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Research Article

Controlling the *in vitro* Contamination of Carnation (*Dianthus caryophyllus* L.) Single Nodes Explant by Nano-Silver

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ABSTRACT

The carnation (*Dianthus caryophyllus*) is one of the top-selling commercial cut-flower crops worldwide, which is very susceptible to infections. Micro propagation is an advanced biotechnological system for producing free of pathogen colonies. Too many factors may limit micro propagation of plants, which the most important of them is contamination (e.g., fungal, bacterial and viral infections). There are some methods and chemicals which are available to control in vitro contaminations. However, the efficiency of some of these methods is low, and/or some of them are too toxic. Mercury chloride as an example is very effective, but it is very toxic. Nanotechnology is relevant to diverse fields of science and technology. Antimicrobial activity of silver nanoparticles (new and nontoxic material) is important to control conventional microbial agents. The aim of the current study was to evaluate the potential of Nano–silver particles on decontaminations of nodal segments of carnation in *in vitro* propagation. In this experiment the effects of application of different rates of Nano-silver (0,100,200 and 300 ppm) through immersion in Nano-silver solution for 10 minutes after surface sterilization were evaluated. Surface sterilization by immersion explants in 70% ethanol for 30 seconds and following by immersion in 2.5% Clorox for 2 minutes were done. The experimental design was a Complete Randomized Design (CRD) with five replications and 3 explants per vessel. Results of disinfestations of Carnation single nodes demonstrated that treatment of 200ppm was effective to control bacterial contaminations and had no harmful effects on regeneration of explants. Higher rates of Nano-silver caused severe injuries to the explants. We observed that using low concentrations of Nano-silver can be used as a low risk bactericide in Carnation single node tissue culture, but, had no effects on controlling fungal contaminations.

Key words: Micro propagation, bacterial contamination, nano-silver particles

INTRODUCTION

Carnation (*Dianthus caryophyllus L.*) is one of the most important top-selling commercial cut-flower crops all over the world. (Casanova *et al*., 2004). Carnation is one of the member of dicotyledonous Caryophyllaceae family. Genus *Dianthus* has more than 100 ornamental species, which some of them like *Dianthus chinensis, Dianthus caryophyllus* and *Dianthus barbatus* has been grown as ornamental cut flower or pot plant wordwild. Most species of Carnation are herbaceous and perennial plants. (Saeed Hassan *et al*., 2011). As a great floricultural crop, carnation is cultivated all-year-round in temperate regions (Nimura *et al.,* 2008). Carnation can be propagated by different types of propagations methods like, seed, cutting, layering and tissue culture. Micro propagation is a modern biotechnological system for producing pathogen-free plants in agriculture and forestry uses. (Arencibia *et al*., 2008). Among the cut flowers and pot plants commercialized in the Alsmeer market which is located in Neatherlands, about 60% are produced by micro propagations. (Ruffoni, 2009). Moreover, tissue culture allows the production of healthy and virus free stock plants and supports breeding activities (Ruffoni, 2009).

So many factors may limit micro-propagation of flowering plants. The most important limiting factor is *in vitro* contamination e.g., fungal and bacterial infections. There are however, some methods and chemical available to reduce these contaminations, but the capability of these chemical are either restricted or toxic. Antibiotics have been tested for their potency to prevent the growth of

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bacteria. However, the use of antibiotics has own confinement. Antibiotics are expensive; their range of efficiency against types of bacteria is not broad, they are usually heat-labile, phytotoxic and only effective against bacteria and not fungi. Otherwise they are capable of altering the behavior of plant tissue cultured by retardation of plant growth. In other hand, resistance of bacteria to bactericides and antibiotics has been arised in recent years due to the development of resistant strains. Therefore, there is much interest in figuring out new ways to formulate new types of safe and cost-effective biocides materials. (Soltanloo, 2010). Mercury chloride, $HgCl_2$, as an another antimicrobial chemical agents, has been used to remove infections in explants, but it is very toxic and should be used with necessary cautions (Al_Ani, 2011; Rostami *et al*., 2009).

