (%)	Number of shoots/sample			Shoot height (cm)	Fresh weight (mg)
		Total	≥ 2 cm	< 2 cm		
(-)	100	3,33 ± 0,06d*	1,33 ± 0,58c	2,00 ± 1,00b	2,40 ± 0,10c	26 ± 6,02e
(+)		7,33 ± 1,53ab	2,67 ± 0,58b	4,67 ± 1,15a	2,77 ± 0,06b	69 ± 6,02d
37,25		4,67 ± 0,58cd	0,33 ± 0,58c	4,33 ± 0,58a	1,60 ± 0,10e	116 ± 17,10bc
74,50		6,00 ± 1,00bc	0,67 ± 0,58c	5,33 ± 1,53a	1,70 ± 0,10e	112 ± 2,89c
149,00		7,00 ± 1,00ab	5,00 ± 1,00a	2,00 ± 1,00b	3,23 ± 0,25a	187 ± 9,71a
223,50		7,00 ± 1,00ab	2,67 ± 0,58b	4,33 ± 0,58a	2,10 ± 0,10d	137 ± 4,73b
298,00		8,00 ± 1,00a	2,67 ± 0,58b	6,00 ± 1,14a	1,63 ± 0,06e	137 ± 23,39b

**The average values ± SD (Standard Deviation) and values with different letters indicate a significant difference between treatments*

Table 3.3. Effect of MoO_3NPs on shoot multiplication of apical shoot explants of Chrysanthemum after 30 days of culture

MoO_3NPs (µg/L)	Number of shoots	Shoot height (cm)	Number of leaves	Number of nodes	Leaf length (cm)	SPAD	Fresh weight (mg)
(-)	1,00 ± 0,00b*	3,17 ± 1,00b	8,67 ± 4,67bc	3,67 ± 0,58c	1,10 ± 0,00ab	14,00 ± 4,36b	23 ± 4,88b
(+)	1,67 ± 0,58b	4,90 ± 0,36a	12,00 ± 7,00ab	4,67 ± 0,58c	1,17 ± 0,15ab	29,07 ± 4,29a	63 ± 14,71ab
37,25	1,67 ± 0,58b	3,67 ± 0,58b	7,67 ± 3,37c	7,00 ± 0,00b	1,17 ± 0,06ab	15,00 ± 5,53b	26 ± 1,64b
74,50	1,67 ± 0,58b	5,13 ± 0,32a	10,33 ± 8,00ab	8,00 ± 0,00ab	1,20 ± 0,20ab	29,60 ± 4,29a	74 ± 12,92a
149,00	3,00 ± 0,00a	5,40 ± 0,17a	14,00 ± 8,67a	8,67 ± 0,58a	1,30 ± 0,17a	31,17 ± 12,97a	98 ± 6,73a
223,50	1,00 ± 0,00b	3,10 ± 0,56b	8,67 ± 0,58bc	5,00 ± 0,00c	1,07 ± 0,06ab	25,93 ± 8,59ab	51 ± 1,64ab
298,00	1,00 ± 0,00b	2,93 ± 0,81b	6,67 ± 0,58c	4,33 ± 0,58c	0,97 ± 0,15b	24,07 ± 6,95ab	19 ± 2,95b

**The average values ± SD (Standard Deviation) and values with different letters indicate a significant difference between treatments*

Figure 3.2. The effect of MoO_3NPs on shoot multiplication of shoot tips of Chrysanthemum after 30 days of culture. (-); (+); 37.25; 74.50; 149,00; 223.50; 298,00 µg/L; left to right. *Scale = 2 cm.*

3.1.2. Shoot multiplication of stem segments and shoot tips of Chrysanthemum cultivated on media replacing $Na_2MoO_4.2H_2O$ with MoO_3NPs

For the stem segment samples, all explants cultured on media supplemented with MoO_3NPs and the control (with/without $Na_2MoO_4.2H_2O$) showed 100% shoot regeneration after 30 days of culture (Table 3.2; Figure 3.1).

Antioxidant Enzymes

In the shoot regeneration stage from leaf explants, the optimal effect of MoO_3NPs in enhancing the activity of antioxidant enzymes was also observed in this study. The antioxidant enzyme activity in the treatment supplemented with 149 µg/L MoO_3NPs was better than the control (+) (except for SOD) and other treatments. Plant growth also decreased similarly when Mo was not supplemented in the nutrient medium, which reflected a significant decrease in the activity of the antioxidant enzyme system. Additionally, the activity of these enzymes was inhibited due to excess Mo (Figure 3.2A).

