Vietnam Journal of Science and Technology ***55*** *(4) (2017) 503-514*

DOI: 10.15625/2525-2518/55/4/9322

THE EFFECTS OF SILVER NANOPARTICLES ON GROWTH OF *Chrysanthemum morifolium* Ramat. cv. "JIMBA" IN DIFFERENT CULTURAL SYSTEMS

Luong Thien Nghia[1], Hoang Thanh Tung[1,2], Nguyen Phuc Huy[1], Vu Quoc Luan[1], Duong Tan Nhut[1,*]

[1]*Tay Nguyen Institute for Scientific Research, VAST, 116 Xo Viet Nghe Tinh, Da Lat, Lam Dong*

[2]*Hue University of Sciences, Hue University, 77 Nguyen Hue, Hue, Thua Thien – Hue*

[*]Email: *duongtannhut@gmail.com*

Received: 14 March 2017; Accepted for publication: 31 July 2017

ABSTRACT

Silver nanoparticles (SNPs) are one of metallic nanoparticles widely applied in many fields. Research of SNPs application in plant tissue culture has been gaining attention in recent years. Moreover, novel plant tissue culture systems have been researched and developed for improving SNPs uptake capability in medium. In this study, we investigated effects of SNPs on *Chrysanthemum morifolium* ramat. cv. "JIMBA" growth and its ability in 3 culture systems: *in vitro* solid medium system, *in vitro* liquid medium system and microponic (combined of micropropagation and hydroponic). The 3 cm *Chrysanthemum* shoots and silver nanoparticles of diameter smaller than 20 nm were used in the experiments. After 4 weeks, the results showed that the SNPs concentration was suitable for growth of *Chrysanthemum in vitro* solid medium system, *in vitro* liquid medium system and microponic was 1.5, 1.5 and 5 ppm, respectively. Microponic system not only improved plant growth but also reduced the succulent phenomenon. SNP uptake was likely dependent on concentrations and culture systems. At low concentrations (1, 5, and 10 ppm), SNPs were completely absorbed after 4 weeks of culture in all systems, but not at high concentrations (20 ppm). The amount of absorbed silver nanoparticles was directly proportional to the culture period and inversely proportional to the concentration of SNPs supplemented to the medium. In all of our investigated systems, the hydroponic system showed the highest capability of SNPs absorption.

Keywords: absorption, *Chrysanthemum* sp., growth, microponic, silver nanoparticles.

1. INTRODUCTION

The rapid growth of nanotechnology make it become one of the greatest impetuses to technological and industrial development in the 21st century. Among the different type of nanomaterials, silver nanoparticles (SNPs) are extensively used in many fields of science and technology. In plant tissue culture technology, SNPs were applied as a factor which may resist some inhibitors in micro-propagation, such as *in vitro* contamination (e.g., fungal and bacterial infections) [1, 2, 3, 4, 5, 6] and ethylene [7, 8, 9], hence, improve *in vitro* plant growth. In recent

years, nanoparticles absorption mechanisms have gained first attentions, experiments demonstrated SNPs can be absorbed via leafs or roots [10] by penetrated or diffused in symplast and apoplast [11]. Otherwise, cultural conditions are the most important factors which directly impact to nanoparticle absorption. Kumari et al. proved that in liquid medium metal nanoparticles are absorbed more effectively than in solid or semi-solid medium [12]. On the other hand, ventilation condition between inside and outside system is an advantage factor for uptake nutrient from medium due to enhancing transpiration capability. Nevertheless, studying about conditions effect on SNPs absorption is still quite limited. Therefore, the targets in this experiment aim to investigate effect of SNPs on *Chrysanthemum* sp. growth in various cultural systems and find out which system is appropriate to SNPs absorption.

2. MATERIALS AND METHODS

2.1. Sample source and materials

Chrysanthemum morifolium shoots with 3 cm in length were used as explant source. These shoots were obtained from a mass of shoots cultured *in vitro* on Murashige and Skoog (MS) medium [13] with 8 g/L agar and 30 g/l sucrose after 40-45 days of culture.

The used silver nanoparticles (SNPs) were of size smaller than 20 nm which had been manufactured using the rate: [$AgNO_3$] = 750 – 1000 ppm, [β-chitosan] = 250 – 300 ppm, [$NaBH_4$] = 200 ppm, mole rate [$NaBH_4$]/[$AgNO_3$] = ¼, $NaBH_4$ drip speed: 10 – 12 droplet/min.

Substrates in microponic and *in vitro* liquid medium systems are tubes of nylon films with 2 cm in height and 1.5 cm in diameter. Substrates were put into the cultural vessels (bottles or plastic box).

2.2. Cultural systems

2.2.1. In vitro with solid or liquid medium systems

Glass bottles with 250 ml in volume, each of bottles contain 40 ml of MS medium, with 30 g/l sucrose (and supplied 8 g/l agar in solid medium system). After that, we supplied SNPs of various concentrations and sterilized by autoclaving at 121 ^oC, 1 atm in 30 minutes.

2.2.2. Microponic system

Microponic system was circular plastic containers with 12 cm of diameter at top, 9 cm of diameter at bottom, and 8.5 cm of height. 40 ml of half-strength sugar-free liquid MS medium was added to system with 15 tubes of nylon film. Top of the system was equipped with one Millipore filter by Milliseal^{TM}, of pore size of 0.2 μm (Nihon Millipore Ltd., Tokyo, Japan).

All experiments were incubated at 25 $\pm$ 2 ^oC with humidity of 55 – 60 % and photoperiod of 12 hours/day under fluorescent light with 45 $\mu mol.m^{-2}.s^{-1}$ of intensity.

