Journal of Biotechnology **18**(2): 307-319, 2020

IRON NANOPARTICLES ON GROWTH AND ACCLIMATIZATION OF *Chrysanthemum morifolium* Ramat. cv. "Jimba" IN DIFFERENT CULTURE SYSTEMS

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Received: 26.11.2019
Accepted: 27.3.2020

SUMMARY

In plant tissue culture, iron nanoparticles (FeNPs) was one of the first types of nano to be used in plants. Previous reports have identified the effect of FeNPs on many different plant species. In this study, FeNPs was used to replace Fe-EDTA in MS (Murashige, Skoog, 1962) medium to assess their effects on growth, chlorophyll (a, b and a+b) accumulation, antioxidant activity of ascorbate peroxidase (APX) and superoxide dismutase (SOD) enzymes, and acclimatization in greenhouse conditions in different culture systems (*in vitro* solid, *in vitro* hydroponic and microponic culture). The obtained results show that FeNPs added to MS medium was higher growth, chlorophyll (a, b and a+b) content, antioxidant activity of SOD and APX enzymes than Fe-EDTA in MS medium as control treatment. The effect of FeNPs are differences between culture systems. *In vitro* solid and microponic culture systems, the optimal concentration is 75 mM FeNPs and *in vitro* hydroponic culture system is 100 mM FeNPs. The optimal activity of the antioxidant enzyme SOD (35.04 $U.mg^{-1}$ prot) obtained in the roots of cultured plants in microponic culture system; meanwhile, the optimal activity of the antioxidant enzyme APX (2.11 $\mu mol.min^{-1}.mg^{-1}$ prot) obtained in leaves cultivated in solid culture system. The plantlets derived from MS medium added FeNPs were transfered into greenhouse conditions, the microponic cultivated plants supplemented with FeNPs at a concentration of 100 mM gave the highest survival rate (94.67%). The results of this study showed that FeNPs can replace Fe-EDTA salt in MS medium, and iron deficiency in culture media will reduce chlorophyll content.

Keywords*:* *Ascorbate peroxidase, Chrysanthemum, in vitro culture, iron nanoparticles, microponic, superoxide dismutase*.

INTRODUCTION

The culture systems differ not only from the ingredients in the culture medium but also different conditions such as medium status, aeration or sterility, etc. Depending on certain conditions, uptake of nutrients will also be affected. There have been several reports on the use of different culture systems in investigation of nanoparticles on plants. Kumari *et al.* (2009) cultivated onion plants on liquid media showed that silver nanoparticles have the ability to seriously affect cell division, other studies have shown that liquid medium has the ability to help increase the absorption of plant growth regulators, and dissolve nutrients better in solid medium (Gurel *et al*., 1998; Klimaszewska *et al*., 2000). Aeration is also a factor that greatly affects the ability to absorb

nutrients from the roots. However, there are currently no studies on the uptake of nanoparticles in aerated and non-aerated culture.

As a micronutrient in plant tissue culture, FeNPs was one of the first types of nano to be used in plants. Previous reports have identified the effect of FeNPs on many different plant species. FeNPs have been reported to significantly increase yield on soybean crops (Roghayyeh *et al*., 2010), Faba beans (Nadi *et al*., 2013), and ginger (Ganesan, Lazer, 2016). Libralato *et al*. (2006) studied the effects of FeNPs compared to other forms of iron, which showed that FeNPs were significantly effective in biomass growth in *Lepidium sativum*, *Sinapis alba* and *Sorghum saccharatum* and could be replaced Fe-EDTA in medium. Recent studies on the addition of FeNPs as a nutritional component in *in vitro* culture have also reported initial results. Soad *et al*. (2016) analyzed the effectiveness of FeNPs compared to other iron forms in minimizing chlorosis and increasing growth of *in vitro* Volkamer lemon. However, the results of research on FeNPs are still limited, especially in different culture system. In this study, the effect of FeNPs on plant growth in different culture systems (*in vitro* solid, *in vitro* hydroponic and microponic) was investigated as well as the acclimatization at the greenhouse.

MATERIALS AND METHODS

Plant material

Chrysanthemum shoots (*Chrysanthemum morifolium* Ramat. cv. "Jimba") in 1-month-old, disease-free *in vitro* originating from Japan, was available at the Department of Molecular Biology and Plant Breeding (Tay Nguyen Institute for Scientific Research).

Nanoparticles solution

FeNPs solution (20 - 60 nm) was made by dehydration method using precursor $FeSO_4.7H_2O$. Sodium borohydride was used as a reducing agent and carboxymethyl cellulose as a stabilizer (Chau *et al*., 2008).

Effect of FeNPs on the growth of *Chrysanthemum* in culture systems

Chrysanthemum shoots (2.0 cm) with 2 pairs of leaves were cultured on modified MS medium that removed Fe-EDTA, and added different concentrations of FeNPs (0, 25, 50, 75, 100 and 200 mM corresponds to Fe0, Fe¼, Fe½, Fe¾, Fe1 and Fe2). MS medium with 100 mM $FeSO_4.7H_2O$ (FeMS) was used as the control.

The culture systems in this study include:

In vitro solid culture: 250 mL glass bottle contained 40 mL of MS medium was supplemented with 30 g/L sucrose and 8 g/L agar.

In vitro hydroponic culture: 250 mL glass bottle contained 40 mL of MS medium was supplemented with 30 g/L sucrose (Nhut *et al*., 2005). The medium after preparation was adjusted to pH = 5.8. *In vitro* solid and *in vitro* hydroponic culture systems were autoclaved at 121°C, 1 atm for 30 min.

Microponic culture: A circular plastic box (500 mL) with 8.5 cm in height, 12 cm in top diameter and 9 cm in bottom diameter (Dai Dong Tien, Vietnam) was contained 40 mL of ½ MS medium (a half mineral) (Tung *et al*., 2018).

Absorption spectrum and chlorophyll content analysis

SOD activity was determined according to Beyer and Fridovich (1987). The specific SOD activity was expressed as enzyme units per mg soluble protein ($U.mg^{-1}$ prot).

APX activity was measured following the H_2O_2-dependent oxidation of ascorbate in a reaction mixture composed of 50 mM K-phosphate buffer (pH 7.0), 1 mM EDTA, 0.5 mM ascorbate, and 0.1 mM H_2O_2. The decrease in absorbance was recorded at 290 nm (Miyak, Asada, 1992). The specific APX activity was expressed as µmol ascorbate per min mg soluble protein ($\mu mol.min^{-1}.mg^{-1}$ prot).

The activities of enzymes were measured in 10 explants and triplicate, at 30°C, using a

Shimadzu UV- 160 Spectro-photometer (Shimadzu UV-160, Kyoto, Japan).

