MINISTRY OF EDUCATION AND TRAINING

VIETNAM ACADEMY OF SCIENCE AND TECHNOLOGY

GRADUATE UNIVERSITY OF SCIENCE AND TECHNOLOGY

Huynh Gia Bao

EFFECT OF SILVER AND COPPER NANOPARTICLES IN STERILIZATION OF EXPLANTS, CULTURE MEDIA, AND MICROPROPAGATION ON SOME ECONOMICALLY VALUABLE PLANT SPECIES

SUMMARY OF DISSERTATION ON BIOTECHNOLOGY

Code: 9 42 02 01

HA NOI – 2023

The research was finished at:Graduate University of Science and Technology, Vietnam Academy of Science and Technology

Supervisors:

1. Supervisor 1: Hoang Thanh Tung, PhD.
2. Supervisor 2: Prof. Dương Tan Nhut, PhD.

Reviewer 1: Assoc. Prof. Pham Van Hien, PhD.

Reviewer 2: Assoc. Prof. Pham Bich Ngoc, PhD.

Reviewer 3: Assoc. Prof. Quach Ngo Diem Phuong, PhD.

The thesis will be defended in front of the Academy-level PhD Thesis Evaluation Council, meeting at the Graduate University of Science and Technology - Vietnam Academy of Science and Technology at …

The dissertation can be found at:

- Graduate University of Science and Technology Library
- National Library of Vietnam

INTRODUCTION

The necessity of the dissertation

Currently, tissue culture or micropropagation are widely used to propagate different plant species (flowers, vegetables, ornamental plants, etc.), and are useful tools used in research on plant growth, physiology and biochemistry. Tissue culture can be used to produce large numbers of disease-free plantlets in a short time and to produce biomass containing secondary compounds,... Besides the advantages, tissue culture still has some limitations such as fungal infections, bacteria, etc., they grow and limit the growth of explants because they use nutrient medium. In addition, the optimal or ideal culture medium for the growth of the culture requires autoclaving. Culture vessels (nylon bags or glass bottles) containing the medium should also be autoclaved, the medium boiled; then sterilize the medium at 121°C with a pressure of 15 psi for a period of 20 to 40 min. In addition, the steps to prepare the media need to go through many different stages and take a lot of labor, electricity, time, etc. for the autoclaving of the media.

Silver nanoparticles (AgNPs) have anti-microbial effects (fungi, bacteria, etc.) and are used in research or applications in many fields such as medicine, pharmacy, biomedicine,... Studies on the effects of AgNPs on plants such as disinfection of explants, germination of some plants, physiology and morphology of plants have also been shown. However, the effect of AgNPs on the sterilization of explants and culture media has not been previously published. In this thesis, AgNPs were added to the culture medium (without autoclaving) to evaluate the sterilization of culture mediato and induce subsequent growth of Chrysanthemum explants. In addition, previous studies have not recorded the role of copper nanoparticles (CuNPs) in the sterilization and induction stages; therefore, this study also evaluated the effects of AgNPs and CuNPs in the explant sterilization and subsequent growth, somatic embryogenesis, etc. on Begonia plants.

In addition, the role of AgNPs in growth ability and abnormal phenomena in the shoot multiplication stage of African violet also pointed out in this thesis. Thesis "**Effect of silver and copper nanoparticles in sterilization of explants, culture media, and micropropagation on some economically valuable plant species"** towards solving some of the limited problems of tissue culture. This is a research direction that has not received much attention in the world as well as in Vietnam. The results of the study will contribute to a new research direction in plant micropropagation.

Objective and requirement of the dissertation

General objective

The thesis was carried out to provide new solutions to overcome some of the limitations encountered in micropropagation such as sterilizating explants and culture media, improving subsequent growth, overcoming overcome some abnormal phenomena of Chrysanthemum, Begonia and African violet cultured *in vitro*.

Detail objective

Determine the effectiveness of AgNPs on the sterilization of culture media instead of autoclaving as well as the subsequent growth of Chrysanthemum. Determination of the effectiveness of AgNPs and CuNPs in replacing traditional disinfectants such as mercury chloride ($HgCl_2$) or calcium hypochlorite ($Ca(ClO)_2$) and their effect on the subsequent growth of Begonia. Determination of the effectiveness of AgNPs on growth and limiting some abnormal phenomena during the shoot regeneration stage of African violet.

Subject and research scope

Subject

Three types of plants (Chrysanthemum, Begonia and African violet) are used as research objects of the thesis.

Two types of nanomaterials (AgNPs and CuNPs) were used as materials for sterilization of explants and culture media or as additions to *in vitro* media.

Research scope

The thesis evaluates the effectiveness of AgNPs and CuNPs on the ability to sterilization of explants, culture media and subsequent growth, physiological and biochemical of Chrysanthemum, Begonia and African violet cultured *in vitro*.

Scientific and practical significance

Scientific significance

This thesis has evaluated the effectiveness of AgNPs or CuNPs on sterilizing the media to replace autoclaving, inducing morphogenesis, subsequent growth and limiting some abnormal phenomena in cultured *in vitro* of Chrysanthemum, Begonia, and African violet.

Practical significance

The thesis used AgNPs as a media disinfectant to replace traditional disinfectants and does not affect the subsequent growth. In addition, AgNPs and CuNPs can be used as sterilization of explants to replace traditional disinfectants and increase the subsequent growth of explants.

The findings of the dissertation

AgNPs added to the culture media and reduced a half agar content were effectively sterilized the culture media and without sterilization by autoclave.

AgNPs and CuNPs can use the the sterilant replaced the traditional disinfectant and improved the subsequent growth of explant.

AgNPs increased the shoot regeneration, reduced abnormal phenomena and reduced ethylene accumulation, and increased antioxidant enzyme activity of the in vitro explant.

Chapter I: LITERATURE REVIEW

Ph.D. thesis has been referred to use in this dissertation related to: (1) Sterilization of culture media; (2) Sterilization of explants; (3)

AgNPs and its applications; (4) CuNPs and and its applications; (5) Abnormal phenomana in micropropagation; (6) General of subjects.

Chapter II: MATERIALS, CONTENTS AND METHODS

2.1. Materials

2.1.1. Plant materials

The 4-week-old *Chrysanthemum morifolium*, 8-week-old *Begonia tuberous* and *Saintpaulia ionantha* with different parts were used as starting material for the experiments.

2.1.2. Nanoparticles solution

AgNPs and CuNPs were chemically synthesized with an initial concentration of 1,000 mg/L and produced by the Institute of Environmental Technology (VAST, Hanoi, Vietnam). AgNPs solution has a particle size of about 20 nm; Meanwhile, CuNPs solution has particle size from 20 to 60 nm.