2.3. Methods

2.3.1. Evaluating effect of SNPs to Chrysanthemum growth on in vitro solid medium system

Shoots were cultured in *in vitro* solid medium system within different SNPs concentrations (0; 0,5; 1; 1,5; 2; 3; 5; 7; 10 ppm) and sterilized in autoclave to investigate effects of SNPs on *Chrysanthemum* growth.

2.3.2. Evaluating effect of SNPs to Chrysanthemum growth on in vitro liquid medium system

Shoots were cultured in *in vitro* liquid medium system within different SNPs concentrations (0; 0.5; 1; 1.5; 2; 3; 5; 7; 10 ppm) and sterilized in autoclave to investigate effects of SNPs on *Chrysanthemum* growth.

2.3.3. Evaluating effect of SNPs to Chrysanthemum growth on microponic system

Shoots were cultured in microponic system within different SNPs concentrations (0; 5; 10; 15; 20 ppm) without sterilization to investigate effects of SNPs on *Chrysanthemum* growth.

2.3.4. Evaluating absorption capability of different systems

Shoots were cultured in different culture systems (*in vitro* solid medium, *in vitro* liquid medium and microponic system) with various SNPs concentrations (1; 5; 10; 20 ppm). After 1, 2, 3, 4 weeks, medium remained was collected and SNPs content in culture medium was examined to determine absorbed-SNPs contents.

2.4. Collecting and analysing data

Data were scored in 4 weeks of culturing and analysis of variance was performed. Investigated growth characteristic include: Plant height (mm); number of shoots; number of leaves; number of roots, root length (mm); SPAD - total chlorophyll content (μg/g), fresh weight (mg); dry weight (mg); net weight rate (%), absorbed-SNPs rate (%).

Total chlorophyll contents in leaves were evaluated by SPAD-502 (Minolta Co., Ltd., Osaka, Japan). SNPs contents in medium were evaluated by UV–vis spectroscopic at 480 nm [14] of wavelength (Shimadzu, UV-2450, Japan). Root morphology were observed by Nikon SMZ 800 (Nikon, Japan) in 20x of magnify rate.

Net weight rates were calculated by the following formula:

$$\text{Net weight rate (\%)} = \frac{\text{Dry weight (mg)}}{Fresh\ weight\ (mg)} \times 100\ \%$$

Absorbed-SNP rates were calculated by the following formula:

$$\text{AgH (\%)} = \frac{\text{Ag0} - \text{AgT}}{\text{Ag0}} \times 100\ \%$$

where: AgH is absorbed-SNPs rates after (1, 2, 3, 4 weeks) (%),

Ag0 is total SNPs content in culture medium at the beginning (mg),

AgT is total SNPs content in culture medium at 1, 2, 3, 4 weeks (mg).

All treatments were in triplicates and each replicate with 10 culture vessel. The means were compared using Duncan's multiple range Test using SPSS (Version 16.0) at $\alpha = 0.05$ [15].

3. RESULTS AND DISCUSSION

3.1. Effects of SNP on *Chrysanthemum* growth in *in vitro* solid medium system

The effects of exposure to different concentrations of SNPs on *Chrysanthemum* plantlets after four weeks of incubation are presented in Table 1 and Figure 1.

Table 1. Effects of SNP on *chrysanthemum* growth in *in vitro* solid medium system.

SNP (ppm)	No. of shoots	Plant height (mm)	No. of leaves	No. of roots	Root length (mm)	SPAD (mg/g)	Fresh weight (mg)	Dry weight (mg)	Net weight rate(%)
0	6.3^{bcd}	39.7^{bc}	10.3^{a}	8.7^{c}	49.7^{bcd}	31.9^{b}	300.3^{b}	14.0^{e}	4.66^{a}
0.5	6.3^{bcd}	39.0^{bc}	8.3^{bcd}	8.0^{c}	45.0^{d}	32.7^{b}	328.0^{b}	19.0^{de}	5.79^{a}
1	7.3^{b}	44.7^{ab}	8.7^{abc}	14.0^{abc}	45.3^{d}	31.7^{b}	531.0^{a}	31.0^{c}	5.84^{a}
1.5	9.0^{a}	48.3^{a}	10.0^{ab}	17.3^{a}	65.0^{a}	37.6^{a}	673.7^{a}	58.7^{a}	8.71^{a}
2	6.7^{bc}	42.0^{ab}	8.0^{bcd}	16.7^{ab}	62.0^{abc}	34.0^{ab}	657.3^{a}	46.0^{b}	7.00^{a}
3	6.7^{bc}	34.7^{cd}	9.7^{abc}	13.7^{abc}	62.2^{abc}	32.5^{b}	354.3^{b}	27.3^{cd}	7.71^{a}
5	5.3^{cd}	32.0^{d}	6.3^{e}	10.3^{bc}	53.7^{bcd}	31.7^{b}	188.7^{b}	15.3^{e}	8.13^{a}
7	5.7^{bcd}	42.7^{ab}	7.7^{cde}	11.7^{abc}	64.3^{ab}	34.9^{ab}	342.7^{b}	22.0^{cde}	6.42^{a}
10	4.7^{d}	31.7^{d}	7.0^{de}	10.7^{bc}	48.3^{d}	32.3^{b}	228.3^{b}	14.3^{e}	6.28^{a}

Different letters within a column indicate significant differences at $\alpha = 0.05$ by Duncan's multiple range tests.