Content of chlorophyll a and chlorophyll b were determined based on maximum absorption spectrophotometer of chlorophyll a (662 nm) and chlorophyll b (645 nm) using UV-2900 spectrophotometer machine (Lichtentaler, Wellburn, 1985).

Chlorophyll a = $(11.75*A_{662} – 2.35*A_{645})$ (µg/g)

Chlorophyll b = $(18.61*A_{645} – 3.96*A_{662})$ (µg/g)

Culture conditions

In vitro: The culture condition was set up fluorescent light with photoperiod of 16 h/day, intensity of 45 µmol.m^{-2}.s^{-1}, relative humidity 50 - 60% at 25 ± 2°C.

Ex vitro: Experimental greenhouse conditions with daytime temperatures of 27 ± 2°C, night temperatures of 14 ± 2°C, humidity of 70 - 80%, light coverage of 50%, the medium was humus added to a plastic pot with a mouth diameter of 10 cm, a bottom of 5 cm, and a height of 7 cm.

Data processing

The experiments were repeated 3 times. All data were processed by MicroSoft Excel 2010 and SPSS 16.0 statistical analysis software by Duncan test method with α = 0.05 (Duncan, 1955).

RESULTS AND DISCUSSION

In vitro solid culture

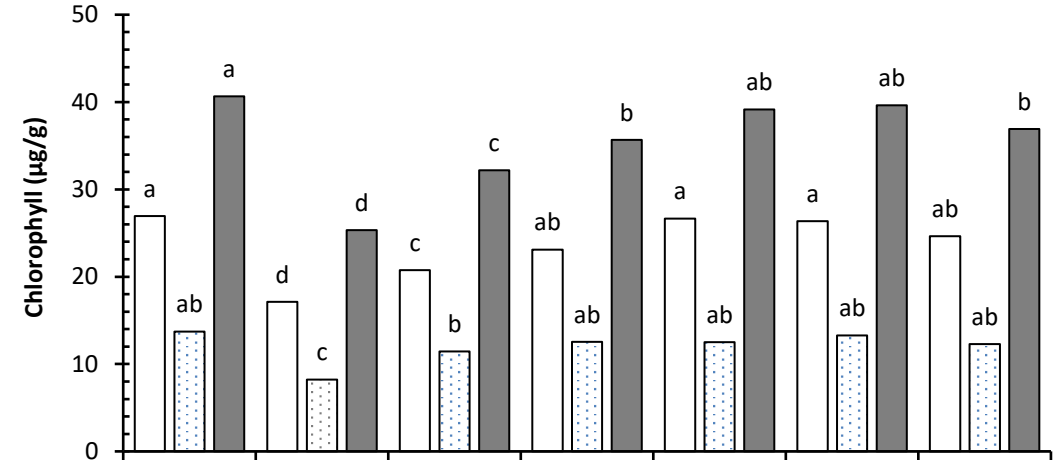
The effect of FeNPs on the growth of shoots cultured on solid medium was recorded after 4 weeks of culture (Table 1 and Fig. 1, 2). Results showed that plant height, number of leaves, number of roots did not have a clear difference between the treatments. However, the fresh weight, dry weight, root length, chlorophyll content index (a, b and a+b) were different (Table 1 and Fig. 1).

In particular, the addition of Fe¾ (75 mM FeNPs) on MS medium resulted in an increase in fresh weight (1.45 g), dry weight (0.16 g), chlorophyll a (26.67 µg/g), chlorophyll b (12.49 µg/g) and chlorophyll a+b (39.16 µg/g) were the best compared to other treatments (Table 1 and Fig. 1). In the treatment without FeNPs (Fe0), the lowest chlorophyll (a, b and a + b) content was observed (17.12 µg/g, 8.22 µg/g, and 25.34 µg/g, respectively), lack of pigment, light green and small leaves. FeNPs concentration exceeded Fe1 (100 mM), most of the indicators such as plant height, fresh weight, dry weight and chlorophyll content tended to decrease again (Table 1 and Fig. 1).

Table 1. Effect of FeNPs on *Chrysanthemum* growth in *in vitro* solid culture after 4 weeks of culture.

Treatment	Plant height (cm)	No. of leaves	No. of root	Root length (cm)	Fresh weight (g)	Dry weight (g)
FeMS	4.34b*	13.33ab	13.67ab	0.33c	0.70c	0.09b
Fe0	4.83ab	11.33ab	12.33ab	0.61a	1.01bc	0.11b
Fe¼	4.21b	11.00b	11.00b	0.40b	0.81bc	0.09b
Fe½	4.22b	11.67ab	13.00ab	0.38bc	0.79bc	0.08b
Fe¾	5.02a	14.67a	15.67a	0.43b	1.45a	0.16a
Fe1	5.17a	11.00b	12.33ab	0.42b	1.08b	0.13b
Fe2	4.53ab	12.33ab	13.33ab	0.36bc	0.94bc	0.10b

** Different letters (a, b, ...) in the same column represent statistically significant differences at α = 0,05 (Duncan's test).*



	FeMS	Fe0	Fe1/4	Fe1/2	Fe3/4	Fe1	Fe2
Chl a	26.93	17.12	20.75	23.11	26.67	26.35	24.62
Chl b	13.71	8.22	11.44	12.55	12.49	13.27	12.30
Chl a+b	40.64	25.34	32.19	35.66	39.16	39.62	36.92

Figure 1. Effect of FeNPs on chlorophyll accumulation of 4-week *Chrysanthemum* in *in vitro* solid culture.

This result shows that iron plays an important role in the growth and synthesis of chlorophyll. Iron not only participates in chlorophyll biosynthesis by being a constituent of proteins [4Fe - 4S], FX, FA, FB (PsbA, PsbB, PsbC) in photosystem I but also participates in proteins Cyt b559 (PsbE, PsbF), Cyt b6 f complex, Rieske [2Fe - 2S] protein (PetC), Cyt f (PetA), etc. with a key role in electronic transport (Mamyandi *et al*., 2009). In addition, iron is the substrate that forms the active center of the protein glutamyl-tRNA reductase, which contributes to the synthesis of 5-aminolevulinic acid as a precursor to chlorophyll (Kumari *et al*., 2009).