2.1.3. Culture systems

The glass bottle culture system (AuM) was a control system consisting of a 250 mL glass bottle (AuM1) containing 30 mL of MS medium and a 500 mL glass bottle (AuM2) containing 60 mL of MS medium. MS medium was supplemented with 30 g/L sucrose and 8 g/L agar. The AuM culture system was autoclaved.

The plastic container culture system (NoM) consists of a 5 L square plastic container (21.5 cm × 21.5 cm in top diameter; 8.5 cm in height) (NoM1) containing 250 mL medium and 15 L rectangular plastic box (top diameter: 24.0 cm × 35.0 cm; bottom diameter: 22.5 cm × 33.0 cm; height: 13.5 cm) (NoM2) containing 500 mL MS medium supplemented with 30 g/L sucrose and agar (according to the experiment on the effect of optimal agar concentration). The NoM culture system was supplemented with AgNPs and not autoclaved.

2.1.4. Equipment – Instruments

2.1.5. Culture conditions

2.2. Research contents

Effect of AgNPs on sterilization of explants, culture media and micropropagation of Chrysanthemum.

Effect of AgNPs and CuNPs on sterilization of explants and subsequent growth of Begonia.

Effect of AgNPs on shoot regeneration and abnormal phenomena of African violet.

2.3. Research methods

2.3.1. Design of experiments

2.3.1.1. Effect of AgNPs on sterilization of explants, culture media and micropropagation of Chrysanthemum

2.3.1.2. Effect of AgNPs and CuNPs on sterilization of explants and subsequent growth of Begonia

2.3.1.3. Effect of AgNPs on shoot regeneration and abnormal phenomena of African violet

2.3.2. Determine of ethylene gas by HPLC

2.3.3. Determine of antioxidant enzyme by ultraviolet spectrum analysis

2.3.4. Determine of sugar and starch content by ultraviolet spectrum analysis analysis

2.3.5. Plant anatomy

2.3.6. Data analysis

2.4. Location and time

Chapter III: RESULTS AND DISCUSSION

3.1. Effect of AgNPs on sterilization of explants, culture media and micropropagation of Chrysanthemum

3.1.1. Effect of AgNPs on sterilization of culture media

The effect of the addition of AgNPs on the sterilization of non-autoclaved medium was shown in Table 3.1.1. Therefore, MS0 medium supplemented with 4 mg/L AgNPs that did not autoclave

autoclave (Fig. 3.1.1) had a similar sterilizing effect on the autoclaved media and was used in the next experiment.

Table 3.1.1. Effect of AgNPs on sterilization of MS0 media after 1, 2, 3 and 4 weeks of culture.

MS0 medium	AgNPs (mg/L)	Contamination of medium (%)			
		1-week	2-week	3-week	4-week
Non-autoclaved	0	33.33a*	76.67a	100.00a	100.00a
	1		60.00b		
	2		50.00c	73.33b	86.67b
	3		20.00d	60.00c	80.00b
	4	-**			
	5		-**	-**	-**
Autocalved (Control)	0				

**Different letters in the same column indicate differences with statistical significance p < 0.05 in the Duncan test; **Non-contamination*

3.1.2. Sterilization of explants by AgNPs and callus induction

The leaf sterilized with AgNPs recorded faster callus induction than that sterilized with 1,000 mg/L $HgCl_2$. Callus was induced when leaf were sterilized with AgNPs after only 2 weeks of culture as compared to those 3 weeks of 1,000 mg/L $HgCl_2$. Leaf sterilized with 250 mg/L AgNPs for 15 and 20 min recorded the highest callus induction (71.67% - 75.00%) compared to that sterilized with 1,000 mg/L $HgCl_2$ (53.3%) after 4 weeks (Fig. 3.1.2).

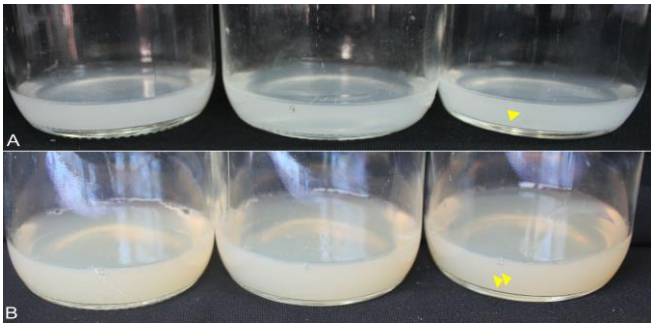

Figure 3.1.1. MS0 medium observed after 4 weeks. **A:** MS0 medium autoclaved; **B:** MS0 medium added to 4 mg/L AgNPs and non-autoclaved. *Single arrow: White medium; Double arrow: Slightly yellow medium.*

The results showed that all leaves washed with distilled water were infected with fungi and bacteria after 4-7 days of culture. In this study, MS medium was isolated and recorded 1 bacterial species (*Pseudomonas* sp.) and 4 fungal species (*Aspergillus aculeatus*, *Fusarium* sp., *Penicillium* sp., and *Trichoderma* sp.) based on the morphology of the bacteria. cells (Table 3.1.2). Besides, 125 mg/L AgNPs in 5 - 15 min, or 500 mg/L AgNPs in 10 - 30 min, did not record callus induction. This could be because explants sterilized with low concentrations of AgNPs are not effective in disinfecting fungi and bacteria, or the AgNPs concentration is too high and causes toxicity and damage to the explants; therefore, the explants become infected or necrotic leading to the non-callus induction (Fig. 3.1.2).

Table 3.1.2. Fungi and bacteria infect the media containing the explants after 1 week of culture.

No.	Characteristics of colonies	Cell morphology	Classify
1	Mycelium: thick and white, yellow-orange interspersed with pink. Spores: pink-green (middle), slightly pink (center). Colonies: blue (top), pinkish-brown with light yellow (bottom). Fiber: white	Hyphae: Branched, with septa. Reproductive organs: Well-proportioned and dense brush shape. Spores: Elliptical to spherical	*Fusarium* sp.
2	Mycelium: thick, white Spores: black	Hyphae: Branched, septate. Reproductive organs: Spherical. Spores: Spherical	*Aspergillus* sp.
3	Mycelium: smooth and milky white. Spores: blue	Hyphae: Branched, septate. Reproductive organs: Branched. Spores: Spherical	*Trichoderma* sp.
4	Mycelium: thick and milky white, protruding blue with lobed (middle)	Hyphae: Thin, many branches, with septa. Spore: Elliptical	*Penicillium* sp.
5	Colonies: round, pink, smooth, glossy surface, convex agar surface, regular borders. Colony Size: <1 mm	Spores: Oval shape. Gram+	*Pseudomonas* sp.