For the shoot multiplication stage from stem segments, similar results were observed in the Chrysanthemum shoot multiplication experiment, where the SOD, CAT, and APX enzyme concentrations decreased due to either excess or deficiency of Mo in the culture medium (Figure 3.2B). In contrast, the optimal concentration (control (+) and 149 µg/L MoO3NPs) showed a positive effect not only on growth but also on physiological and biochemical indicators (Figure 3.2B).

Mo residue in Chrysanthemum cultivated on media supplemented with MoO_3NPs

Mo residue increased gradually with the concentration of Mo in the culture medium. Mo residue was still detected even when the samples were cultured on media without $Na_2MoO_4.2H_2O$ (treatment (-)), indicating that Mo accumulation remained from the previous culturing stage. However, this Mo concentration was insufficient for the growth and development of the Chrysanthemum shoots. Additionally, this study demonstrated that nutrients (Ca, Mg, K, Cu, Fe) were absorbed more quickly by plant samples when in nanoparticle form. These results show that replacing MoO_3NPs for $Na_2MoO_4.2H_2O$ in the culture medium enhanced the absorption capacity, providing micronutrient sources for plants to support growth and other vital activities.

Mineral absorption of chrysanthemum cultivated on media supplemented with MoO_3NPs

The optimal results were recorded in the treatment with 149 µg/L MoO_3NPs. Although the Mo concentration in the control treatment was equivalent to that in the 149,00 µg/L MoO_3NPs treatment, the nutrient absorption efficiency in this treatment was not optimal. Therefore, at the nano level, Mo had a more optimal effect on the plant's mineral absorption capacity. However, excessively high concentrations of MoO_3NPs (223.50 and 298,00 µg/L) were toxic to the Chrysanthemum plants, causing a decline in nutrient absorption capacity.

The Chrysanthemum shoots in the treatment (-) showed the lowest mineral absorption compared to the other treatments. In this study, the mineral absorption capacity (Ca, Mg, K, Cu, Fe) increased with the increasing MoO_3NPs in the culture medium (37.25 – 149,00 µg/L). In the 149,00 µg/L MoO_3NPs treatment, the mineral absorption was higher than in the other treatments (Figure 3.6). At excessively high MoO_3NPs concentrations (223.50 or 298,00 µg/L), the nutrient absorption capacity decreased and impaired growth.

Subsequent growth of Chrysanthemum plants

After 15 days of cultivation, the remaining MoO_3NPs in the samples affected the *in vitro* rooting stage of the Chrysanthemum plants (Table 3.4; Figures 3.3). In all parameters (plant height, number of leaves, number of roots, root length, SPAD, fresh weight, and dry weight), the treatment with 149,00 µg/L MoO_3NPs still showed an advantage at the rooting stage. Interestingly, although the Mo residue in the positive control sample was higher than in the other treatments (except for 149,00 µg/L MoO_3NPs), the rooting ability and growth of these plants were not as good as those from the MoO_3NPs-supplemented environments (74.50 - 223.50 µg/L). After 15 days of cultivation, the remaining MoO_3NPs in the shoot samples affected the *in vitro* rooting stage of the Chrysanthemum plants (Figures 3.4 and 3.5).

Table 3.4. Rooting of Chrysanthemum plants derived from stem cuttings cultivated in MoO_3NPs-supplemented medium after 15 days

MoO_3NPs (µg/L)	**Rooting rate (%)**	**Plant height (cm)**	**No of leaves /plant**	**No of roots/plant**	**Root length (cm)**	**SPAD**	**Fresh weight (g)**
(-)	100 ± 0,00a*	9,17 ± 0,15e*	10,33 ± 0,58cd	6,67 ± 0,58e	4,77 ± 0,31e	32,9 ± 1,97de	0,26 ± 0,03cd
(+)		10,3 ± 0,44d	11,67 ± 1,15b	10,33 ± 0,58d	5,63 ± 0,15d	40,97 ± 0,72b	0,35 ± 0,01b
37,25		9,3 ± 0,20e	7,33 ± 0,58e	10 ± 1,00d	5,37 ± 0,21d	31,1 ± 0,70e	0,28 ± 0,00cd
74,50		14,03 ± 0,21b	9,33 ± 0,58d	14,33 ± 0,58b	6,53 ± 0,21c	41,43 ± 1,19b	0,35 ± 0,03b
149,00		16,83 ± 0,25a	13,67 ± 0,58a	16,33 ± 0,58a	11,57 ± 0,21a	57,93 ± 1,80a	0,41 ± 0,05a
223,50		12,57 ± 0,15c	11,33 ± 0,58bc	12,67 ± 0,58c	8,07 ± 0,21b	35,17 ± 1,55cd	0,32 ± 0,02bc
298,00		10,23 ± 0,25d	7,67 ± 0,58e	10,33 ± 0,58d	6,37 ± 0,25c	36,5 ± 1,6c	0,23 ± 0,06d