The chlorophyll content in Fe-EDTA and FeNPs treatments were higher than those in the non-nanoparticles (Fe0) treatment. The concentration of Fe¾ (75 mM) is more effective when using Fe-EDTA (100 mM), showing that FeNPs are more effective than iron in the form of ions. FeNPs have a higher surface penetration and interaction efficiency than iron in the form of Fe-EDTA, thus optimizing the effect of iron in culture media. This result was consistent with the study of Lopez-Moreno *et al*. (2010), FeNPs were significantly effective in biomass growth in *L. sativum*, *S. alba* and *S. saccharatum* and could be substituted for Fe-EDTA. Plantlets in MS medium supplemented with FeNPs showed a significant increase in fresh weight, dry weight, chlorophyll content compared to the control (Syu *et al*., 2014).

In vitro hydroponic culture

In vitro hydroponic culture, Fe1 (100 mM) supplemented in MS medium showed the best effect on plant height (7.51 cm), fresh weight (1.49 g), and dry weight (0.16 g) compared to other treatments (Table 2 and Fig. 4).

Especially, the chlorophyll (a, b and a+b) content (14.67 µg/g, 6.68 µg/g and 21.35 µg/g) was the lowest in Fe0 treatment and the highest in Fe1 (100 mM) supplementation were 29.85 µg/g, 14.39 µg/g and 44.24 µg/g (Fig. 3). This demonstrated the important role of iron in the pigmentation of *Chrysanthemum*, and the addition of FeNPs was more effective than the ionic form in the same concentration.

The results also showed that the root length was inversely proportional to the concentration of replacement FeNPs in the culture medium. All treatments with FeNPs showed lower root length than the FeMS (Fe-EDTA in MS medium). Liquid conditions cause the roots to release some of the oxygen in the respiration, which oxidizes the metal layer around the root area and forms an insoluble metal oxide layer, which precipitates in the surrounding area. Around the roots reduces the ability to absorb other nutrients and inhibits root growth (Hinsinger, 2001). The phenomenon of FeNPs inhibiting root growth is also observed in rice (Darioush, Akihiro, 2014), and sunflower (Musante, White, 2012). In this study, replacing Fe-EDTA with FeNPs (100 mM) gave the best growth but FeNPs made roots shortened in *in vitro* hydroponic.

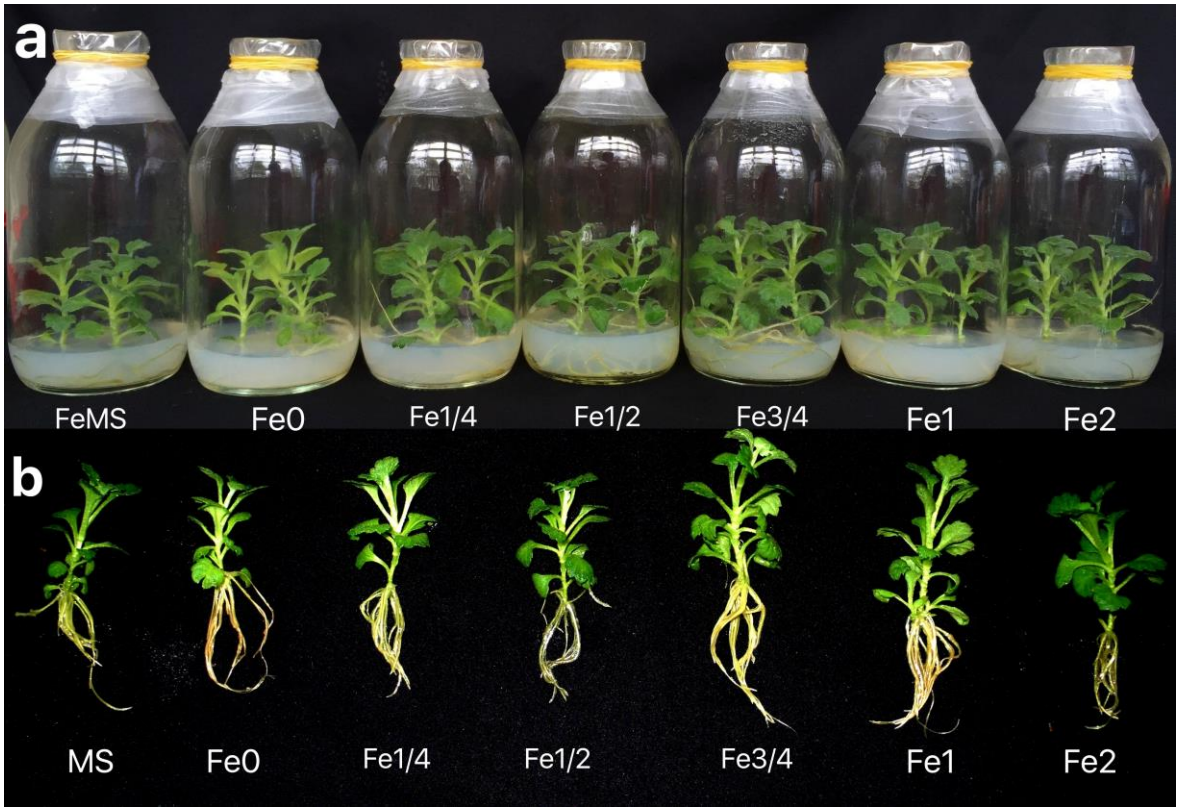


Figure 2. Effect of FeNPs on *Chrysanthemum* growth in *in vitro* solid culture after 4 weeks of culture.

Microponic culture

Results of cultivation in microponic with Fe – EDTA replacement with FeNPs at different concentrations are shown in Table 3, and Figure 5, 6. In this study, the results showed that plant height, number of leaves and number of roots wasn't significant difference; however, dry weight, fresh weight and root length, chlorophyll were the best in Fe¾ (75 mM) treatmnet.

Cultivation of plants in a microponic condition, which inhibits the extension of roots in liquid medium, has been overcome.

The root length achieved in Fe½ was 3.4 times longer than in the same treatment in the same liquid medium (Table 2 and 3). This result is explained by the better nutrient uptake combined with photoautotrophic conditions in microponic condition (open systems) that promote root growth, causing them to grow longer while culture in closed *in vitro* condition. Experiments show that the most suitable concentration of FeNPs to replace Fe-EDTA in microponic condition is Fe¾ .

Table 2. Effect of FeNPs on *Chrysanthemum* growth in *in vitro* hydroponic culture after 4 weeks of culture.