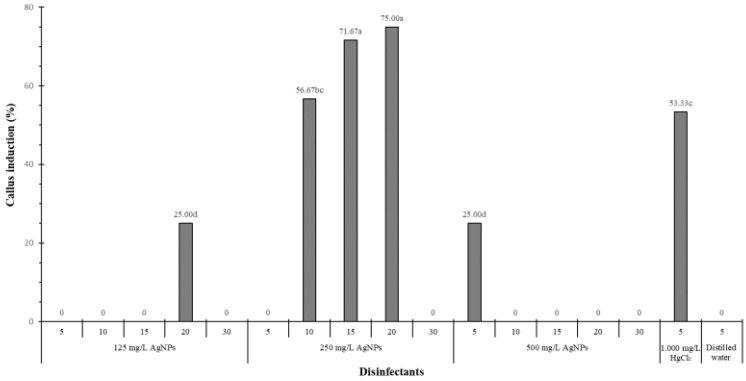

Figure 3.1.2. Callus induction of leaf sterilized with disinfectants after 4 weeks.

3.1.3. Shoot regeneration on non-autoclaved medium supplemented with AgNPs

Total shoots were recorded when calli were cultured on non-autoclaved MS0 medium supplemented with 4 mg/L and MS0 medium without AgNPs (autoclaved) containing 0.2 mg/L BA. There was no difference after 4 weeks. However, other growth indicators have clear differences (Fig. 3.1.3). In this study, it was found that leaf cultured on medium supplemented with 4 m/L AgNPs increased callus induction and shoot regeneration (Fig. 3.1.2; Table 3.1.3).

Table 3.1.3. Shoot regeneration on non-autoclaved/autoclaved medium supplemented with AgNPs (adding 4 mg/L AgNPs) after 4 weeks of culture.

Treatment	Shoot regener-ation (%)	No. of shoots/explant			Shoot height (cm)	Fresh weight (g)
		Total shoots	< 1 cm	> 1 cm		
MS0 + 4 mg/L AgNPs + 0,2 mg/L BA (non-autoclaved)	100.00a *	10.00a	3.67b	6.33a	1.50a	1.32a
MS0 + 0,2 mg/L BA (autoclaved)		9.33ab	7.00a	2.33b	0.46b	1.16b
MS0 (autoclaved)	-**	-**	-**	-**	-**	-**

**Different letters in the same column indicate differences with statistical significance p < 0.05 in the Duncan test; **Data not record.*

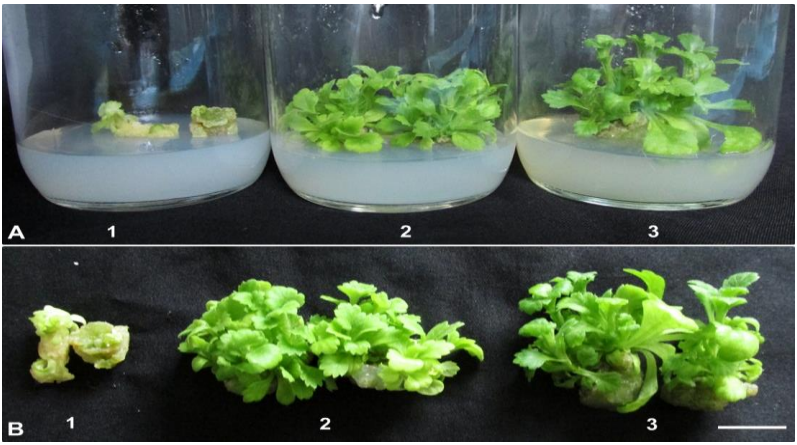

Figure 3.1.3. Shoot regeneration on MS0 medium with/without 4 mg/L AgNPs after 4 weeks. **A_1, B_1:** Shoot on MS0 medium without AgNPs, BA and autoclaved; **A_2, B_2:** Shoot on MS0 medium containing 0,2 mg/L BA and autoclaved; **A_3, B_3:** Shoot on MS0 medium containing 0,2 mg/L BA, 4 mg/L AgNPs and non-autoclaved. *Bar: 1 cm.*

3.1.4. Effect of agar content on the growth of plant in non-autoclaved medium added to AgNPs

Plantlets obtained from 1.5 cm shoots cultured on MS0 medium supplemented with 4 mg/L AgNPs with different agar contents were obtained after 4 weeks of culture (Table 3.1.4 and Fig. 3.1.4).

Table 3.1.4. The growth of plant in non-autoclaved medium added to 4 mg/L AgNPs with different agar contents after 4 weeks.

AgNPs (mg/L)	Agar (g/L)	Plantlet height (cm)	No. of leaves/ plantlet	Leaf width (cm)	No. of roots/ plantlet	Root length (cm)	Fresh weight (g)	SPAD
0^x	8	4.33a*	8.33a	1.23ab	12.33a	2.57c	0.39a	36.53a
4^y	3	3.87bc	7.67ab	1.13bc	7.67c	3.83a	0.35ab	30.8c
	4	4.11ab	7.67ab	1.30a	9.67b	3.23b	0.36ab	37.63a
	5	4.17ab	6.67bc	1.33a	10.00b	2.40c	0.37ab	33.07b
	6	3.63cd	7.00bc	1.27ab	10.33b	2.36c	0.29c	32.7bc
	7	3.47cd	6.67bc	1.23ab	8.33bc	2.23c	0.25c	34.3ab
	8	3.00d	8.00ab	1.13bc	9.67bc	2.13c	0.27c	33.77ab

**Different letters in the same column indicate differences with statistical significance $p < 0.05$ in the Duncan test; x: Autoclaved; y: Non-autoclaved.*

Figure 3.1.4. The growth of plantlets on non-autoclaved MS0 medium added to 4 mg/L AgNPs and different agar content after 4 weeks. **0:** Plantlet on autoclaved MS0 medium containing 8 g/L agar (without AgNPs) (control); **3, 4, 5, 6, 7, 8:** Plantlet on agar treatments (3, 4, 5, 6, 7, 8 g/L; respectively), 4 mg/L AgNPs and non-autoclaved. *Bars: 2 cm*

Among different agar concentrations (3 - 8 g/L), plantlets obtained on MS0 medium containing 4 - 5 g/L agar showed higher plant growth compared to other agar concentrations (Table 3.1.4).

3.1.5. Micropropagation in different culture systems

In all three culture systems, all stem nodes recorded shoot regeneration (100%) after 2 weeks of culture. In addition, growth parameters such as fresh weight, shoot height and number of leaves were also not different between the 3 culture systems (data not shown). However, the shoot tips recorded higher fresh weight, plant height, number of roots and SPAD in the NoM1 and NoM2 systems than these parameters in the AuM2 system after 4 weeks of culture (Table 3.1.5; Fig. 3.1.5), this shows the positive effect of AgNPs on the growth of chrysanthemum at the rooting stage in all 3 culture systems.