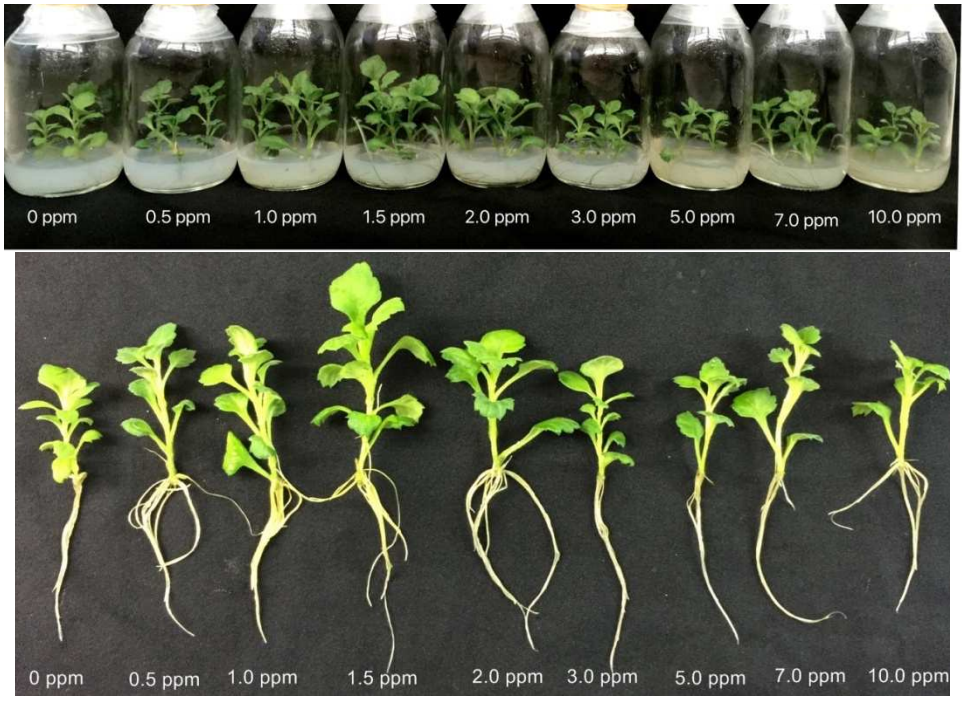

Figure 1. Effects of SNPs on *Chrysanthemum* morphology in *in vitro* solid medium system.

Most of values observed such as number of shoots, plant height, number of leaves, number of roots, root length, SPAD (Soil-Plant Analysis Development), fresh weight and dry weight showed a significant change (Supplementary Table 1; Figure 1). The mean net weight rate of the plantlets showed no significant difference in various SNPs concentrations. Plants cultured on

medium with 1.5 ppm SNPs grew well with almost investigated characteristics reached best as compared to remain SNPs concentrations. When adding higher SNPs concentration - 1.5 ppm in medium, plant growth was slightly lower, severely at 10 ppm, number of shoots, plant height, number of leaves were decreased on the contrary SNPs free. Therefore, in *in vitro* with solid medium system, SNPs with concentration 1.5 ppm is appropriate to *Chrysanthemum* plant growth.

Some reports confirmed the positive response of SNPs on many plant species. For instance, *in vitro Chrysanthemum* growth improved at 10 ml/l of SNPs dose [16], *Araucaria excelsa* explants grown in MS medium supplemented with SNPs demonstrated that explants grown on media supplemented with SNPs were fresher, had a suitable growth, and maintained their green color on the contrary to SNPs free-MS medium. The authors hypothesized that SNPs indirectly affect plant growth but its inhibitory effects of plant phytohormone ethylene - an inhibitor caused senescence, malformation or some phenomenon as hyperhydicity [8]. According to what reported by Sarmast, findings in *Tecomella undulata* (Roxb.) Seem. micropropagation demonstrated that the ethylene present in culture vessels during its micropropagation caused shedding of leaves, decreased in chlorophyll content and finally would result in the demise of explants. Providing SNPs in MS medium of *T. undulata* improved survival percentage of explants and increased mean number of shoot and length of explants [17]. Ethylene resistance mechanism of SNPs due to blocking 1-Aminocyclopropane-1-carboxylic acid (ACC) gene - ethylene precursor synthesized gene, subsequently, inhibit ethylene synthesis [17]. On the other hand, in higher concentrations of SNPs negative effects, including seed germination, shoots and roots growth, late flowering and low yield, were observed [18, 19, 20]. Especifically, at 20 ppm of SNPs concentration, yield and antioxidant gene expressions were decreased in *Arabidopsis* [21], or inhibition of early development at 73.4 ppm [22].

3.2. Effects of SNPs on *Chrysanthemum* growth in *in vitro* liquid medium system

After 4 weeks of treatment with different concentrations of SNPs in *in vitro* liquid medium, the results showed that mean of plant height, number of roots, root length, fresh weight, dry weight were statistically different in various concentrations of SNPs (Table 2, Fig. 2). Among them, 1.5 ppm SNPs reached best effects to *Chrysanthemum* growth which manifested in the best of plant height, number of shoots, number of roots, root length, fresh weight, dry weight and net weight rate as compared to control (1.12; 1.45; 3.3; 1.3; 1.3; 1.75 times, respectively).

Table 2. Effects of SNPs on *Chrysanthemum* growth in *in vitro* liquid medium system.