**The average values ± SD (Standard Deviation) and values with different letters indicate a significant difference between treatments*

Figure 3.3. Effect of Mo residue on *in vitro* rooting ability after 15 days of culture.

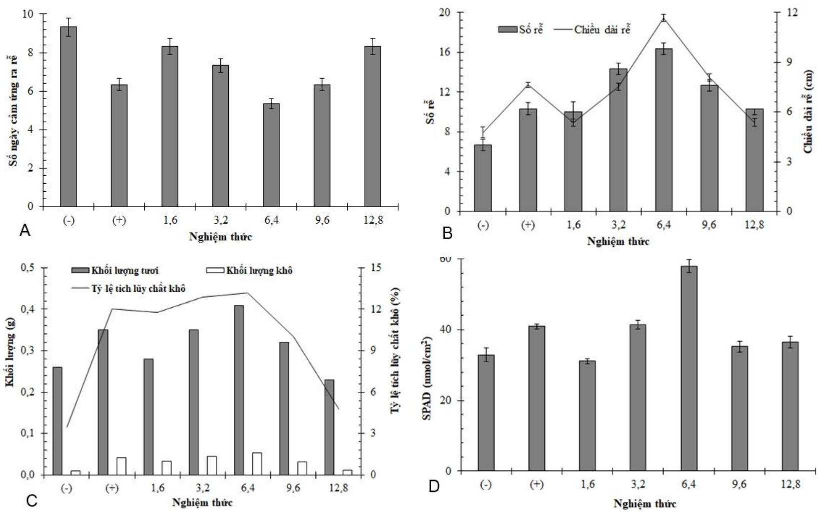

Figure 3.4. Effect of MoO_3NPs residue on *in vitro* rooting ability of Chrysanthemum after 15 days of culture.

Figure 3.5. Effect of MoO_3NPs residue on *in vitro* rooting ability of Chrysanthemum after 15 days of culture.

3.2. Study of replacing $CoCl_2.6H_2O$ with CoNPs in *in vitro* propagation of African violet, Gerbera, and Chrysanthemum

3.2.1. Rapid shoot regeneration from leaf explants of African violet on media supplemented with CoNPs replacing $CoCl_2.6H_2O$

After 60 days of culture, CoNPs proved effective in promoting shoot regeneration, addressing some abnormal phenomena, and regulating ethylene fluctuations and antioxidant enzyme activities (Table 3.5; Figures 3.6 and 3.7).

Table 3.5. Effect of CoNPs on shoot regeneration and growth of shoot clusters after 60 days of culture.

CoNPs (µg/L)	Number of shoots/explant		Shoot height(cm)	SPAD	Fresh weight of shoot cluster (mg)	Dry weight of shoot cluster (mg)
	Total number of shoots	Shoots > 1 cm				
(+)**	7,33 ± 0,58c*	1,33 ± 0,58e	0,67 ± 0,06cd	27,00 ± 2,00b	1.047 ± 10c	118 ± 2e
(-)	6,67 ± 0,58c	2,67 ± 0,58d	0,43 ± 0,11e	27,67 ± 2,51b	760 ± 54d	124 ± 11de
1,55	9,33 ± 0,58b	4,33 ± 0,58c	0,57 ± 0,06de	29,00 ± 1,00ab	1.360 ± 70b	162 ± 3c
3,10	10,67 ± 1,15b	6,33 ± 0,58b	0,80 ± 0,10bc	28,33 ± 0,58b	1.384 ± 106b	187 ± 8b
4,65	13,00 ± 1,0a	7,67 ± 0,58a	1,37 ± 0,11a	31,33 ± 1,15a	1.535 ± 32a	212 ± 12a
6,20	6,67 ± 1,5c	4,67 ± 0,58b	0,97 ± 0,15b	26,67 ± 1,53b	800 ± 100d	136 ± 11d

**The average values ± SD (Standard Deviation) and values with different letters indicate a significant difference between treatments*

Figure 3.6. The effect of CoNPs on shoot regeneration in African violet after 60 days of culture.