Table 3.1.5. The growth of plantlets in different culture systems after 4 weeks.

Growth parameters \ Culture systems		NoM1	NoM2	AuM2
Plantlet height (cm)		4.02a*	3.95a	3.76b
No. of leaves/ plantlet		7.33a	7.20a	7.35a
Leaf width (cm)		2.30a	2.27a	2.22a
No. of roots/ plantlet		13.17a	13.50a	11.33b
Root length (cm)		2.88a	3.04a	2.45b
Fresh weight (g)		0.55a	0.52a	0.45b
SPAD		38.45a	37.22a	34.37b
APX (U/g)	**Root**	1.21a	1.38a	0.54b
	Leaf	1.53a	1.67a	0.44b
SOD (U/g)	**Root**	21.01a	24.27a	11.61b
	Leaf	12.69a	13.32a	7.54b

** *Different letters in the same row indicate differences with statistical significance $p < 0.05$ in the Duncan test.*

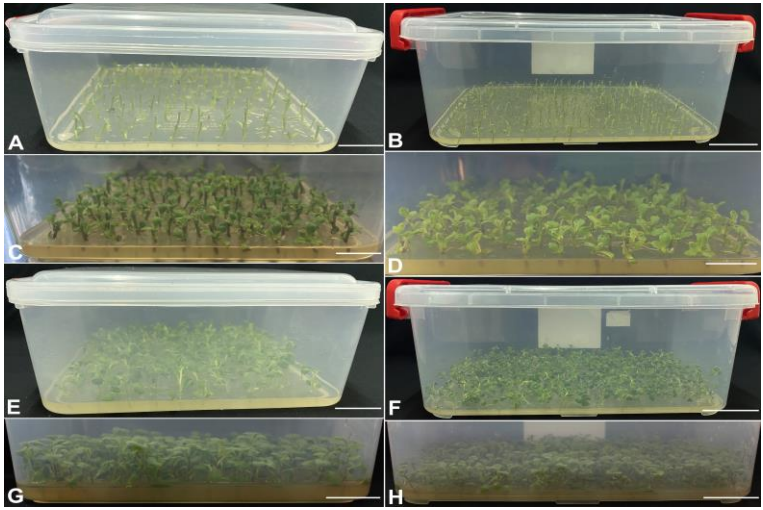

Figure 3.1.5. The growth of shoots in NoM1 and NoM2 system. **A:** Stem nodes cultured on 5 L NoM1 system; **B:** Stem nodes cultured on 15 L NoM2 system; **C:** 1-week-old shoots in 5 L NoM1 system; **D:** 1-week-old shoots in 15 L NoM2 system; **E:** Shoots in 5 L NoM1 system; **F:** Shoots in 15 L NoM2 system; **G:** 2-week-old plantlets in 5 L NoM1 system; **H:** 2-week-old plantlets in 15 L NoM2 system. *Bar: A, C, E, G - 3 cm; B, D, F, H - 5 cm.*

3.1.6. Acclimatization in the greenhouse

The results obtained in Table 3.1.6 and Figure 3.1.6 show that plantlet height, number of leaves, leaf width, leaf length and survival rate of plantlets were higher in NoM1 and NoM2 systems as compared to those in AuM2 system after 16 weeks of planting in the greenhouse. Plantlets obtained from the NoM1 and NoM2 systems in the greenhouse grew faster. In addition, flowers started to bloom one week earlier than flowers in the 500 mL AuM2 system (Table 3.1.6; Fig. 3.1.6C). After 16 weeks of planting, the plantlets from the NoM1 and NoM2 systems began to bloom (Fig. 3.1.6D).

Table 3.1.6. The ggwowth of plantlets derived from diferent culture systems after 16 weeks in the greenhouse.

Culture systems / Growth parameters	NoM1	NoM2	AuM2
Survival rate (%)	100.00a*	100.00a	85.67b
Plantlet height (cm)	14.71a	14.23a	12.38b
Fresh weight (g)	3.42a	3.67a	2.61b
No. of roots/ plantlet	18.33ab	20.67a	17.33ab
Root length (cm)	6.97a	7.33a	6.87a
No. of leaves/ plantlet	14.33ab	14.67ab	12.33b
Leaf length (cm)	3.79a	3.93a	3.24b
Leaf width (cm)	3.21ab	3.47a	2.97b
Flower bud (week)	12.21ab	12.47ab	13.14a
Budding flower (week)	14.68b	14.76b	15.53a
Flowering bloom (week)	16.13b	15.91b	17.01a

** *Different letters in the same row indicate differences with statistical significance $p < 0.05$ in the Duncan test.*

3.1.7. Estimated economic efficiency of AgNPs on the sterilizeation explant, culture media and micropropagation of Chrysanthemum

Chrysanthemum plantlets includes different stages such as explant sterilization, morphogenesis and acclimatization. Currently, the tissue culture facilities in Lam Dong give the cost of each tissue cultured plantlets ranging from 500 to 1,000 VND. In this study, it is estimated that the cost of producing 10,000 plantlets was about 5 million VND (Table 3.1.7).

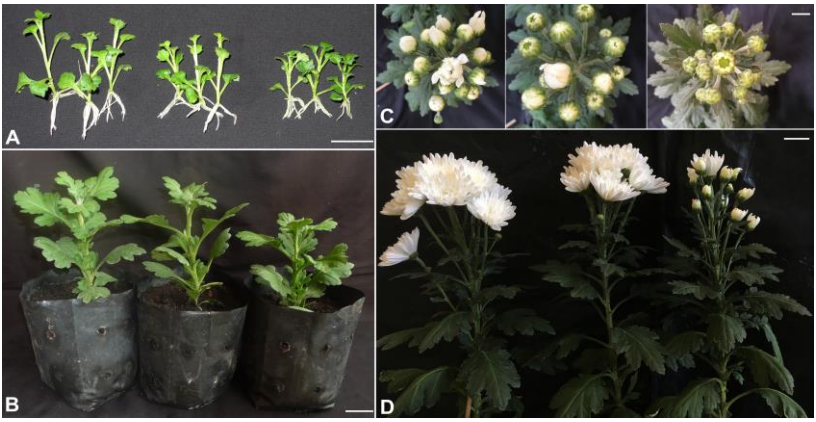

Figure 3.1.6. The growth and flowering of plantlet derived from diferent culture systems in the greenhouse. **A:** 4-week-old *in vitro* plantlets; **B, C, D:** 4, 12, 16-week-old plantlets in the greenhouse (5 L NoM1, 15 L NoM2 and 500 mL AuM2 system, left to right; respectively). *Bars: 2 cm.*

Table 3.1.7. Estimated production cost of 10,000 Chrysanthemum plantlets.