SNP (ppm)	No. of shoots	Plant height (mm)	No. of leaves	No. of roots	Root length (mm)	SPAD (mg/g)	Fresh weight (mg)	Dry weight (mg)	Net weight rate (%)
0	10.0^{ab}	71.7^{b}	13.0^{a}	16.0^{b}	16.0^{d}	39.2^{ab}	1221.0^{b}	32.0^{cd}	2.62^{b}
0.5	8.0^{cd}	72.7^{b}	8.3^{c}	16.0^{b}	17.7^{d}	41.3^{a}	790.7^{c}	23.7^{d}	2.99^{b}
1	8.3^{c}	78.3^{ab}	9.7^{bc}	16.7^{b}	57.0^{a}	34.8^{de}	1022.0^{b}	34.7^{abc}	3.39^{b}
1.5	8.7^{bc}	81.0^{a}	9.3^{bc}	23.3^{a}	53.0^{ab}	37.7^{bc}	1590.7^{a}	74.0^{a}	4.65^{a}
2	11.0^{a}	83.0^{a}	13.0^{a}	17.7^{b}	42.3^{bc}	35.9^{cd}	1470.3^{ab}	46.3^{b}	3.15^{b}
3	6.7^{de}	77.0^{ab}	9.0^{bc}	17.8^{b}	63.0^{a}	35.7^{cd}	1308.3^{ab}	38.3^{bc}	2.93^{b}
5	6.3^{e}	52.7^{c}	8.7^{c}	17.0^{b}	53.3^{ab}	37.9^{bc}	1202.0^{b}	30.0^{cd}	2.50^{b}
7	6.0^{e}	41.3^{d}	11.0^{ab}	15.3^{b}	39.3^{c}	33.6^{e}	836.3^{c}	27.0^{cd}	3.23^{b}

10	6.3^{e}	31.7^{e}	8.0^{c}	15.0^{b}	36.0^{c}	34.2^{de}	1005.3^{bc}	29.3^{cd}	2.92^{b}

Different letters within a column indicate significant differences at $\alpha = 0.05$ by Duncan's multiple range tests.

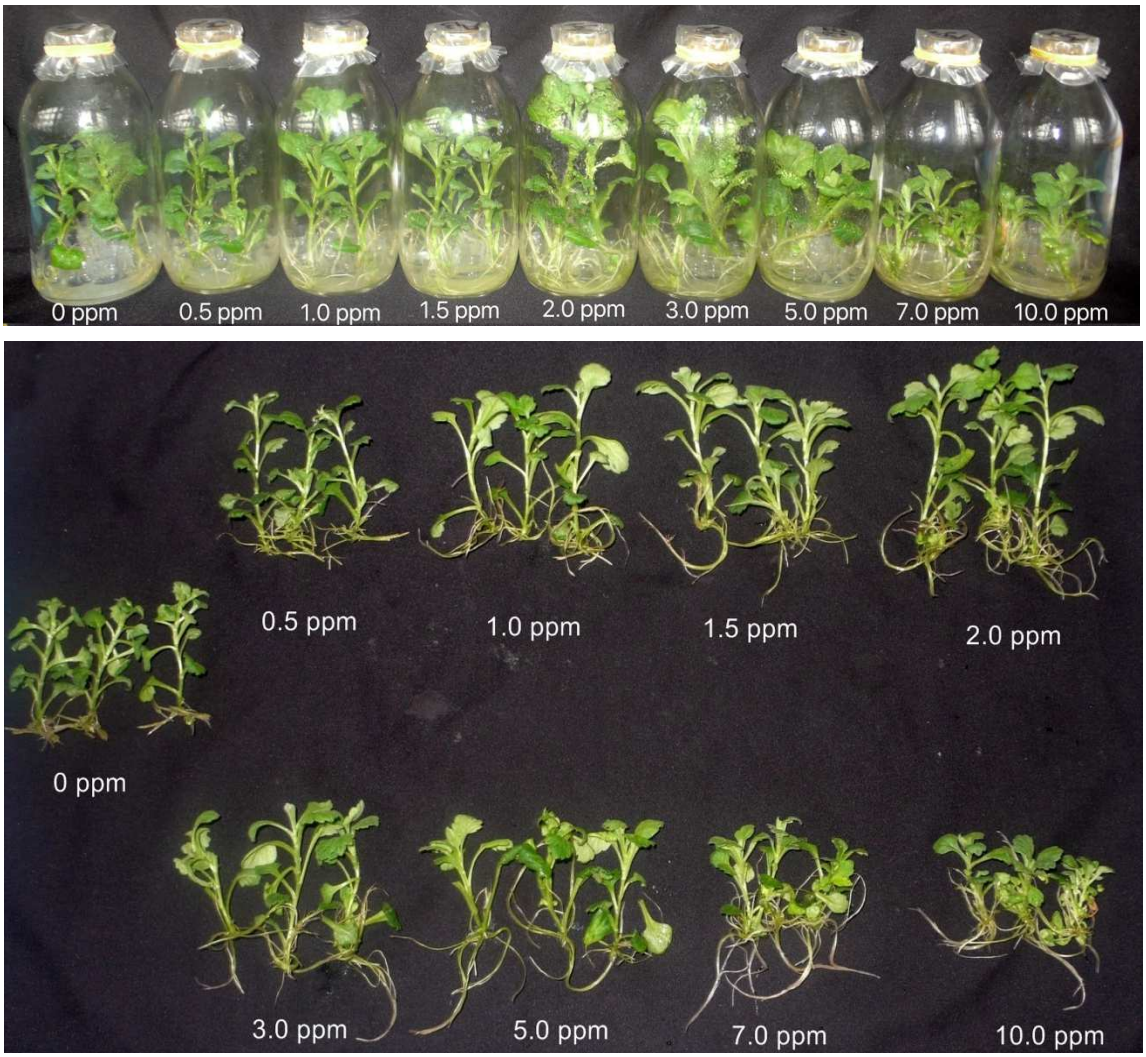

Figure 2. Effects of SNP on *Chrysanthemum* morphology in *in vitro* liquid medium system.