Treatment	Plant height (cm)	No. of leaves	No. of root	Root length (cm)	Fresh weight (g)	Dry weight (g)
FeMS	7.17ab*	13.00a	16.00a	1.60a	1.22b	0.13ab
Fe0	5.97d	12.33a	11.67b	1.41ab	0.76c	0.09c
Fe¼	6.63c	14.00a	16.67a	1.45ab	0.68d	0.07cd
Fe½	6.45c	13.00a	14.33ab	1.41b	0.96b	0.11b
Fe¾	7.23ab	13.00a	15.33a	1.29b	1.36b	0.14ab
Fe1	7.51a	11.67ab	10.00d	1.13bc	1.49a	0.16a
Fe2	5.48d	9.00b	11.00cd	1.25bc	0.68e	0.06d

** Different letters (a, b, ...) in the same column represent statistically significant differences at α = 0,05 (Duncan's test).*

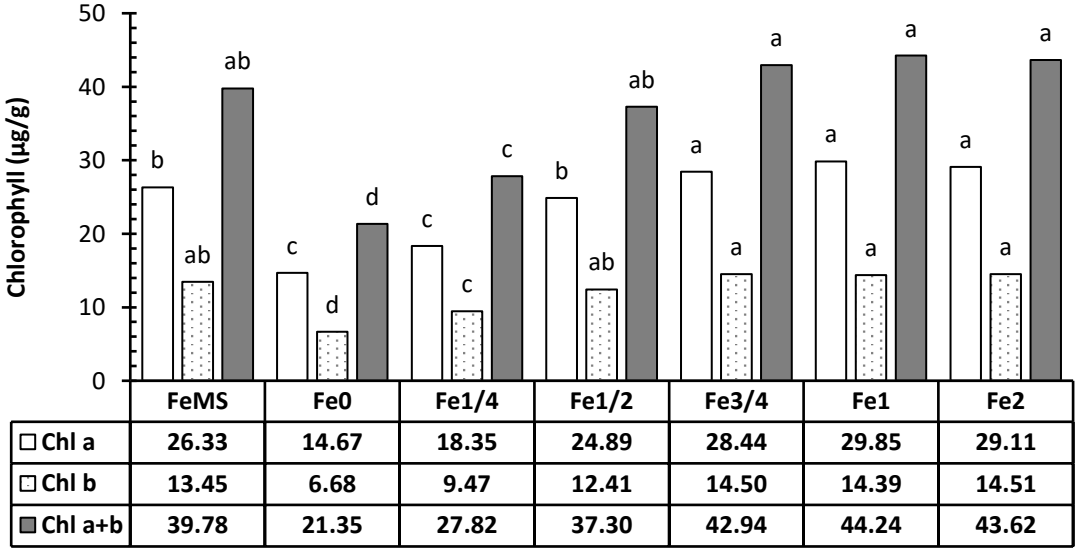


Figure 3. Effect of FeNPs on chlorophyll accumulation of 4-week *Chrysanthemum* in *in vitro* hydroponic culture.

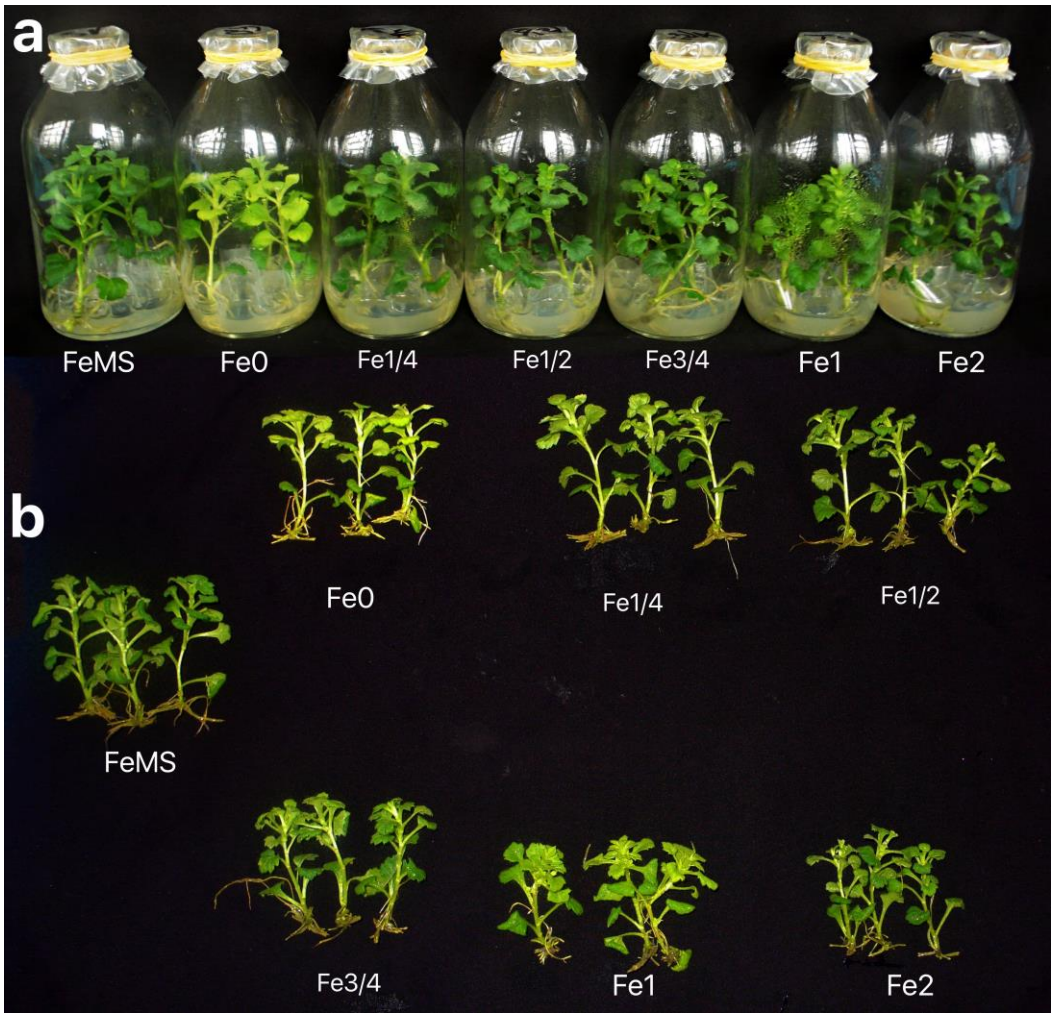
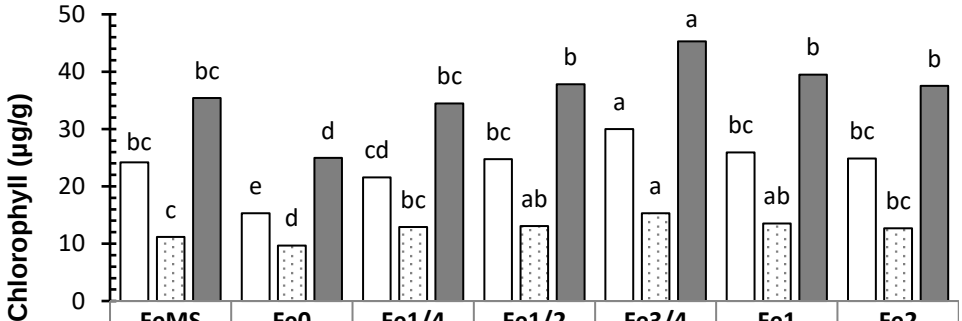


Figure 4. Effect of FeNPs on *Chrysanthemum* growth in *in vitro* hydroponic culture after 4 weeks of culture.