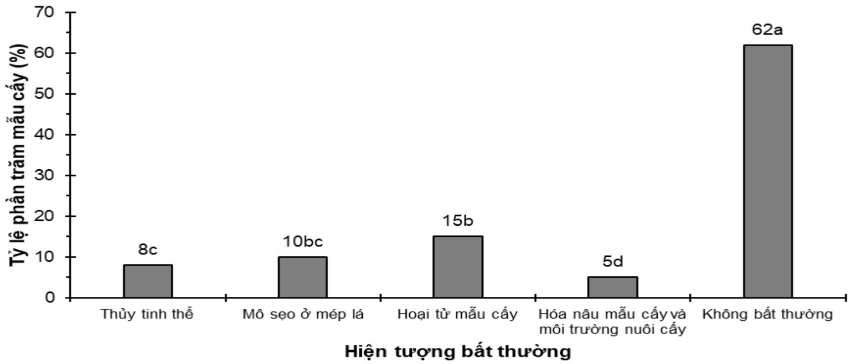

Figure 3.7. The effect of 4,65 µg/L CoNPs on the ability to overcome certain abnormalities during the shoot regeneration phase of African violet after 60 days of culture

Table 3.6. Ethylene gas content in the culture plate and antioxidant enzyme activity of shoot clusters on media supplemented with CoNPs after 60 days of cultivation.

CoNPs (µg/L)	Ethylene (ppm)	CAT activity (U/g)	APX activity (U/g)
(+)	1,37 ± 0,02a	80,00 ± 1,00d	0,55 ± 0,01e
(-)	1,28 ± 0,02b	87,33 ± 2,08c	0,93 ± 0,03d
1,55	1,19 ± 0,01c	97,33 ± 1,52b	1,26 ± 0,03c
3,10	1,11 ± 0,02d	98,33 ± 2,08b	1,57 ± 0,06b
4,65	0,87 ± 0,06e	105,67 ± 1,15a	2,00 ± 0,04a
6,20	1,31 ± 0,02b	80,33 ± 2,51d	1,27 ± 0,47c

** Different letters (a, b,…) in the same column indicate a significant statistical difference at $p < 0.05$ (Duncan's test).*

3.2.2. Rapid shoot proliferation of Gerbera shoot samples cultured on medium substituting $CoCl_2.6H_2O$ with CoNPs

Abnormal phenomena in Gerbera micropropagation

During the shoot proliferation and root induction *in vitro*, abnormal phenomena such as vitrification, leaf yellowing, and browning were

observed and recorded after 30 days of culture. The results showed that vitrification (23.33% during the shoot proliferation stage and 27.67% during root induction *in vitro*) and leaf yellowing (23.33% during shoot proliferation and 20.00% during root induction *in vitro*) were higher than the browning ratio in both stages of shoot proliferation and root induction *in vitro*.

The recorded results indicated that the medium DC0 supplemented with CoNPs had an effect on the shoot proliferation process and reduced vitrification and leaf yellowing after 30 days of culture. The highest number of shoots was obtained from shoots cultured on the DC0 medium supplemented with 6,20 µg/L CoNPs, showing a greater number of shoots over 2 cm (6.67 shoots), with higher growth characteristics compared to other treatments.

3.2.3. In vitro rooting of Gerbera shoot samples cultured on medium substituting $CoCl_2.6H_2O$ with CoNPs

The *in vitro* rooting ability of Gerbera shoots reached 100% in all treatments supplemented with CoNPs and the control (without CoNPs) after 7 days of culture (Figure 3.8). However, the *in vitro* rooting time (5.33 days) of shoots cultured on DR0 medium supplemented with 4,65 µg/L CoNPs occurred 2 days earlier than the control medium (7.33 days). Additionally, 4,65 µg/L CoNPs reduced the occurrence of vitrification, leaf yellowing, and browning compared to the control (without CoNPs), with the 4,65 µg/L CoNPs treatment reducing these abnormal phenomena five times more than the control.