Nanotechnology is a new and modern field of science and technology and many aspects of its abilities to control environmental contamination has been proved. Nano-silver is a new classes of material with different physiochemical and biological characteristics. It has antibacterial, antifungal and antiviral effects. (Tahmasbi *et al*., 2011). The use of nano-sized silver particles as antimicrobial agents has become more prevalent. (Safavi *et al*., 2011). This capability of Nano-silver is due to release of very small particles of silver ions, so it is capable of destroying not only bacteria, fungus but also the viruses (Al_Ani, 2011). Application of Nano-silver in *Valeriana officinalis* can lessen the contamination rate of tissue culture and had no effects on leaf number, proliferation and number and length of explant roots. (Abdi *et al*., 2008). In our study, we focused on the evaluation of antimicrobial effects of Silver nano particle and the best concentrations of the treating Nano-silver solutions on a highly contaminated plant, *Dianthus caryophyllus*.

MATERIALS AND METHODS

The experiment was conducted at tissue culture laboratory of the Department Horticultural Science of Tarbiat Modares University (Iran) in autumn 2012. Mother plants were purchased from a commercial greenhouse in Tehran province, Pakdasht city ,then transported to the greenhouse of Department Horticultural Science of Tarbiat Modares University. Stem cutting of about 10 cm length, were picked out from mother plants. Leaves were removed and single nodes with the length of relatively 1.5 cm were separated. After that, the explants were pre washed under running water, which is supplemented with 0.2-0.3% of a commercial detergent (Rika-Iran) and 2-3 drops of tween-20 for 20 min. Then explants were submerged in a solution of 2 grL^{-1} Benomyl, to control fungal contaminations.

Surface sterilization

Explants were submerged in 70% ethylic ethanol for 30 seconds and after 3 times rising in sterile distilled water, they were submerged in 2.5% Clorox (Kija-Iran) for 2 min. The Clorox has 0.1% active ingredient. After Clorox treatments, explants were washed 3 times in sterile distilled water.

Treatments by Nano-silver:

Nano-silver particles are available in Iran, as keloids solution which is mixed by tween-20 to increase their contact surfaces, and were used as disinfecting treatment after surface sterilization. Nano-silver treatments included submersion of explants in different concentration of Nano-silver, (0, 100, 200 and 300 ppm) which are prepared in sterile distilled water. Thirty days after culture the percentages of infected and developed explants were recorded**.**

Medium and culture conditions

The explants were placed on Murashige and Skoog media supplemented with 30 gr sucrose and 7.1 gr Agar, without any plant grow regulator. The pH of medium adjusted to 5.8 by KOH prior to adding 7.1 gr agar and then autoclaved at 121°C for 20 min.

Culture room conditions were 25°C , 16 hour photoperiod at 40 Wm^{-2} irradiation and 40% humidity rate.

Statistical analysis

The experiment was conducted in Complete Randomized Design (CRD) with five replications and 3 explants per vessel. Data were analyzed with MSTAT_C test/.

RESULTS AND DISCUSSION

The results of the analysis of variance table showed that using Nano-silver after surface sterilization can be diminish surface and inner bacterial contaminations significantly. (Table: 1), (Fig: 1). Treatment of 200 ppm for 10 min decreased bacterial contaminations significantly, and in all of the replications of this treatment we only observed 12.5% bacterial contaminations. The survival rate in this concentration was 81.25% (Fig: 2, 3). In the treatment of immersion of explants in solution of 100 ppm Nano-silver, we observed 37.5% bacterial contaminations. The survival rate was 87.5%. The highest rate of explants which were survived (100%) was belonged to control treatment, but we saw 50% bacterial contaminations. It can be concluded that, because of not applying Nano-silver, the rate of contaminations were very high. (Fig: 4). Treatment of 300 ppm did not show any bacterial contaminations, however the high rate of Nano-silver caused more than 75% of

Table 1: The effects and the comparison of the different concentrations of Nano_Silver on bacterial contaminations and regeneration rate of carnation explants.

Concentrations of Nano_Silver	Bacterial contaminations Mean ± S.E.	F- Value	Regeneration rate Mean ± S.E.	F- Value
0	50±10.20a	4.99*	100±0a	14.73**
100	37.5±16.13ab		87.5±7.21a	
200	12.5±7.21bc		81.25±11.96a	
300	0±0c		25±10.26b	

* and **, significant at 5% and 1% levels, respectively; Mean in each column with differenc letter show significant differences according to Dancan,s Multiple($P \leq 0.05$).