Table 3. Effect of FeNPs on *Chrysanthemum* growth in microponic culture after 4 weeks of culture.

Treatment	Plant height (cm)	No. of leaves	No. of root	Root length (cm)	Fresh weight (g)	Dry weight (g)
FeMS	4.52bc*	6.67b	18.67a	2.50d	0.64bc	0.07d
Fe0	4.34c	10.00a	16.00a	3.37c	0.59c	0.06e
Fe¼	5.05b	8.00b	16.67a	3.24c	0.73ab	0.10ab
Fe½	5.31a	8.33ab	18.00a	4.83a	0.83a	0.11ab
Fe¾	5.26a	7.67b	18.33a	4.01ab	0.80ab	0.10ab
Fe1	5.15ab	8.00b	17.67a	3.44c	0.73ab	0.08bc
Fe2	4.53bc	7.67b	17.67a	2.83cd	0.70b	0.08bc

** Different letters (a, b, ...) in the same column represent statistically significant differences at α = 0,05 (Duncan's test).*



	FeMS	Fe0	Fe1/4	Fe1/2	Fe3/4	Fe1	Fe2
Chl a	24.21	15.33	21.58	24.73	29.98	25.93	24.87
Chl b	11.18	9.66	12.89	13.10	15.33	13.55	12.67
Chl a+b	35.39	24.99	34.47	37.83	45.31	39.48	37.54

Figure 5. Effect of FeNPs on chlorophyll accumulation of 4-week *Chrysanthemum* in microponic culture.

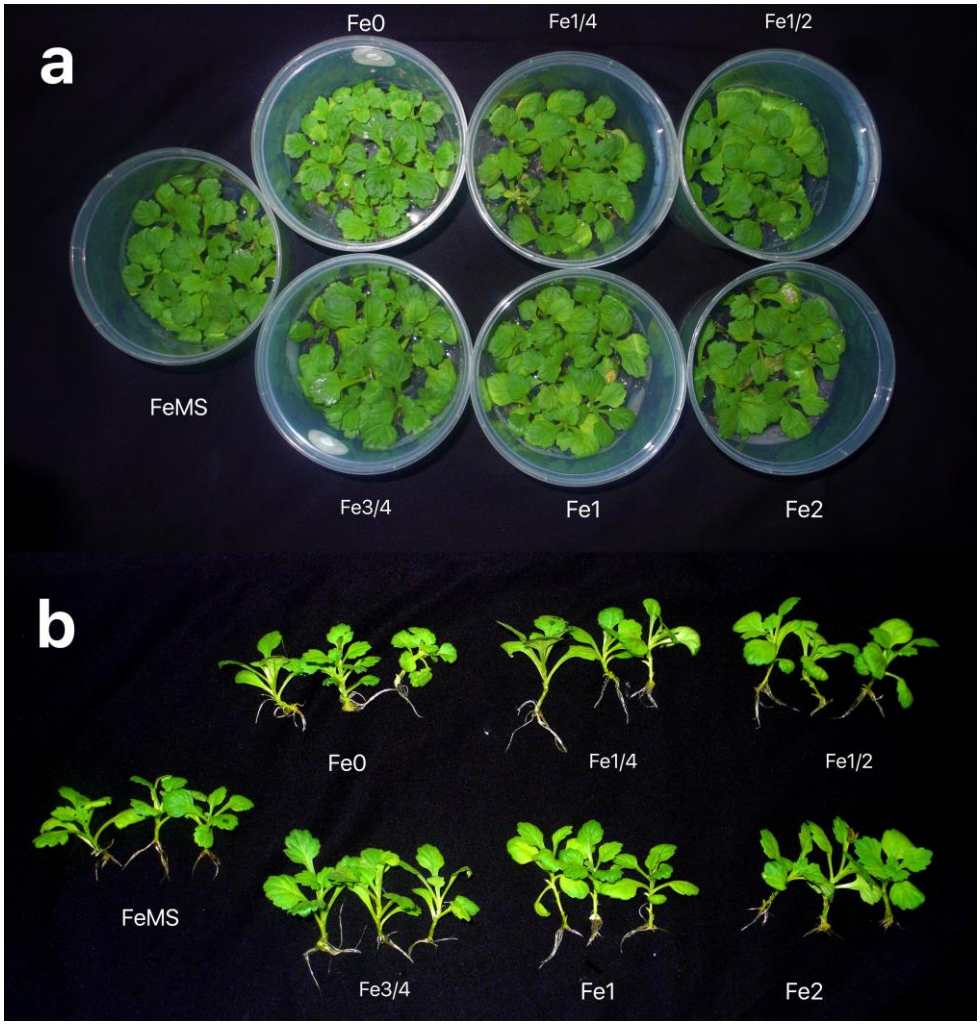


Figure 6. Effect of FeNPs on *Chrysanthemum* growth in microponic culture after 4 weeks of culture.

So far, studies on microroponic have been limited. Microponic systems have many advantages over traditional breeding systems because of inheriting the advantages of micropropagation and culture systems (Hahn *et al*., 1996, 1998, 2000; Nhut *et al*., 2005; Tung *et al*., 2018). In a microponic system, it only takes 2 weeks for the plant to transfer the plants to the greenhouse. This explains why the growth parameters of plants in microponic systems are lower than in other culture systems. Plantlets cultured in a microponic system (an open system, plantlets grown in non-sterile conditions) are easily adapted to external conditions because the plants are trained in both rooting processes and adapt during culture.

The activities of enzymes

The results of antioxidant activity analysis are presented in Figure 7 and 8. In general, the addition of FeNPs to the culture media showed that the SOD and APX enzymes in the roots and leaves of the plants were always highly active, while in the control treatments supplemented with Fe-EDTA, the enzymes were low change. For the control treatment using Fe-EDTA, the content of the antioxidant enzymes [SOD (7.68 and 7.47 U.mg^{-1} prot) and APX (0.36 and 0.32 µmol.min^{-1}.mg^{-1} prot)] in the roots and leaves were low (Figure 7 and 8). The activity of both SOD and APX enzymes of cultured plants in different systems (FeNPs) increased.