The optimal concentration of CoNPs not only increased the *in vitro* rooting efficiency and antioxidant activity (SOD, CAT, and APX), but also reduced abnormal phenomena, ethylene gas accumulation, and the activity of hydrolytic enzymes (cellulase and pectinase) after 30 days of culture. The seedlings observed in the 4,65 µg/L CoNPs treatment showed improvements in seedling height, the number of roots per seedling, the number of leaves per seedling, fresh and dry weight of seedlings, along with a reduction in abnormal phenomena (vitrification, leaf yellowing, and browning), ethylene accumulation, and antioxidant activity (SOD, CAT, and APX).

Figure 3.8. *In vitro* rooting of Gerbera (*Gerbera jamesonii* Revolution Yellow) on medium supplemented with CoNPs after 30 days of culture.

Subsequent growth in nursery conditions

After 15 days of planting in the nursery, the results showed that the survival rate of seedlings (originating from 4,65 µg/L CoNPs) was higher compared to the control treatment. Moreover, seedling height, the number of roots per seedling, root length, leaf number, leaf size, SPAD value, and fresh and dry weight of seedlings in the 4,65 µg/L CoNPs treatment were all better compared to the control. Additionally, the formation of flower buds and flowering in seedlings originating from 4,65 µg/L mg/L CoNPs occurred approximately 7 days earlier than in the control plants.

3.2.4. In vitro rooting of Gerbera shoot samples cultured on medium substituted with $CoCl_2.6H_2O$ by CoNPs

After 15 days of culture, the results observed indicate that CoNPs at various concentrations influenced the rooting ability and growth of Gerbera shoot samples (Figure 3.9).

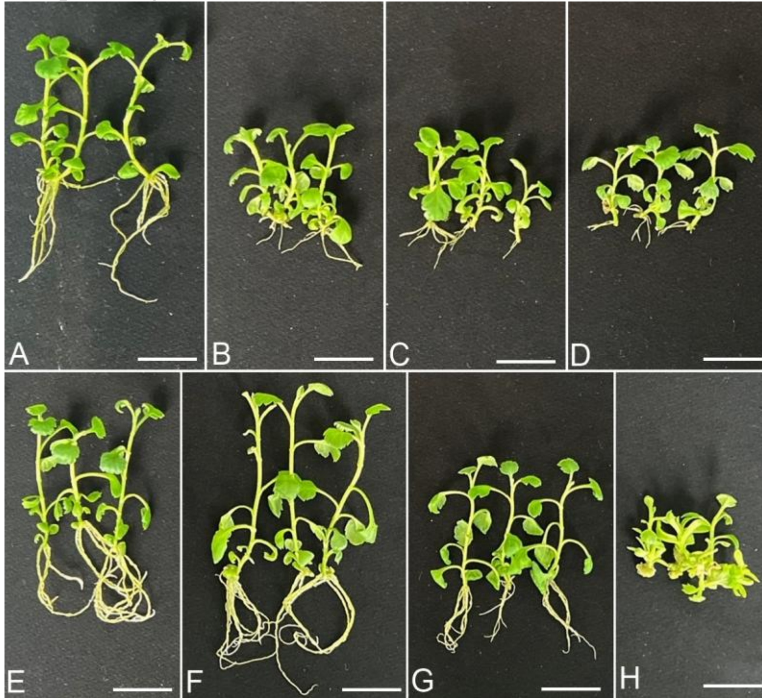

Figure 3.9. Morphology of Gerbera plants at the rooting stage *in vitro* on medium supplemented with various concentrations of CoNPs after two weeks of culture.

The results show that the shoot samples cultured on basic MS medium (positive control), MS medium devoid of $CoCl_2.6H_2O$, and MS medium supplemented with CoNPs at concentrations of 0,775 to 6,20 µg/L all showed a 100% rooting rate. In contrast, the treatment supplemented with 12,4 µg/L CoNPs did not exhibit rooting *in vitro*, and the shoot samples also showed the formation of callus tissue at the base (Figure 3.9H). Supplementing CoNPs into the culture medium affected changes in the activity of SOD and CAT enzymes. The enzyme activity increased from the

control group (without supplementation) to the 4,65 µg/L CoNPs treatment and then decreased (Table 3.7). Meanwhile, the activity of the antioxidant enzyme APX did not show significant differences among treatments. The DPPH activity gradually decreased from the control (-) treatment to the 3,1 µg/L CoNPs treatment and then gradually increased from 4,65 to 12,40 µg/L CoNPs.