	Unit price (VND)	**Control**		**4 mg/L AgNPs**		**Effective**
		No.	*Amount (1)*	*No.*	*Amount (2)*	*(2) – (1)*
I. Shoot regeneration						
Coefficient of shoot regeneration		2.33		6.33		
(1) MS medium (liter)	12,000	86	1,032,000	32	384,000	-648,000
(2) PGRs (mg)						
(a) NAA (0,5 mg/L)	73	43	3.139	16	1,168	-1,971
(b) BA (2 mg/L)	2,057	172	353,804	64	131,648	-222,156
(3) Energy (kW)						
(a) Autoclave	2.200	25	55.000	0	0	-55.000
(b) Box	2.200	16	35.200	16	35.200	0

(c) Lighting 1 electric bulb 12 hours/day for 28 days	2,200	121	266,200	121	266,200	0
(4) AgNPs (mL)	200	0	0	128	25,600	25,600
(5) Agar (g)	400	688	275,200	128	51,200	-224,000
(6) Labor	250,000	2.5	625,000	2.5	625,000	0
II. Growth						
(1) MS medium (liter)	12,000	80	960,000	80	960,000	0
(2) PGRs (mg)						
(3) Energy (kW)						
(a) Autoclave	2,200	25	55,000	0	0	-55,000
(b) Box	2,200	16	35,200	16	35,200	0
(c) Lighting 1 electric bulb 12 hours/day for 28 days	2,200	121	266,200	121	266,200	0
(4) AgNPs (mL)	200	0	0	320	64,000	64,000
(5) Agar (g)	400	640	256,000	320	128,000	-128,000
(6) Labor	250,000	2.5	625,000	2.5	625,000	0
Production cost of 10,000 plantlets (excluding equipment depreciation costs and infection loss rate)			**4.842,943**		**3.598,416**	**-1,244,527**
Average cost for 1 plantlet			**484.29**		**359.84**	**-124.45**

Note: Prices are based on current prices provided by chemical companies.

3.2. Effect of AgNPs and CuNPs on sterilization of explant and subsequent growth of Begonia

3.2.1. Effect of AgNPs and CuNPs on sterilization of explant

Survival rates of 3 types of explants (petioles, flower stalks and stems) sterilized with AgNPs, CuNPs, $HgCl_2$ and $Ca(ClO)_2$ were obtained after 1 - 4 weeks of culture. The results showed that, depending on the type of explant and the disinfectant used, the survival rate of the explants differed. Overall, 50 - 300 mg/L AgNPs recorded high disinfection efficiency for all three types of explants. These concentrations of AgNPs showed disinfection efficacy equal to or greater than that of 1,000 mg/L $HgCl_2$ or 60,000 mg/L $Ca(ClO)_2$ after 4 weeks. On the other hand, when increasing the concentration of AgNPs to 400 mg/L, many explants were not contaminated with microorganisms but turned dark brown or necrotic. Therefore, the survival rates of petioles (62.20%), flower stalks (73.33%), and stems (73.33%) explants were low at high concentrations of AgNPs. For disinfection of explants with CuNPs, a concentration of 50 - 200 mg/L CuNPs disinfects petioles, a concentration of 200 - 300 mg/L CuNPs disinfects flower stalks, and a concentration of 100 - 300 mg/L CuNPs disinfects stems showed similar effects compared to optimal AgNPs concentrations.

3.2.2. Effect of AgNPs and CuNPs on somatic embryogenessis

The somatic embryogenesis of sterilized explants with AgNPs, CuNPs, $HgCl_2$ and $Ca(ClO)_2$ was different after 4 weeks (Table 3.2.1). In general, all sterilized explants induced embryogenesis; In which, 200 - 300 mg/L AgNPs to sterilize all explants gives a higher somatic embryogenesis than other concentrations; in which, 200 mg/L CuNPs also gives the same effect. In addition, analysis using atomic absorption spectroscopy showed that in petiole, stem and flower stalk, the Cu content was recorded from 0.10 - 0.18 mg/Kg. After disinfection with CuNPs, Cu residue in petioles, flower stalks

and stem increased proportionally to the concentration of CuNPs from 0 to 400 mg/L (Fig. 3.2.1). Among the three types, Cu residue in stem and petiole was higher than in flower stalk (Fig. 3.2.1). This suggests that different cultures absorb CuNPs differently.

Table 3.2.1. Effect of disinfectants on somatic embryogenesis from different explant sources after 4 weeks.

Chất khử trùng	**Concentration (mg/L)**	**The percentage of somatic embryogenesis (%)**		
		Peptiole	*Flower stalk*	*Stem*
AgNPs	50	22.20bc	20.00bc	13.33e
	100	37.80a	24.47b	22.20cd
	200	37.80a	40.00a	40.00a
	300	35.53a	35.53a	42.20a
	400	28.87b	20.00bc	28.87bc
CuNPs	50	31.13b	20.00bc	11.13e
	100	35.53a	22.20bc	26.67bc
	200	40.00a	42.20a	46.67a
	300	31.13b	40.00a	42.20a
	400	28.87b	22.20bc	26.67bc
$HgCl_2$	1.000	17.80c	13.33c	20.00de
$Ca(ClO)_2$	60.000	22.20bc	22.20bc	31.13b

**Different letters in the same column indicate differences with statistical significance $p < 0.05$ in the Duncan test.*

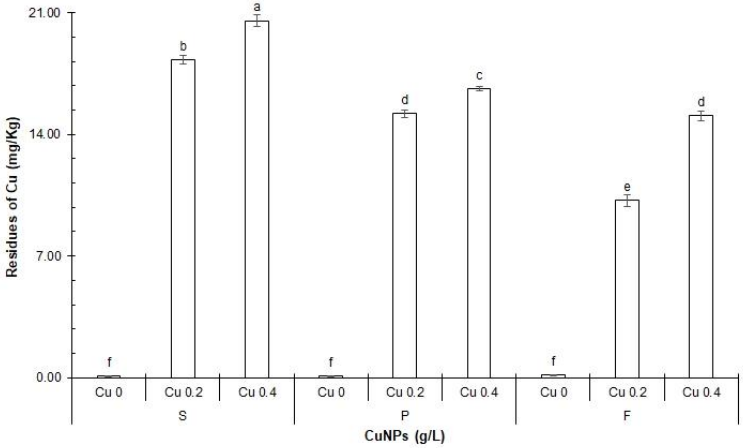

Figure 3.2.1. Cu residue in Begonia explants sterilizated with CuNPs

3.2.3. Effect of AgNPs and CuNPs on embryo growth

Different shapes of somatic embryos obtained from explants sterilized with AgNPs, CuNPs and $HgCl_2$ were recorded after 8 weeks (Table 3.2.2). After the first week, all treatments formed somatic embryos (100%). However, there are differences in the number of somatic embryos in each type of explant with different explant disinfectants (Table 3.2.2). Antioxidant enzymes (CAT and APX) of embryos obtained from explant sterilization with $HgCl_2$, AgNPs and CuNPs were not the same after 8 weeks (Table 3.2.3).