In microponic culture with Fe¾, SOD enzyme activity in roots (35.04 U.mg^{-1} prot) is higher than other culture systems and 4.5 times higher than the control (FeMS). Meanwhile, the addition of FeNPs to culture media in culture systems increased approximately 2 times the activity of the SOD enzyme compared to the control. APX (2.11 µmol.min^{-1}.mg^{-1} prot) enzyme activity in leaves increased by 6.5 times compared to the control (0.36 µmol.min^{-1}.mg^{-1} prot) in solid culture systems.

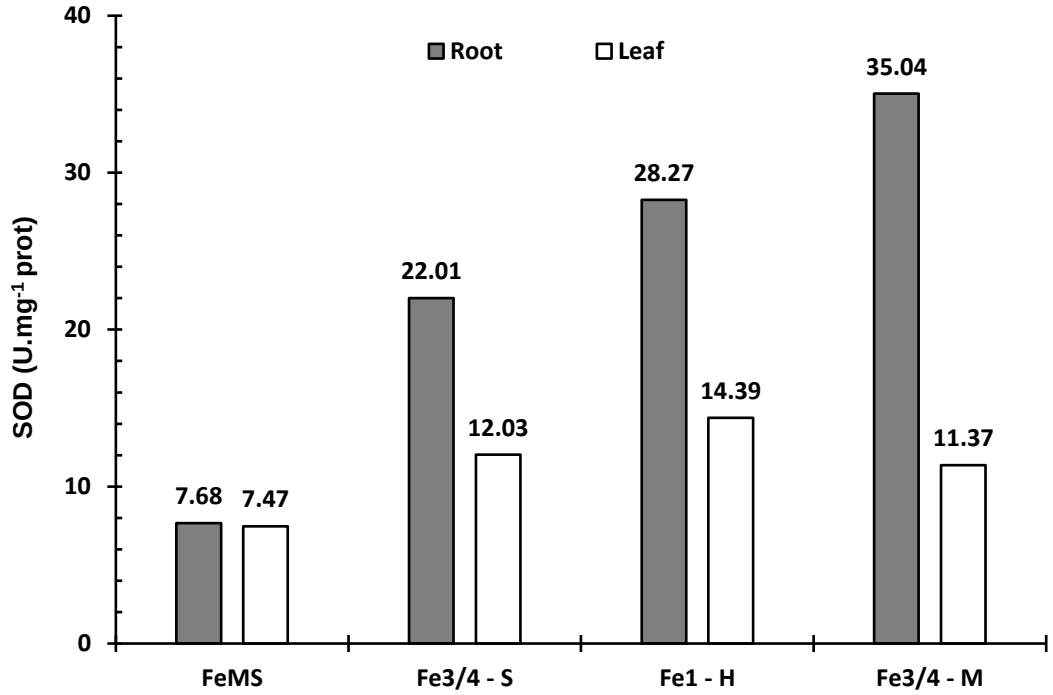


Figure 7. Effect of FeNPs on antioxidant activity of superoxide dismutase.

A number of previous studies have mainly assessed the effects of silver nanoparticles in increasing the activity of enzymes SOD, APX, CAT, etc. (Sharma *et al*., 2012; Nair, Chung, 2014). In this study, FeNPs with different concentrations (75 - 100 mM) plays a role in increasing the activity of SOD and APX enzymes in different culture systems. With the function of breaking down H_2O_2 to H_2O and O_2, the activity of APX and SOD increases, reducing the amount of H_2O_2 which is toxic to the roots. Thus, the increase of SOD, APX activity at 75 - 100 mM FeNPS shows that the plant's antioxidant ability increases, reduces oxidative radicals of O_2 and H_2O_2 in plants, contributing to increased resistance tolerance of plants in culture medium.

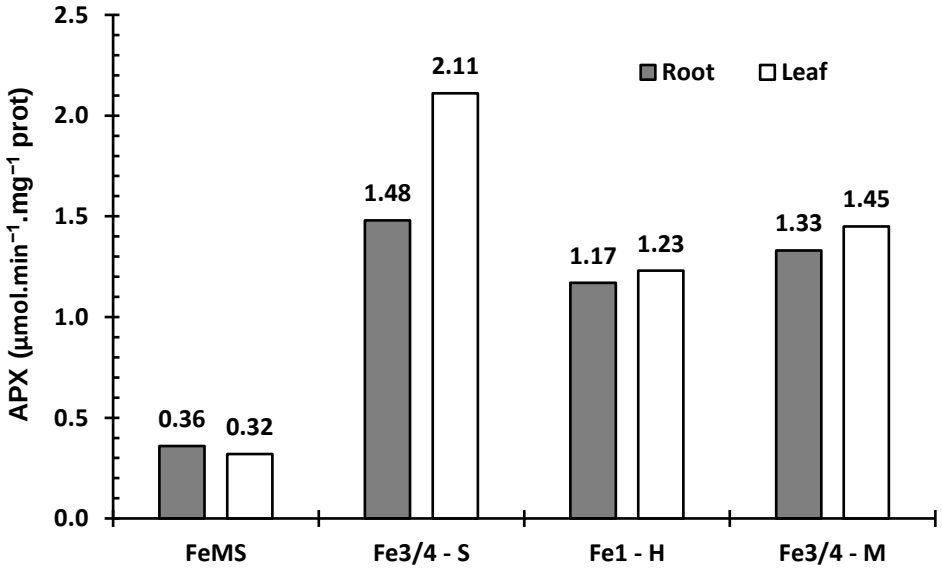


Figure 8. Effect of FeNPs on antioxidant activity of ascorbate peroxidase.

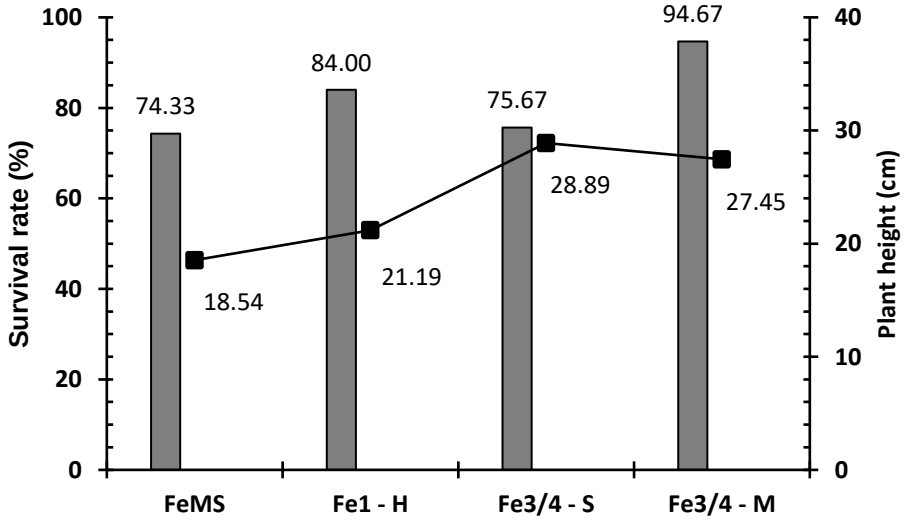


Figure 9. Acclimatization of *Chrysanthemum* grown in different systems in the greenhouse after 8 weeks.