When supplying 1 ppm SNPs into medium, although root length reached highest value, others still lower as compared with those cultured on medium supplemented with 1.5 ppm SNPs. When SNPs concentration over 1.5 ppm, growth index was suppressed. Particularly, supplied 10 ppm SNPs gained inhibited response in plant. Plant height, number of leaves, number of roots, root length, SPAD, fresh weight, dry weight decreased. In this experiment, hyperhydricity phenomenon on *in vitro* plantlet in closure vessel was appeared and expressed in lower net weight rate. Exposed SNPs *Chrysanthemum* at different concentrations were resisted hyperhydricity phenomenon. Especially, 1.5 ppm SNPs help *Chrysanthemum* gained best as compared to others concentrations and control.

3.3. Effects of SNPs on *Chrysanthemum* growth in microponic system

Table 3. Effects of SNP on *Chrysanthemum* growth in microponic system.

SNP (ppm)	No. of shoots	Plant height(mm)	No. of leaves	No. of roots	Root length (mm)	SPAD (mg/g)	Fresh weight (mg)	Dry weight (mg)	Net weight rate (%)
0	10.0^{ab}	49.0^{b}	8.7^{a}	21.0^{a}	11.3^{ab}	31.0^{c}	619.0^{b}	37.3^{b}	6.03^{b}
5	11.0^{a}	56.0^{a}	8.0^{a}	14.2^{ab}	13.3^{a}	39.3^{a}	708.3^{a}	45.3^{a}	6.40^{a}
10	8.3^{c}	47.0^{bc}	10.0^{a}	12.0^{b}	6.7^{bc}	35.2^{b}	654.7^{ab}	44.0^{ab}	6.72^{a}
15	8.7^{bc}	45.0^{bc}	8.3^{a}	8.7^{c}	4.7^{c}	34.1^{b}	569.3^{bc}	37.0^{b}	6.50^{b}
20	7.3^{c}	41.7^{d}	9.0^{a}	8.3^{c}	4.0^{c}	29.8^{c}	472.7^{c}	31.3^{c}	6.63^{ab}

Different letters within a column indicate significant differences at $\alpha = 0.05$ by Duncan's multiple range tests.

The effects of exposure to different concentrations of SNPs on *Chrysanthemum* growth are shown in Table 3 and Fig. 3. The overall results indicated that plants which were exposed to different concentrations of SNPs showed significant change in growth characteristics. 5 ppm of SNPs-exposed plant growth is best as compared to the other concentrations. Like in aforementioned *in vitro* systems, at high concentrations of SNPs, plant growth quite be inhibited. Particularly, 10 ppm of SNPs-exposed plant showed as plants of lowest quality. This result is appropriate with Tung et al. report in 2006, in this study, the authors determined suitable concentration of SNPs in microponic culture on *Chrysanthemum* is 7.5 ppm, if SNPs concentrations were higher than this value, plant growth would become suppressed [23].

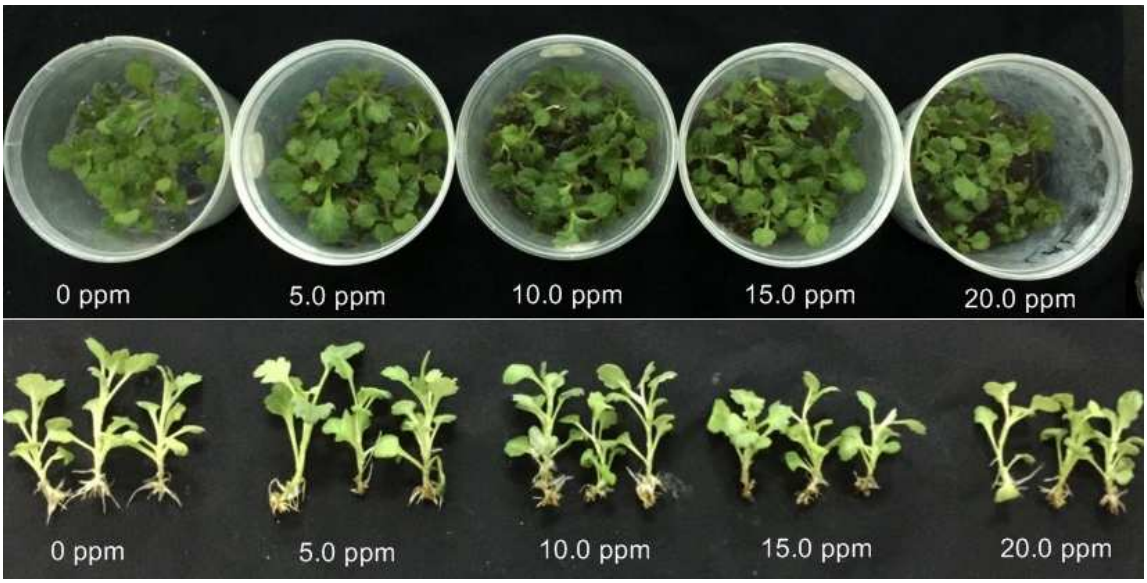

Figure 3. Effects of SNP on *Chrysanthemum* morphology in microponic system.

When comparing growth and morphology of *Chrysanthemum* which were cultured in 3 different systems (*in vitro* solid medium, *in vitro* liquid medium and microponic system). The results showed that *in vitro* liquid medium seem was the best medium for *in vitro* plant growth due to some of characteristics reach highest as compared to other systems. Due to in aqueous condition, medium can dilute and translocate nutrients easier than in hard condition. Moreover, supported nutrient content (mineral, carbon source) in *in vitro* liquid medium system was higher in microponic system, consequently, plant growth in *in vitro* liquid medium system was better. Nevertheless, *in vitro* liquid medium system with closure vessel condition causes high humidity and hyperhidricity phenomenon expressed in net weight rate lower than *in vitro* solid medium and microponic system (3.7 and 13.5 times, respectively).