Table 3.7. Antioxidant enzyme activity of Gerbera after 15 days of culture.

CoNPs (µg/L)	SOD (U/g)	CAT (U/g)	APX (U/g)
(+)	141,03 ± 7,72a	330,60 ± 9,38b	0,47 ± 0,06
(-)	92,02 ± 2,56e	295,27 ± 16,70de	0,45 ± 0,04
0,775	89,79 ± 7,01e	278,95 ± 6,60e	0,43 ± 0,07
1,55	102,18 ± 11,13cd	321,17 ± 3,65bc	0,54 ± 0,06
3,10	116,80 ± 17,20bc	335,98 ± 13,38b	0,49 ± 0,04
4,65	132,11 ± 8,12ab	367,61± 9,33a	0,54 ± 0,06
6,20	124,04 ± 6,72b	276,93± 14,05e	0,46 ± 0,07
12,40	100,86 ± 3,98cd	306,71± 7,75cd	0,43 ± 0,07
			ns

**Mean ± SD; **Different letters (a, b,...) in the same column indicate statistically significant differences with $p < 0.05$ (Duncan's test); ns: non-significant.*

3.3. Study on the substitution of $FeSO_4.7H_2O$ with FeNPs in the micropropagation of Chrysanthemums, Gerberas and African violets

3.3.1. In vitro root formation of shoot tips of Chrysanthemums cultured on media substituting $FeSO_4.7H_2O$ with FeNPs

The results in Table 3.8 and Figure 3.10 show the effect of FeNPs on the growth of Chrysanthemum shoots after 30 days of culture. There were no significant differences in plant height, leaf length, leaf width, and root number. However, significant differences were observed in the parameters

such as fresh weight, dry weight, root length, and SPAD values. Among the treatments, the one supplemented with 4.2 mg/L FeNPs showed the best results for leaf number, fresh weight, and dry weight compared to the control sample.

Table 3.14. Effect of FeNPs on *in vitro* root formation of Chrysanthemum shoot tips after 30 days of culture.

FeNPs (mg/L)	**Plant height (cm)**	**Number of leaves**	**Number of roots**	**Root length (cm)**	**Fresh weight (mg)**	**Dry weight (mg)**
(+)	4,40 ± 0,30cd*	13,33 ± 0,58ab	13,33 ± 0,58b	3,30 ± 0,30d	694 ± 5g	11,67 ± 0,58c
(-)	4,80 ± 0,30ab	11,33 ± 1,53c	12,33 ± 0,58bc	6,20 ± 0,10a	1016 ± 7c	14,33 ± 1,52b
1,4	4,20 ± 0,30d	11,00 ± 1,00c	11,00 ± 1,00c	4,10 ± 0,40bc	806 ± 5e	11,67 ± 0,58c
2,8	4,20 ± 0,20d	12,67 ± 0,58bc	13,00 ± 1,00b	3,90 ± 0,30bc	786 ± 5f	10,33 ± 0,58c
4,2	5,00 ± 0,20ab	14,67 ± 0,58a	15,67 ± 0,58a	4,3 ± 0,30b	1445 ± 13a	24,33 ± 0,58a
5,6	5,20 ± 0,40a	11,00 ± 1,00c	12,33 ± 1,15bc	4,20 ± 0,30b	1078 ± 7b	11,67 ± 0,58c
11,2	4,50 ± 0,30bc	12,33 ± 1,49bc	13,33 ± 1,51b	3,60 ± 0,30cd	934 ± 5d	11,67 ± 0,58c

**Note: The experiment used an improved MS medium with Fe-EDTA removed, while the control treatment (FeMS) used the standard MS medium.*