Table 3.2.2. The somatic embryogenesis of begonia explants sterilized with AgNPs, CuNPs and $HgCl_2$ after 8 weeks.

Explant	Disinfectant	Total embryos	Shapes of somatic embryos			
			Globular	Heart	Toperdo	Cotyledon
Peptiole	$HgCl_2$	29.33bc*[x]	8.67b	7.67b	6.33c	6.67c
	AgNPs	32.00b	8.33b	5.00d	7.67ab	9.00b
	CuNPs	33.00b	10.33a	6.00cd	8.00ab	8.67b
Flower stalk	$HgCl_2$	29.33bc	7.67bc	9.67b	6.33c	5.67cd
	AgNPs	36.33a	4.33e	9.67a	8.00ab	14.33a
	CuNPs	38.00a	5.33e	10.00a	8.67ab	14.00a
Stem	$HgCl_2$	30.67bc	10.33a	7.00bc	6.67bc	7.00c
	AgNPs	34.33ab	6.33d	5.00d	10.67a	12.33a
	CuNPs	36.67a	7.00bc	4.67d	9.33ab	12.67a

** Different letters in the same column indicate differences with statistical significance $p < 0.05$ in the Duncan test;*

[x] Total embryos were sum of all the different Shapes of somatic embryos (Globular, heart, toperdo and cotyledon).

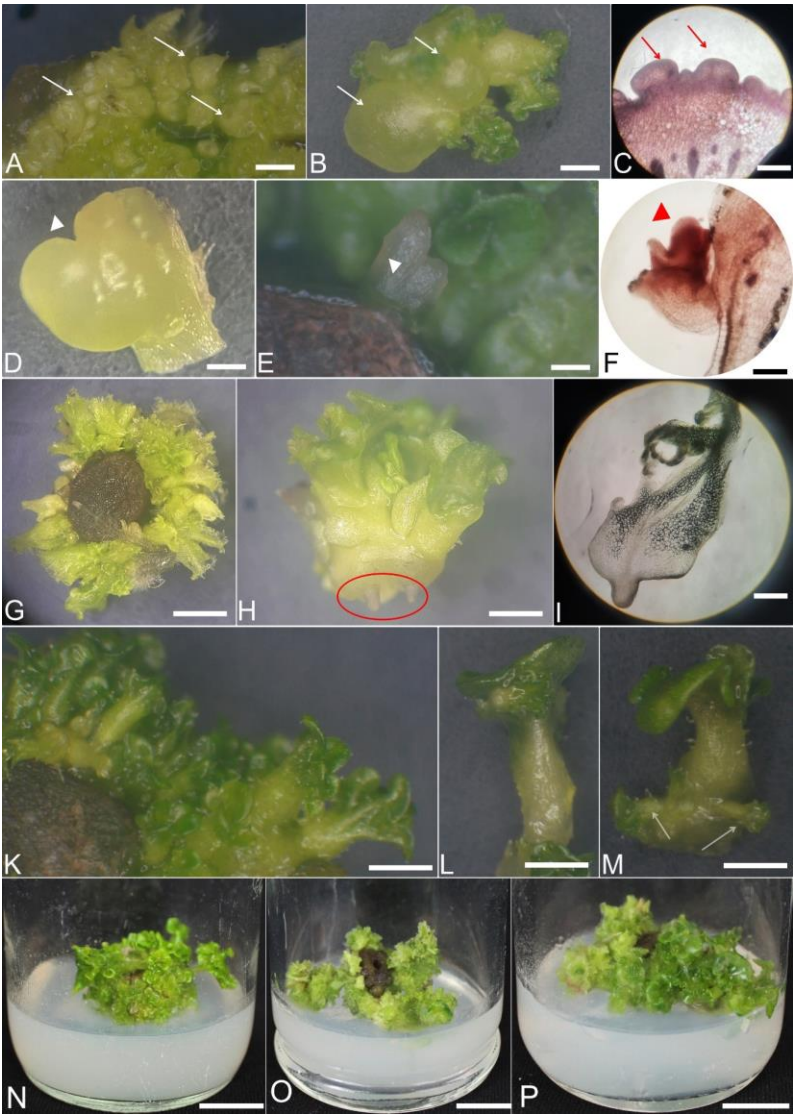

Figure 3.2.7. Somatic embryogenesis of begonia explants sterilized with CuNPs. **A, B, C:** Globular embryo; **D, E, F:** Heart embryo; **G, H, I:** Torpedo embryo; **K, L, M:** Cotyledon embryo; **N, O, P:** Somatic embryo cluster obtained from peptiole, flower stalk and stem (*A - F, I: Bars 0.5 cm; G, H, K - P: Bars 1 cm*).

Table 3.2.3. Antioxidant enzymes (CAT and APX), starch and sugar content of Begonia embryo clusters after 8 weeks of culture.

Explant \ Disinfectant		$HgCl_2$	AgNPs	CuNPs
Peptiole	**CAT (U/g)**	95.19b*	96.08b	100.06a
	APX (U/g)	0.40b	0.55a	0.58a
	Starch (%)	33.24b*	36.11ab	39.26a
	Sugar (mg/g)	75.98ab	68.45b	80.16a
Flower stalk	**CAT (U/g)**	100.12a	102.33a	104.86a
	APX (U/g)	0.21c	0.50b	0.70a
	Starch (%)	34.28b	38.23ab	40.87a
	Sugar (mg/g)	90.24a	81.36b	70.09c
Stem	**CAT (U/g)**	102.44b	94.97b	112.88a
	APX (U/g)	0.24c	0.45b	0.66a
	Starch (%)	37.24c	41.27b	45.58a
	Sugar (mg/g)	90.24a	86.19ab	75.24b

** Different letters in the same column indicate differences with statistical significance $p < 0.05$ in the Duncan test.*

3.2.4. In vitro rooting and acclimatization

The embryo-derived plantlets developed well and had no morphological abnormalities during *in vitro* rooting (Table 3.2.4). After 16 weeks of planting in the greenhouse, the plantlets recorded a good survival rate and there were no significant differences in growth such as plantlet height and number of leaves per plantlet (Table 3.2.5).

Table 3.2.4. The growth embryo-derived plantlets after 16 weeks.