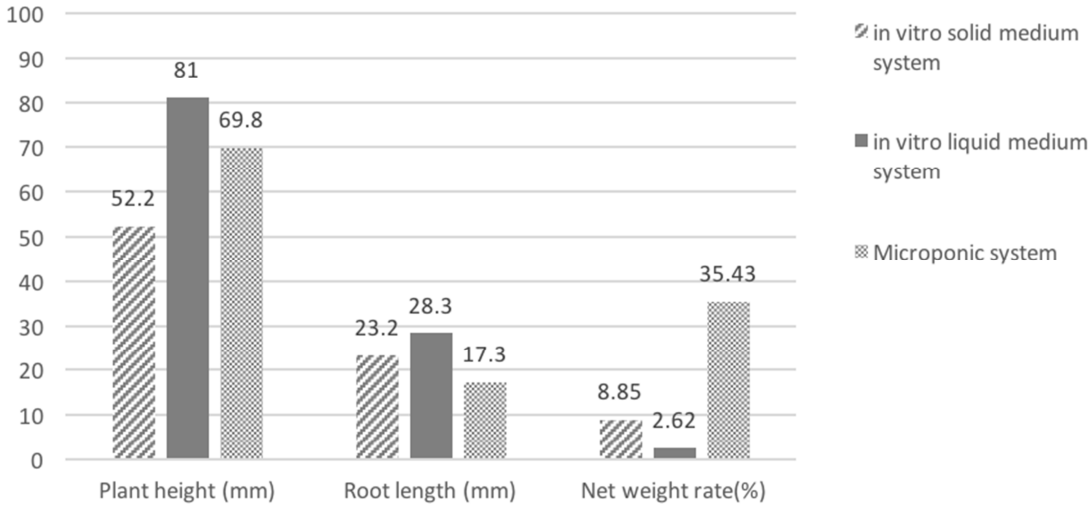

Figure 4. SNPs-exposed *Chrysanthemum* growth in different cultural systems.

Figure 5. SNPs-exposed root morphology in different cultural system (left to right: *in vitro* solid medium, *in vitro* liquid medium and microponic system, respectively).

Beside of growth values, the alteration in plant morphology under SNP-exposed in different cultural conditions were recognized (Fig. 4). When observed with microscope, root morphologies in different systems have significant change. In liquid medium conditions (*in vitro* liquid medium and microponic), root-hair developed denser than in solid medium (Fig. 5). Moreover, it was observed that exposure to different concentrations of SNPs has resulted in root tip browning in microponic system. Similar phenomenon was realized in Tung et al. research, they hypothesized that this phenomenon be intensified when SNPs concentration increased and cause root necrosis [23]. Prakash and Ill Min Chung also confirmed root browning by culturing Arabidopsis thaliana in a medium supplemented with SNPs, which occurs due to the accumulation of silver nanoparticles at the root tips [24].

3.4. SNPs absorption in different cultural systems

In the first two weeks of culture, the SNPs absorbed capability of *Chrysanthemum* were very low in all cultured systems. The SNPs uptake was improved then in subsequent culture weeks. Under *in vitro* solid medium, the ability to absorb SNPs at 20 ppm was only 15.8 % in the first two weeks, but within the next two weeks absorbed-SNPs increased up to 71 % (Fig. 6). This can be explained at the beginning, *Chrysanthemum* root system still not formed which makes the absorbed-SNPs capacity be limited and only gradually improved in the next stages.

Graphs of absorbed-SNPs rate in *in vitro* the solid medium system also showed that silver were not completely absorbed at all concentrations over a 4-week period. From 71.7 to 95.9 % and inversely proportional to the SNPs concentration added to the culture medium (Fig. 6).

In *in vitro* liquid medium systems, at low concentrations (1, 5, 10 ppm), silver nanoparticles are completely absorbed after 4 weeks. Especially, 1 ppm SNPs were completely absorbed only for 3 weeks. In this system, absorbed-SNPs capability is also directly proportional to the culture times and inversely proportional to the SNP concentrations. Due to the liquid medium is capable of absorbing growth regulators, dissolving nutrients is better in the solid medium [25]. The study by Suthar et al. also showed that cultured *in vitro Boswellia serrata* Roxb shoots in liquid medium gave better performance than in solid medium [26]. The authors explained this based on the solubility and flexibility of the environment, which makes the nutrient in the medium easier to be absorbed. In this study, the image was observed under a microscope showing that in a liquid medium, root hairs grow stronger in a solid medium, consequently increases the uptake of nutrients (Fig. 5).

In the microponic system after 4 weeks of culture, except the treatment with 20 ppm SNPs-supplementation (only 80.9 % SNPs absorbed), silver nanoparticles were completely absorbed in other treatments. Remaining in all treatments, silver nanoparticles were completely absorbed, in treatments 1 and 5 ppm silver nanoparticles were completely absorbed only after 2; 3 weeks. Silver nanoparticle absorption in this system was directly proportional to the culture time and inversely proportional to the initial silver nanoparticles added to the culture medium, too.

As compared to *in vitro* liquid medium systems, it was found that in microponic system (aeration filter equipped), SNPs absorbed capability were better at all concentrations. Ventilation is also a condition that enhances the uptake of nutrients from the medium, Ventilation condition enhances air, moisture exchange, promotes water and nutrients uptake from the medium. As compared with other systems, the micro hydroponics system included both growth helpful conditions: liquid medium and ventilated, furthermore saved materials (no sugar added, less than 1/2) and energy (medium not through sterilize by autoclaving) and still guaranteed the utility of silver nanoparticles.