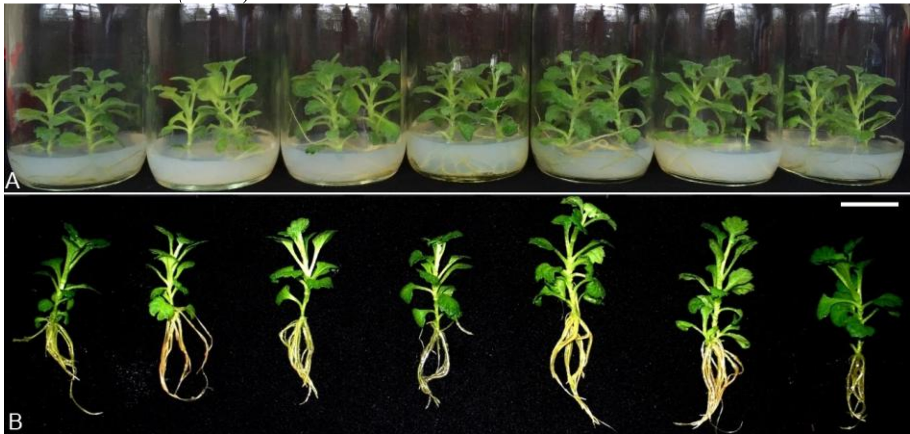

Figure 3.10. *In vitro* rooting of Gerbera plants on media supplemented with FeNPs after 30 days of cultivation.

3.3.2. *In vitro* rooting of Gerbera shoots cultivated on media replacing $FeSO_4.7H_2O$ with FeNPs

The results indicate that the rooting percentage of Gerbera shoots reached 100% in all treatments after 30 days of culture. However, the root induction time for shoots on media supplemented with 5.6 mg/L FeNPs (5.00 days) was earlier compared to shoots on the control medium (7.33 days). Supplementation with FeNPs affected the rooting ability and growth of Gerbera after 30 days of culture (Figures 3.11A-C).

Plant height (5.83 cm) and SPAD (41.47) observed in plants grown on media supplemented with 5.6 mg/L FeNPs were higher than plants grown on media with other concentrations of FeNPs, and were similar to the control plants (5.83 cm and 33.27, respectively) after 30 days. Fresh and dry weight both increased with higher concentrations from 0 to 4.2 mg/L FeNPs in the *in vitro* medium, and were higher than in the control group. No significant differences were observed in plants originating from 4.2 and 5.6 mg/L FeNPs in terms of fresh and dry weight; however, the number of roots (13.00 roots), root length (5.27 cm), leaf length (2.07 cm), and leaf width (1.83 cm) were higher in plants originating from the 5.6 mg/L FeNPs concentration compared to plants from the 4.2 mg/L FeNPs concentration. The antioxidant enzyme activities (SOD, CAT, and APX) in plants originating from the 5.6 mg/L FeNPs treatment during *in vitro* rooting were higher (41.32 U/g; 308.70 U/g; 0.55 U/g, respectively) than in plants originating from media MR0 (control) and MR0 media with $FeSO_4.7H_2O$ removed after 30 days. Meanwhile, the hydrolytic enzyme activity showed the opposite results. This indicates that supplementation with FeNPs increased antioxidant enzyme activity and decreased hydrolytic enzyme activity, thus promoting *in vitro* rooting and growth of Gerbera plants after 30 days of cultivation.

Figure 3.11. Effect of FeNPs on root formation and stomatal morphology of Gerbera plants after 30 days of culture.

K, Ca, and Mg absorption capacity

The results after 30 days of cultivation show that 5.6 mg/L FeNPs increased the absorption of potassium (K) (436.04 mg), magnesium (Mg) (60.33 mg), and calcium (Ca) (42.33 mg) in Gerbera plants grown *in vitro*, compared to the MS-Fe and MR0 treatments.

3.3.3. In vitro rooting of African violet shoots on media replacing $FeSO_4.7H_2O$ with FeNPs

The rooting rate of the shoots of the African violet reached 100% in all treatments after 30 days under nursery conditions. Using FeNPs instead of FeSO4.7H2O did not show any differences in plant growth traits such as: plant height, number of roots, root length, number of leaves, leaf length, as well as fresh and dry weight.

However, the antioxidant and hydrolytic enzyme activities after 30 days showed differences. SOD, CAT, and APX levels in the 5,6 mg/L FeNPs treatment were higher (41,32 U/g; 308,70 U/g; 0,55 U/g, respectively) in the *in vitro* rooting treatment compared to the control and MR0 medium with FeSO4.7H2O removed after 30 days. Meanwhile, the hydrolytic enzyme activity showed the opposite results. This indicates that the addition of FeNPs increased antioxidant enzyme activity and decreased hydrolytic enzyme activity; thereby promoting *in vitro* rooting and seedling growth after 30 days.