Traetment	Plantlet height (cm)	No. of roots/ plantlet	Root length (cm)	No. of leaves/ plantlet	SPAD	Fresh weight (mg)	Dry weight (mg)
$HgCl_2$	9.83ab*	6.67b	6.00a	6.00a	43.72a	2019.55b	209.31c
AgNPs	10.35a	8.00a	5.10a	6.00a	44.34a	2316.05ab	227.23b
CuNPs	10.52a	7.67ab	5.21a	6.33a	46.89a	2395.13a	252.56a

** Different letters in the same column indicate differences with statistical significance $p < 0.05$ in the Duncan test.*

Table 3.2.5. Acclimatization of begonia plantlets in the greenhouse after 16 weeks.

Treatment	Survival rate (%)	Planlet height (cm)	No. of leaves/ plantlet	SPAD	Flowering (%)	Blooming (day)	Flower diameter (cm)
$HgCl_2$	87.67ab*	21.83b	14.33ab	45.35c		92.67a	2.51b
AgNPs	90.00a	23.15ab	13.67b	48.54b	100a	86.67b	2.70a
CuNPs	90.33a	23.96a	15.00a	52.14a		85.33bc	2.68a

** Different letters in the same column indicate differences with statistical significance $p < 0.05$ in the Duncan test.*

3.3. Effect of AgNPs on shoot regeneration and abnormal phenomena of African violet cultured *in vitro*

3.3.1. Shoot regeneration and abnormal phenomena

The results showed that the main vien recorded more optimal shoot regeneration than the whole leaf with the rate of shoot regeneration (66.00%), number of shoots (5.33 buds) and shoot height (0.73 cm) (Table 3.3.1). Therefore, using the main vein explants gives a higher ability to regenerate shoots than the whole leaf. Some abnormal phenomena during the shoot regeneration stage of African violet are recorded in Table 3.3.1.

Table 3.3.1. The growth and abnormal phenomena during shoot regeneration of African violet after 8 weeks.

Parameters	Main vien	Whole leaf	$LSD_{0,05}$
Shoot regeneration (%)	40.00 ± 1.15*	66.00 ± 2.08	9.96
No. of shoots/explant	2.67 ± 0.33	5.33 ± 0.33	1.72
Shoot height (cm)	0.50 ± 0.60	0.73 ± 0.06	0.10
Vitriffication (%)	20.00 ± 1.15	19.67 ± 1.20	Ns
Callus at the edge leaf (%)	21.67 ± 1.67	22.33 ± 1.45	Ns
Necrosis (%)	14.67 ± 0.88	12.33 ± 1.45	0.50
Browing of explant and medium (%)	20.33 ± 0.88	10.67 ± 0.67	4.43

**Data in the table represent mean data ± SE (standard error) in LSD test at $p < 0.05$; Ns: The difference is not statistically significant.*

In addition, in shoot regeneration stage on both whole leaf and main vein, vitrification obtained 20.00% and 19.67%; respectively, and callus at the edge leaf (21). 0.67% and 22.33%; respectively), necrosis (14.67% and 12.33%; respectively), browning of the explants and culture medium (20.33% and 15.67%, respectively)

(Table 3.3.1 and Fig. 3.3.1). The abnormal phenomenona recorded at the shoot regeneration stage was about 20% on both types of explants with characteristics such as succulent buds and leaves and deformation (Fig. 3.3.1D-F). In this study, the callus at the edge leaf was observed in tissue culture, which may be due to the fact that during the shoot regeneration stage, the shoots formed were small in size and the leaves were located close to and adjacent to each other. exposure to culture medium (MS medium containing PGRs such as BA and NAA); Therefore, the leaf explants form callus (Fig. 3.3.1G-I).

3.3.2. AgNPs enhanced shoot regeneration and overcame abnormal phenomena

After 4 and 8 weeks of culture, AgNPs were effective on shoot regeneration, limiting some abnormal phenomena, ethylene gas, antioxidant enzymes (Table 3.3.2, 3.3.3, 3.3.4). and Fig. 3.3.2, 3.3.3).

Figure 3.3.1. Abnormal phenomena in shoot regeneration of African violet after 8 weeks of culture. **A, B, C:** Normal explant; **D, E, F:** Vitriffication; **G, H, I:** Callus at the edge leaf; **K, L, M:** Browing of explant and medium (*Bars: 1 cm; Excepted A: 0.5 cm – Explants were photographed under a stereo microscope at 10x magnification – except A and K*).

Table 3.3.2. Effect of AgNPs on shoot regeneration and the growth of shoot cluster after 4 weeks of culture.

AgNPs (mg/L)	Shoot regeneration (%)	No. of shoots/ explant	Shoot height (cm)	Fresh weight (mg)	Dry weight (mg)
0	67.67d*	5.67b	0.47bc	282.67c	36.00d
1	79.00bc	6.00b	0.53ab	467.67b	60.67b
2	89.33a	6.00b	0.67a	581.33a	72.67a
3	82.33b	7.67a	0.43bc	409.00b	50.33c
4	76.33c	5.00b	0.30c	409.33b	42.33cd

** Different letters in the same column indicate differences with statistical significance $p < 0.05$ in the Duncan test.*

Table 3.3.3. Effect of AgNPs on shoot regeneration and the growth of shoot cluster after 8 weeks of culture.

AgNPs (mg/L)	No. of shoots/ explant		Shoot height (cm)	SPAD	Fresh weight (mg)	Dry weight (mg)
	Total	> 1 cm				
0	7.33bc*	1.67bc	0.73c	27.33a	1020.33d	120.00c
1	10.67a	2.33b	0.80bc	27.67a	1198.33c	134.33b
2	10.00a	5.67a	1.30a	30.00a	1476.00a	160.67a
3	8.00b	1.33d	0.93b	28.00a	1358.00b	147.00ab
4	6.00c	1.00e	0.73c	25.33a	977.67d	110.67c

** Different letters in the same column indicate differences with statistical significance $p < 0.05$ in the Duncan test.*

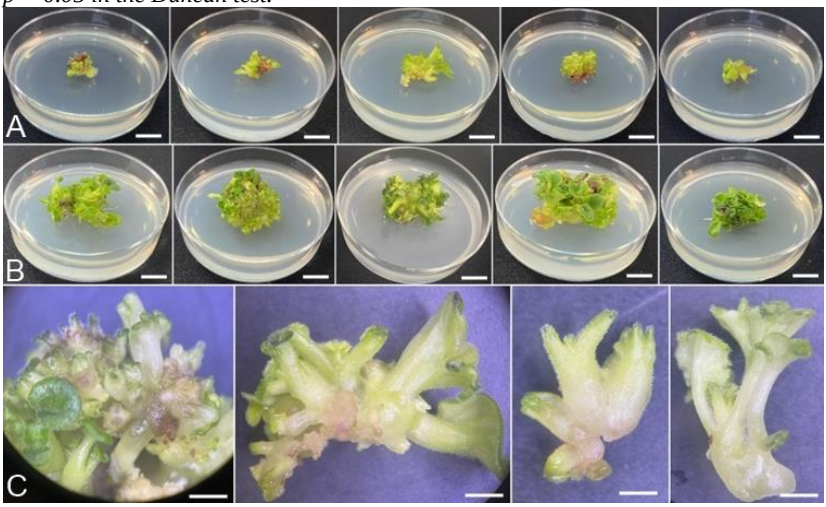

Figure 3.3.2. Effect of AgNPs on shoot regeneration after 4 and 8 weeks of culture. **A:** Shoot cluster after 4 weeks of culture; **B:** Shoot cluster after 8 weeks of culture; **C:** Shoot morphology (*Bars: 2 cm*).