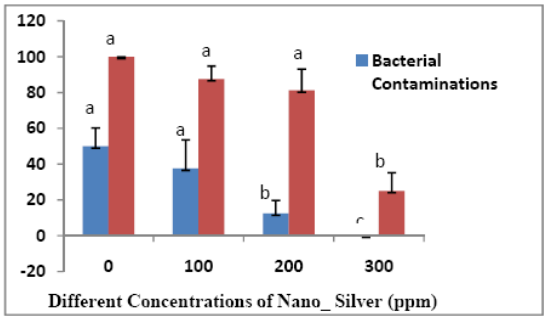

Fig: 1: The table of application of Nano-silver through immersion of explants after surface sterilization on percentages of bacterial contaminations and survival rate.

Fig. 2: Explants were submerged in 200 ppm Nano-silver solution.

Fig. 3: Explants grew normally after treatment by 200 ppm Nano-silver solution

Fig. 4: Control treatment. Explants showed high bacterial contaminations.

Fig. 5: Treatment of 300 ppm. Most of explants were not developed.

Fig 6: Fungal contaminations which are not controlled by Nano-silver treatmen.

explants were not able to regenerate. (Fig: 5). We saw that Nano-silver could not have any positive effects on controlling fungal contaminations in carnation tissue culture. (Fig: 6).

Conclusion

Generally, making a sterile establishment is the most considerable step of micro propagation system. Since, one of the most significant advantages of micro propagation is reduction in producing expenditures, there will be a growing demand for this system. As a result, application of new chemical which are not expensive and toxic will be highly noticed. Various types of antimicrobial chemical agents have been tested in plant tissue cultures. In current experiment, we investigated the potential of using Nano-silver as a non-toxic and environmentally friendly material in tissue culture of carnation.

Some researchers were conducted to evaluate the effects of Nano-silver in reduction of bacterial contaminations in tissue culture system. Nano-silver by the concentration of 200 ppm had a significane effects in controlling bacterial contaminations in Gerbera capitulum explants. (Fakhrfeshani *et al*., 2012). Surface sterilization of explants with nano-sized silver particle had considerable influence on the bacterial contaminants reduction, without any adverse effects on growth rate in micro propagation of valerian (Abdi, *et al*, 2008). Using Nano-silver for eliminating bacterial contaminations of *Araucaria excelsa* R. Br. var. Glauca in tissue culture was useful. (Sarmast, *et al*., 2011). Submerging the olive explants of cultivar Mission, in Nano-silver solution after surface sterilization, showed that Nano-silver is very useful in controlling bacterial and fungal contaminations. (Rostami, *et al*., 2009). The open question is how Nano-silver particles function as biocide material against contaminations. The mechanism of inhibitory action of silver on microorganisms is not fully known, however silver ions do not have only a single mode of action. They interfere with a broud range of molecular processes within microorganisms which is resulting from inhibition of growth or loss of infection through cell death. The mechanism completely rely on the concentration of silver ions and the sensitivity of the microbial species to silver. In addition, contact time and temperature can have impact on both the rate antimicrobial activity (Dibrov, *et al*, 2002) (Soltanloo, *et al*., 2010). Due to their unique physicochemical characteristics of Nano-silver such as catalytic activity, optical properties, electronic properties, anti microbial activity, magnetic properties, high specific surface area and a high fraction of surface atoms, it can be

expected that Nano-silver particles will lead to high antimicrobial activity. (Cho *et al*., 2005).

In the current experiment the detrimental effects of Nano-silver particles in high concentrations (300 ppm) may due to the effects of Ag^+ on cell membrane of explants. This result is completely compatible to the result of Abdi *et al.* (2008). The results of current study demonstrated that Nano-silver eliminates the bacterial contaminations of carnation single nodes explants. It is very amazing that working by Nano-silver does not have any toxic effects on human and environment. Immersion of explants in higher rate of Nano-silver solution, inhibit the regeneration of explants. It is suggested that using low concentration of Nano-silver can be used as a low risk bactericide in tissue culture of carnation. We suggest more and more study to evaluate the potential of using Nano-silver for other species plants.

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