CHAPTER 4. CONCLUSIONS AND RECOMMENDATIONS

4.1. Conclusions

The thesis "study on replacing some micronutrient metallic salts with metallic nanoparticles in the micropropagation of ornamental plants (chrysanthemum, gerbera, and african violet)" evaluated the effectiveness of replacing trace metal salts ($FeSO_4.7H_2O$, $Na_2MoO_4.2H_2O$, $CoCl_2.6H_2O$) in MS culture medium with corresponding NPs (FeNPs, MoO_3NPs, and CoNPs) in three plant species: chrysanthemum, gerbera, and lily of the valley. The NPs used in this study improved growth and development indicators in the plant species (chrysanthemum, gerbera, and lily of the valley), and also helped overcome some abnormalities in in vitro propagation such as yellowing leaves and vitreous humor. The results showed the potential of using NPs to replace commonly used trace metal salts in MS culture medium.

In chrysanthemum, the process of callus induction and shoot regeneration from leaf samples achieved optimal efficiency with a callus induction rate of 100% and shoot regeneration rate of 33.33% after 30 days of culture on a medium where $Na_2MoO_4.2H_2O$ was replaced with MoO_3NPs (223.50 µg/L), the rapid shoot multiplication and subsequent growth were significantly improved at a concentration of 149.00 µg/L MoO3NPs, as evidenced by increased rooting ability, shoot morphological development, and the efficiency of absorbing essential nutrients, including macronutrients (Ca, Mg, K) and micronutrients (Cu, Fe). The activity of the

antioxidant enzyme system was significantly enhanced during shoot regeneration from leaf samples, while remaining at a level equivalent to the control during rapid multiplication from stem segments. In addition, the in vitro rooting ability of chrysanthemum plants was also improved when cultured on a medium where Na2MoO4.2H2O was replaced with MoO_3NPs. MoO_3NPs (149.00 µg/L), $CoCl_2.6H_2O$ as CoNPs (4.65 µg/L), or $FeSO_4.7H_2O$ as FeNPs (4.2 mg/L), along with an increase in SOD and CAT enzyme activity, contributed to improved growth and physiological stability of the plants.

For Gerbera , using CoNPs (6.20 µg/L) to replace $CoCl_2.6H_2O$ strongly promoted shoot multiplication, improved morphological indicators (number of shoots > 2 cm, shoot height), and significantly reduced abnormal physiological phenomena such as vitreous humor and yellowing leaves by 2-3 times. At the rooting stage, shoots in the CoNPs (4.65 µg/L) treatment showed earlier rooting time, increased rooting efficiency, plant growth, and antioxidant activity (SOD, CAT, and...). CoNPs (4.6 mg/L) reduced abnormal phenomena, ethylene accumulation, and hydrolytic enzyme activity (cellulase and pectinase) after 30 days. Plants from this treatment showed better adaptability, growth, flowering, and flower quality than other treatments under nursery conditions. Besides CoNPs, the use of FeNPs (5.6 mg/L) also showed better efficacy than $FeSO_4.7H_2O$ in the rooting stage, resulting in approximately 2 days earlier in vitro root induction, higher growth, SOD, CAT, and APX levels, and lower cellulase and pectinase levels compared to other treatments after 30 days.

In African Violet, the treatment with 4.65 µg/L CoNPs significantly improved shoot regeneration from leaf samples, while also recording a reduction in endogenous ethylene accumulation (0.87 ppm compared to 1.37 ppm in the control) and an increase in the activity of resistant enzymes. Oxidative activity, particularly CAT (104.67 U/g) and APX (1.98 U/g), was observed. During the in vitro rooting phase, replacing $FeSO_4.7H_2O$ with FeNPs (5.6 mg/L) helped maintain high activity levels of SOD, CAT, and APX enzymes, while reducing the activity of hydrolytic

enzymes. This contributed to improved root formation efficiency and enhanced seedling quality.

In summary, the results show that replacing metal salts with NPs in MS culture media not only improves in vitro propagation efficiency but also regulates the physiological and biochemical state of plants by strengthening the antioxidant system, improving nutrient uptake, and limiting physiological disturbances. These improvements play a crucial role in enhancing seedling quality and ensuring stable growth and development in subsequent stages, especially in nursery and production conditions.

4.2. Recommendations

Further research is needed on the impact of FeNPs, CoNPs, and MoO3NPs on different growth stages of chrysanthemums, gerbera, and African Violet.

More in-depth studies are required on the role, absorption, transport, and utilization of NPs in plants, as well as the influence of NPs on the absorption of macronutrients, micronutrients, and trace elements in plants.

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