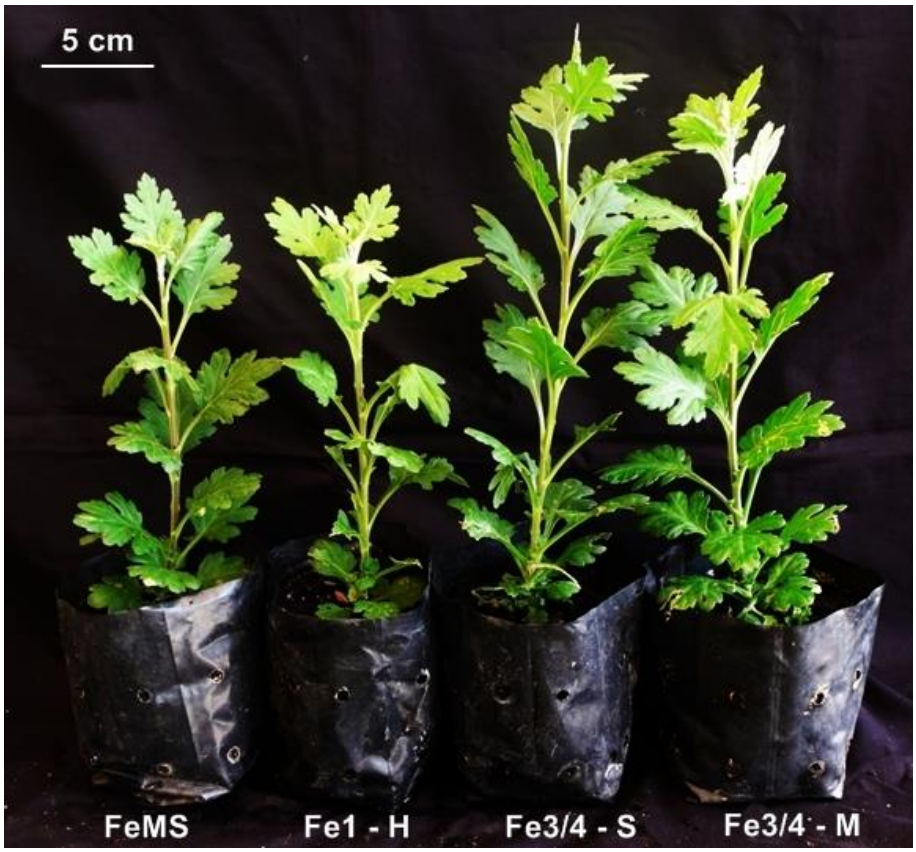


Figure 10. Plant grown in different systems in the greenhouse after 8 weeks.

Acclimatization in the greenhouse

The effectiveness of FeNPs supplementation in culture systems was assessed in the greenhouse after 8 weeks of acclimation (Fig. 9 and 10). The recorded results showed that the highest survival rate (94.67%) of microponic (Fe¾ – M) was higher than in *in vitro* solid (Fe¾ – S) and hydroponic (Fe1 – H) systems (84.00% and 75.67%, respectively). The microponic system is a well-ventilated, reduced nutrient system that helps plants get accustomed to the *ex vitro* conditions, increasing the nursery survival rate.

CONCLUSION

The results of this study showed that FeNPs can replace Fe-EDTA in MS medium. Iron deficiency in culture media will reduce chlorophyll content. The FeNPs concentrations replaced Fe– EDTA in media of *in vitro* solid, *in vitro* hydroponic and microponic culture systems were 75 mM, 100 mM and 75 mM, respectively.

Acknowledgement: *This research is funded by Vietnam National Foundation for Science and Technology Development (NAFOSTED) under Grant number 106.01-2019.301 and Tay Nguyen Institute for Scientific Research under Grant number 30/QĐ-NCKHTN.*

REFFERENCES

Beyer WF, Fridovich I (1987) Assaying for superoxide dismutase activity: some large consequences of minor changes in conditions. *Anal Biochem* 161: 559-566.

Chau HN, Bang LA, Buu NQ, Dung TTN, Ha HT, Quang DV (2008) Some results in manufacturing of nanosilver and investigation of its application for

disinfection. *Adv Nat Sci: Nanosci Nanotech* 9(2): 241-248.

Darioush A, Akihiro I (2014) Phytotoxicity assessment of c-Fe_2O_3 nanoparticles on root elongation and growth of rice plant. *Envir Earth Sci* 71: 5173-5182.

Duncan DB (1955) Multiple range and multiple F test. *Biometrics* 11(1): 1-42.

Ganesan VS, Lazer FJB (2016) Iron oxide nanoparticles promote agronomic traits of ginger (*Zingiber officinale* Rosc.). *Inter J Adv Res in Biol Sci* 3(3): 230-237.

Gurel S, Gulsen Y (1998) The effects of different sucrose, agar and pH levels on *in vitro* shoot production of almond (*Amygdalus communis* L.). *Turk J Bot* 22(1): 363-373.

Hahn EJ, Bae JH, Lee YB, Beom Y (1998) Growth and leaf-surface characteristics of *Chrysanthemum* plantlets between hydroponic and microponic system. *J Kore Soci Hortic Sci* 39(6): 838-842.

Hahn EJ, Bea JH, Lee YB (2000) Growth and photosynthetic characteristics of *Chrysanthemum* plantlets as affected by pH and EC of nutrient solution in microponic culture. *J Kore Soci Hortic Sci* 41(1): 12-15.

Hahn EJ, Lee YB, Ahn CH (1996) A new method on mass-production of micropropagated *Chrysanthemum* plants using microponic system in plant factory. *Acta Hortic* 440: 527-532.

Hinsinger P (2001) *Bioavailability of trace elements as related to root-induced chemical changes in the rhizosphere*. In: Gobran GR, Wenzel WW, Lombi E (Eds), Trace elements in the rhizosphere. *CRC Press LCC*, Boca Raton, Florida, US, pp. 25-41.