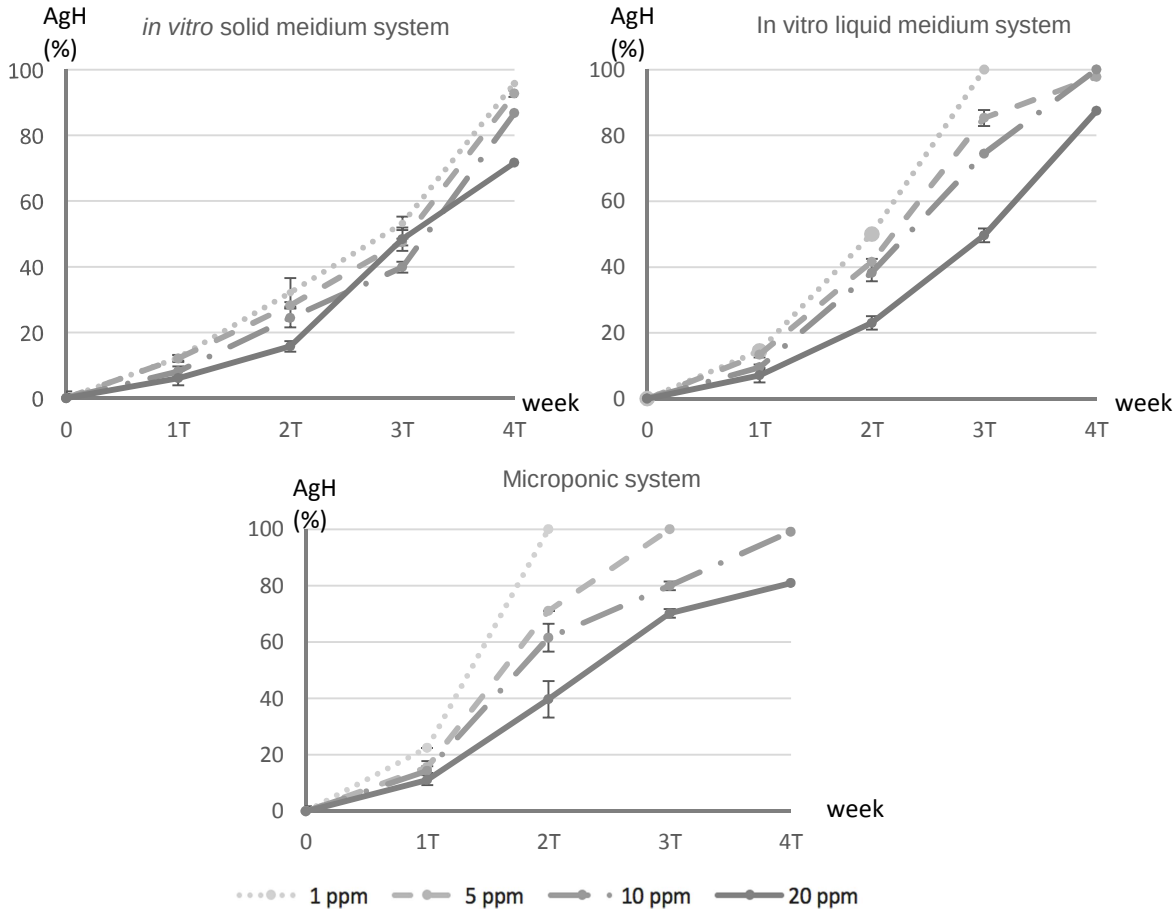

Figure 6. Absorbed-SNPs rate in different culture systems.

4. CONCLUSION

Silver nanoparticles are suitable for *in vitro Chrysanthemum* growth in various culture systems: *in vitro* solid medium, *in vitro* liquid medium and microponic system are 1.5; 1.5; 5 ppm, respectively. Different culture systems have different SNPs absorbability which directly proportional to the culture time and inversely proportional to the SNPs concentration in the medium. Microponic culture system with liquid medium, ventilated conditions, reduced mineral content and no sugar gained the best results on SNPs absorption, *chrysanthemum* growth and cost savings.

Acknowledgements: This work was supported by "Research effect of metal nanoparticles to regeneration capability, growth, development and metabolite accumulating" project by Vietnam Academy of Science and Technology under project no. VAST.TĐ.NANO.04/15-18.