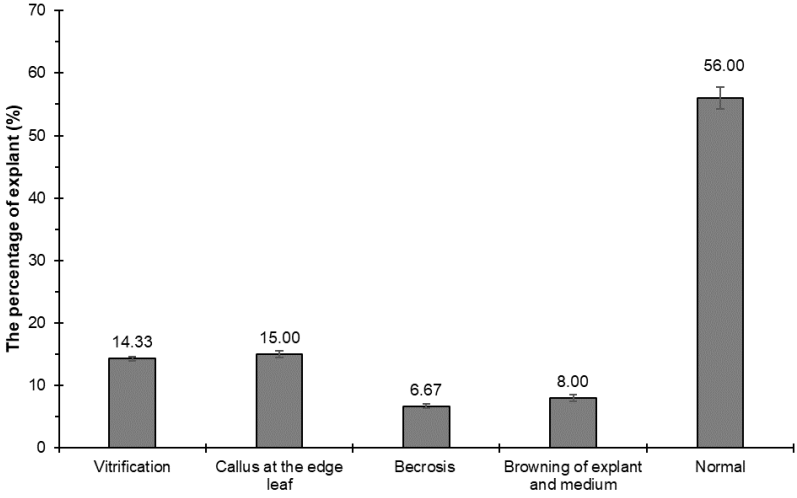

Figure 3.3.3. Effect of 2 mg/L AgNPs on abnormal phenomena in shoot regeneration of African violet after 8 weeks of culture.

Table 3.3.4. Ethylene content in petri dish and antioxidant enzymes of shoot cluster on medium containing AgNPs after 8 weeks of culture.

AgNPs (mg/L)	Ethylene (mg/L)	CAT (U/g)	APX (U/g)
0	1.36ab	77.31e	0.29e
1	1.45a	82.32d	1.11b
2	1.15c	101.51a	2.37a
3	1.32bc	88.09b	0.94c
4	1.44a	86.90c	0.40d

** Different letters in the same column indicate differences with statistical significance $p < 0.05$ in the Duncan test.*

Chapter 4: CONCLUSIONS AND RECOMMENDATIONS

4.1. Conclusions

AgNPs sterilized culture media: Adding 4 mg/L AgNPs to the culture medium and halving the agar content (4 g/L) effectively disinfects the culture medium and without autoclaving.

AgNPs and CuNPs sterilized explant and subsequent growth: Chrysanthemum leaf sterilized with 250 mg/L AgNPs in 15 -20 min obtained optimal callus induction as compared to that with 1.000 mg/L $HgCl_2$. Meanwhile, Begonia petiole, flower stalk and stem sterilized with 200 mg/L AgNPs or CuNPs had optimal explant sterilization efficiency and somatic embryogenesis compared with other disinfectants. In addition, the highest number of somatic embryos and the highest number of somatic embryos with dicotyledonous shape were obtained with sterilization of explants with AgNPs or CuNPs as well as increased antioxidant enzyme (CAT and APX).

AgNPs in shoot regeneration stage: Main vien of African violet culutured on medium supplemented with 2 mg/L AgNPs enhanced shoot regeneration and và the growth of shoot cluster; reduced vitrification, callus at the edge leaf, necrosis, browning of explant and medium; reduced ethylene and increased antioxxidant emzymes (CAT và APX).

4.2. Recommendations

Further study the role of AgNPs and CuNPs on other *in vitro* plants.

Further study the role CuNPs replacing copper salts in culture media.

LIST OF THE PUBLICATIONS RELATED TO THE DISSERTATION

1. H.T. Tung, **H.G. Bao**, D.M. Cuong, H.T.M. Ngan, V.T. Hien, V.Q. Luan, B.V.T. Vinh, H.T.N. Phuong, N.B. Nam, L.N. Trieu, N.K. Truong, P.N.D. Hoang, D.T. Nhut (2021) Silver nanoparticles as the sterilant in large-scale micropropagation of chrysanthemum. *In Vitro* Cell. Dev. Biol. - Plant 57: 897-906.doi.org/10.1007/s11627-021-10163-7.
2. **H.G. Bao**, H.T. Tung, H.T. Van, L.T. Bien, H.D. Khai, N.T.N. Mai, V.Q. Luan, D.M. Cuong, N.B. Nam, B.V.T. Vinh, D.T. Nhut (2022) Copper nanoparticles enhanced surface disinfection, induction and maturation of somatic embryos in Tuberous begonias (*Begonia* × *tuberhybrida* Voss) cultured *in vitro*. Plant Cell Tiss. Org Cult. https://doi.org/10.1007/s11240-022-02360-y.
3. H.T. Tung, **H.G. Bao**, Ngo Quoc Buu, Nguyen Hoai Chau, D.T. Nhut (2022) The use of silver nanoparticles as a disinfectant and media additive in plant micropropagation. In: D.T. Nhut, H.T. Tung, E.C. Yeung (Eds.), Plant tissue culture: New techniques and application in horticultural species of tropical region. Springer, Singapore.
4. H.T. Tung, H.T. Van, **H.G. Bao**, L.T. Bien, H.D. Khai, V.Q. Luan, D.M. Cuong, T.H. Phong, D.T. Nhut (2021) Silver nanoparticles enhanced efficiency of explant surface disinfection and somatic embryogenesis in *Begonia tuberous* via thin cell layer culture. Vietnam Journal of Biotechnology 19(2): 337-347.
5. **H.G. Bao**, H.T. Tung, N.T.N. Mai, H.D. Khải, D.M. Cuong, D.T. Nhut (2022) Siver nanoparticles enhanced shoot regeneration and limited some abnormal phenomena of *African violet* (*Saintpaulia ionantha* Wendl.) cultured *in vitro*. Vietnam Journal of Science and Technology B – Accepted.