Klimaszewska K, Bernier-Cardou M, Cyr D, Sutton B (2000) Influence of gelling agents on culture medium gel strength, water availability, tissue water potential, and maturation response in embryogenic cultures of *Pinus strobus* L. *In Vitro Cell Devl Biol - Plant* 36(4): 279-289.

Kumari M, Mukherjee A, Chandrasekaran N (2009) Genotoxicity of silver nanoparticles in *Allium cepa*. *Sci Total Envir* 407(19): 5243-5246.

Libralato G, Costa DA, Zanella M, Sabbioni E, Mičetić I, Manodori L, Pigozzo A, Manenti S, Groppi F, Volpi GA (2006) Phytotoxicity of ionic, micro- and nano-sized iron in three plant species. *Ecot Envir Saf* 123: 81-88.

Lichtentaler HK, Wellburn AR (1985) Determination of total carotenoids, chlorophyll a and b of leaf in different solvents. *Biochem Soc Trans* 11: 591-592.

Lopez-Moreno M, De La Rosa G, Hernandez-Viezcas JA, Castillo-Michel H, Botez CE, Peralta-Videa JR, Gardea-Torresdey JL (2010) Evidence of the differential biotransformation and genotoxicity of ZnO and CeO_2 nanoparticles on soybean (*Glycine max*) plants. *Envir Sci Technol* 44(19): 7315-7320.

Mamyandi D, Sinha SK, White JC (2009) Assay dependent phytotoxicity of nanoparticles to plants. *Envir Sci Technol* 43(24): 9473-9479.

Miyake C, Asada K (1992) Thylakoid-bound ascorbate peroxidase in spinach chloroplasts and photoreduction of its primary oxidation product monodehydroascorbate radicals in thylakoids. *Plant Cell Physiol* 33: 541-553.

Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue culture. *Physiol Plant* 15: 473-497.

Musante C, White JC (2012) Toxicity of silver and copper to *Cucurbita pepo*: differential effects of nano and bulk-size particles. *Envir Toxic* 27(9): 510-517.

Nadi E, Aynehband A, Moajaddam M (2013) Effect of nano-iron chelate fertilizer on grain yield, protein percent and chlorophyll content of Faba bean (*Vicia faba* L.). *Inter J Biosci* 3(9): 267-272.

Nair PMG, Chung IM (2014) Physiological and molecular level effects of silver nanoparticles exposure in rice (*Oryza sativa* L.) seedlings. *Chemosphere* 112:105–113.

Nhut DT, Don NT, An TTT, Van TPT, Vu NH, Huyen PX, Khiem DV (2005) Microponic and hydroponic techniques in disease-free *Chrysanthemum* (*Chrysanthemum* sp.) production. *J Appl Hortic* 7(2): 67-71.

Roghayyeh S, Mohammad S, Mehdi TS (2010) Effects of nano-iron oxide particles on agronomic traits of soybean. *Not Sci Biol* 2(2): 112-113.

Sharma P, Jha AB, Dubey RS, Pessarakli M (2012) Reactive oxygen species, oxidative damage, and antioxidative defense mechanism in plants under stressful conditions. J Bot: 217037: https://doi.org/10.1155/2012/217037

Soad S, Mousa M, Mohamad HGM (2016) *In vitro* analysis of the efficacy of Fe oxid nanoparticles in

prevention of iron deficiency chlorosis in citrus rootstock (*Citrus volkameriana*). *J Exp Biol Agri Sci* 4(5): 485-492.

Syu Y, Hung JH, Chen JC, Chuang HW (2014) Impacts of size and shape of silver nanoparticles on *Arabidopsis* plant growth and gene expression. *Plant Physiol Biochem* 83C: 57-64.

Tung HT, Nam NB, Huy NP, Luan VQ, Hien VT, Phuong TTB, DT Le, Nhut DT (2018) A system for large scale production of *Chrysanthemum* using microponics with the supplement of silver nanoparticles under light-emitting diodes. *Sci Hortic* 232: 153-161.

ẢNH HƯỞNG CỦA NANO SẮT LÊN SỰ SINH TRƯỞNG VÀ THÍCH NGHI CÂY CÚC (*Chrysanthemum morifolium* Ramat. cv. "Jimba") TRONG CÁC HỆ THỐNG NUÔI CẤY KHÁC NHAU

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TÓM TẮT

Trong nuôi cấy mô tế bào thực vật, sắt là một trong những nano kim loại được sử dụng đầu tiên trên cây trồng. Trong nghiên cứu này, nano sắt (FeNPs) được sử dụng để thay thế muối Fe-EDTA trong môi trường nuôi cấy MS nhằm đánh giá ảnh hưởng của chúng lên sự sinh trưởng, khả năng tích lũy chlorophyll (a, b và a+b), hoạt tính các enzyme chống oxy hóa SOD, APX và khả năng thích nghi ở điều kiện vườn ươm trong các hệ thống nuôi cấy khác nhau (nuôi cấy *in vitro* môi trường rắn, thủy canh *in vitro* và vi thủy canh). Kết quả nhận được cho thấy, bổ sung FeNPs vào môi trường nuôi cấy MS cho hiệu quả sinh trưởng, tích lũy chlorophyll, hoạt tính các enzyme chống oxy hóa SOD và APX tốt hơn so với bổ sung muối Fe-EDTA. Hiệu quả tác động của FeNPs có sự khác biệt giữa các hệ thống nuôi cấy khác nhau. Trong hệ thống nuôi cấy *in vitro* môi trường rắn và vi thủy canh nồng độ tối ưu là 75 mM và hệ thống thủy canh *in vitro* là 100 mM FeNPs. Hoạt tính của enzyme chống oxy hóa SOD (35,04 $U.mg^{-1}$ prot) tối ưu nhất thu nhận trong rễ cây nuôi cấy trong hệ thống vi thủy canh; trong khi đó, hoạt tính của enzyme chống oxy hóa APX (2,11 $\mu mol.min^{-1}.mg^{-1}$ prot) tối ưu nhất thu nhận trong lá của cây nuôi cấy trong môi trường rắn. Khi chuyển cây ra điều kiện vườn ươm, cây có nguồn gốc nuôi cấy vi thủy canh bổ sung FeNPs ở nồng độ 100 mM cho tỷ lệ sống sót cao nhất (94,7%). Kết quả của nghiên cứu này cho thấy FeNPs có thể thay thế được ion sắt Fe-EDTA trong môi trường MS và thiếu sắt trong môi trường nuôi cấy sẽ làm giảm hàm lượng chlorophyll.

Từ khóa: *Ascorbate peroxidase, cúc, nuôi cấy in vitro, nano sắt, superoxide dismutase.*