REFERENCES

1. Gholamreza A., Hassan S., Morteza K-K. - Nano silver: a novel nanomaterial for removal of bacterial contaminants in valerian (*Valeriana officinalis* L.) tissue culture. Acta Physiologiae Plantarum **30** (2008) 709-714.
2. Guggenbichler J. P., Boswald M., Lugauer S., Krall T. - A new technology of microdispersed silver in polyurethane induces antimicrobial activity in central venous catheters, Journal of Infection **27** (1999) 16-23.
3. Jo Y. K., Kim B. H. and Jung G. - Antifungal activity of silver ions and nanoparticles on phytopathogenic fungi, Plant Disease Journal **93** (2009) 1037-1043.
4. Kim S. W., Jung J. H., Lamsal K., Kim Y. S., Min J. S., Lee Y. S. - Antifungal Effects of Silver Nanoparticles (AgNPs) against Various Plant Pathogenic Fungi, Mycobiology Journal **40** (1) (2012) 53-58.
5. Kora A. J., Arunachalam J. - Assessment of antibacterial activity of silver nanoparticles on *Pseudomonas aeruginosa* and its mechanism of action, World Journal of Microbiology and Biotechnology **27** (2011) 1209-1216.
6. Sahu N., Soni D., Chandrashekhar B. - Synthesis and characterization of silver nanoparticles using *Cynodon dactylon* leaves and assessment of their antibacterial activity, Bioprocess and Biosystems Engineering **36** (7) (2012) 999-1004.
7. Rodrıguez F. I., Esch J. J., Hall A. E., Binder B. M., Schaller G. E., Bleecker A. B. - A copper cofactor for the ethylene receptor ETR1 from *Arabidopsis*, Science **283** (5404) (1999) 996-998.
8. Sarmast M. K., Salehi H., Khosh-Khui M. - Nano silver treatment is effective in reducing bacterial contaminations of *Araucaria excelsa* R. Br. var. *glauca* explants, Acta Biologica Hungarica **62** (4) (2011) 477-84.
9. Zhao X. C., Mathews D. E., Schaller G. E. Effect of ethylene pathway mutations upon expression of the ethylene receptor ETR1 from *Arabidopsis*, Plant Physiology **130** (4) (2002) 1983-1991.
10. Zhang P., Ma Y., Zhang Z. - Interactions Between Engineered Nanomaterials and Plants: Phytotoxicity, Uptake, Translocation, and Biotransformation. Nanotechnology and Plant Sciences, Springer, Switzerland, 2015, pp. 87.
11. Larue C., Castillo M. H., Sobanska S. - Foliar exposure of the crop *Lactuca sativa* to silver nanoparticles: evidence for internalization and changes in Ag speciation, Journal of Hazardous Materials **264** (2014) 98-106.
12. Kumari M., Mukherjee A., Chandrasekaran N. - Genotoxicity of silver nanoparticles in *Allium cepa*, Science of The Total Environment **407** (2009) 5243-5246.
13. Murashige T. and Skoog F. - A revised medium for rapid growth and bioassays with tobacco tissue cultures, Plant Physiology Journal **15** (1962) 473-497.
14. Bhui D. K., Bar H., Sarkar P., Sahoo G. P., De S. P., Misra A. - Synthesis and UV–vis spectroscopic study of silver nanoparticles in aqueous SDS solution, Journal of Molecular Liquids **145** (1) (2009) 33-37.
15. Duncan D. B. - Multiple range and multiple F test. Biometrics **11** (1955) 1-42.
16. Duong Tan Nhut, Ho Thanh Tam, Nguyen Thi Thanh HIen, Le Kim Cuong, Vu Quoc Luan, Nguyen Ba Nam, Nguyen Phuc Huy, Vu Thị Hien, Trinh Thi Huong, Nguyen Hong Hoang, Nguyen Xuan Tuan, Nguyen Thanh Sang, Nguyen Viet Cuong, Do Manh Cuong,

Nguyen Hoai Chau, Ngo Quoc Buu - Effects of nanosilver on growth of *Chrysanthemum* sp., *Fragaria* sp. and *Gerbera* sp. cultured *in vitro*, Journal of biotechnology **12** (1) (2014) 103-111 (in Vietnamese).

17. Sarmast M. K., Niazi A., Salehi, H., Abolimoghadam, A. - Silver nanoparticles affect ACS expression in Tecomella undulata *in vitro* culture. Plant Cell, Tissue and Organic Culture **121** (1) (2015) 227-236.
18. El-Temsah Y. S., Joner E. J. - Impact of Fe and Ag nanoparticles on seed germination and dif- ferences in bioavailability during exposure in aqueous suspension and soil, Environment Toxicology **27**(1) (2012) 42–49.
19. Lee W. M., An Y. J., Yoon H., Kweon H. S. - Toxicity and bioavailability of copper nanoparticles to the terrestrial plants mung bean (Phaseolus radiatus) and wheat (Triticum aestivum): plant agar test for water-insoluble nanoparticles, Environment Toxicology Chemical **27**(9) (2008) 1915–1921.
20. Lin S., Reppert J., Hu Q., Hudson J. S., Reid M. L., Ratnikova T. A., Rao A. M., Luo H., Ke P. C. - Uptake, translocation, and transmission of carbon nanomaterials in rice plants, Small **5** (10) (2009) 1128–1132.
21. Kaveh R., Li Y. S., Ranjbar S. - Changes in Arabidopsis thaliana gene expression in response to silver nanoparticles and silver ions. Environment Science Technology **47** (2013) 10637 - 10644.
22. Pokhrel L. R., Dubey B. - Evaluation of developmental responses of two crop plants exposed to silver and zinc oxide nanoparticles, Science Total Environment **452–453** (2013) 321–332.
23. Hoang Thanh Tung, Nguyen Phuc Huy, Nguyen Ba Nam, Vu QUoc Luan, Vu Thi Hien, Le Thị Thu Hien, Truong Thi Bich Phuong, Nguyen Hoai Chau, Ngo Tan Buu, Duong Tan Nhut - Effect of silver nanoparticle on Chrysanthemum's growth in microponic system, Journal of Biotechnology **14** (3) 20160 (Accepted) (in Vietnamese).
24. Prakash M., Ill Min Chung - Assessment of silver nanoparticle-induced physiological and molecular changes in *Arabidopsis thaliana*. Environmental Science and Pollution Research **21** (2014) 8858-8869.
25. Klimaszewska K., Bernier-Cardou M. CyrB. D. R., Sutton C. S. - 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* Cellular & Developmental Biology – Plant **36** (4) (2000) 279 – 286.
26. Suthar R. K., Habibi N., Purohit S. D. - Influence of agar concentration and liquid medium on *in vitro* propagation of Boswellia serrata Roxb. - Indian Journal of Biotechnology **10** (2011) 224-227.