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ORIGINAL ARTICLE

# Effects of antimicrobial activity of silver nanoparticles on in vitro establishment of G × N15 (hybrid of almond × peach) rootstock

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Nano-silver;
Single node

**Abstract** In the present investigation were evaluated the antifungal and antibacterial activities of Nano-silver (NS). Two separate experiments were done to evaluate the potential of silver nanoparticles in controlling the contamination of G × N15 micro-propagation. In the first experiment, a factorial experiment based on a completely randomized design with 15 treatments including five different NS concentrations (0, 50, 100, 150 and 200 ppm) and three soaking time of explants (3, 5 and 7 min) with five replications was conducted. In the other experiment, NS was added to Murashige and Skoog (MS) medium with concentrations of 0, 50, 100, 150 and 200 ppm in a completely randomized design. Results showed that the application of 100 and 150 ppm NS both as an immersion and as added directly to the culture medium significantly reduces internal and external contaminations compared with the control group. Using NS in culture medium was more efficient to reduce fungal and bacterial contamination than immersion. High concentrations of NS had an adverse effect on the viability of G × N15 single nodes and this effect was more serious in immersed explants in NS than directly added NS ones regarding the viability of buds and plantlet regeneration. In conclusion, due to high contamination of woody plants especially fruit trees and also adverse environmental effects of mercury chloride, the NS solution can be used as a low risk bactericide in micro-propagation of G × N15 and can be an appropriate alternative to mercury chloride in the future.



## 1. Introduction

Preventing or avoiding microbial contamination of plant tissue cultures is critical to successful micropropagation. Epiphytic and endophytic organisms can cause severe losses

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to micropropagated plants at each stage of growth [3,6]. Bacterial contaminants are often difficult to detect because they remain mostly within the plant tissue [34]. Contaminated plants may have no visible symptoms, reduce multiplication and rooting rates, or may die [6].

G × N15 (Garnem) is a hybrid of *Prunus amygdalus* (Garfi) × *Prunus persica* (Nemared). Garnem is the most commonly used rootstock for almond and peach and has exhibited total graft compatibility with the whole range of peach and almond cultivars as well as some plum and apricot cultivars [4]. Other appropriate attributes of garnem include good vigor and resistance to root-knot nematodes, adaptation to calcareous soils and other Mediterranean agroecological conditions [37,47,18]. This rootstock is also compatible with some diploid plums (*Prunus salicina* Lindl. and related plums) such as 'Santa Rosa' and 'Golden Japan'. Compatibility has also been observed with some apricot cultivars belonging to the more compatible apricot group such as 'Paviot' but not with the apricot cultivars of the more exigent group such as 'Moniqui'. Pattern be used for almond, peach and Japanese plum irrigated and for rainfed almond by showing good overall compatibility with the varieties of these species [4]. It has shown a good propagation capacity through the different system in use. 'Garnem' is propagated well by hardwood and herbaceous cuttings in aerated and well-drained soils [15]. The commercialization of traditional peach, nectarine and almond orchards is considered of great importance in order to enhance yield; and to achieve this objective the micropropagation of G × N15 as a vigorous rootstock through tissue culture in a high scale and short period of time is essential for high density peach, nectarine and almond orchards. Eliminating or reducing exogenous and endogenous contaminating microorganisms is one of the most important steps of a successful micropropagation process [46,35].

The success and commercial micropropagation rely on many factors such as culture medium, plant growth regulators, explants age and donor plant. One of the most important limiting factors, especially in woody plants is in vitro fungal and bacterial contamination [48,16,9,29]. This problem is a bottleneck in the single node culture establishment stage of G × N15 as in other woody plants. At disinfection stage, several chemical materials have been used to control in vitro contamination. However, the efficiency of some of chemical materials is low and some of them are too toxic. Sodium hypochlorite and mercury chloride ($HgCl_2$) are widely used to control in vitro contamination of woody plants. Many investigations have been carried out to evaluate the effect of different disinfectants on *Prunus* rootstock micropropagation. In the in vitro propagation of wild cherry (*Prunus avium* L.), Izadpanah [27] reported that the use of 1.25% sodium hypochlorite for 20 min has given the best disinfecting result. Using 0.5 mg $L^{-1}$ of $HgCl_2$ for 15 min in in vitro propagation of *Prunus insititia* (variety Adesoto 101) resulted in the lowest contamination [31]. In in vitro disinfection stage of dwarf rootstocks of cherry, the best result was obtained using 1.5 mg $L^{-1}$ of $HgCl_2$ for 15 min [24]. Using silver and its components like silver nitrate or silver sulfadiazine was common in the past centuries. The use of these materials dropped due to production of new antibiotic substances [28]. In order to eliminate bacterial contamination various antibiotics such as nalidixic acid, gentamicin, tetracycline, streptomycin, vancomycin, etc. or a combination of these substances have been used. In some investigation, the use of silver nanoparticles has been suggested to reduce bacterial contamination [8,39].

Nano-biotechnology is a new field of modern science and applications of metal nanoparticles, especially of silver, gold and platinum are emerging rapidly. Nano-silver (NS) is a new non-toxic material and has good capability to eliminate fungal, bacterial and virus contamination without adverse effects on plant growth and development [8,19,22,29]. Many aspects of its abilities to control environmental contamination have been proved, but NS mechanism of action is not fully understood. It exhibits broad-spectrum antimicrobial activity in vitro by binding to microbial DNA, that prevents bacterial replication and to the sulfhydryl groups of the metabolic enzymes in the bacterial electron transport chain, causing their inactivation [38]. Nanoparticles have eccentric properties depending on the size. As the size is smaller, catalytic activity is stronger [11].

Not only NS is able to reduce or eradicate fungal and bacterial infection, but is also able to destroy the viruses because this potential of NS is due to release of tiny particles of silver [12]. To eradicate bacterial contamination in plant micro samples obtained from the valerian (*Valeriana officinalis* L.), Abdi et al. [8] used 25, 50 and 100 mg $L^{-1}$ of NS, before and after surface sterilization. Their result showed that 100 mg $L^{-1}$ of NS solution after surface sterilization resulted in the highest (89%) disinfecting effect. NS in combination with amoxycillin has been used in medium culture by Li et al. [33]. They found that combination of NS and amoxycillin was more efficient than using separately in reducing contamination. Moreover, NS is toxic to several antibiotic resistant bacterial strains such as *Streptococcus* sp., *Pseudomonas* sp., and others [41].

According to the aforesaid reports NS can be used as an alternative for toxic chemical compounds for the disinfestation of the environment, prevention and cure of diseases and pests. The objective of the present study is to develop a reliable protocol for disinfection of G × N15 vegetative rootstock explants for in vitro culture that is a new *Prunus* rootstock in Iran.

## 2. Materials and methods

### 2.1. Plant material and culture initiation

Plant material was obtained from 2-year-old G × N15 or 'Garnem' vegetative rootstock grown at the greenhouse of the Tarbiat Modares University (TMU), Tehran, Iran. Shoots with length of 15–20 cm of 'Garnem' were collected and transferred to the fruit tree micro-propagation laboratory. First of all, shoots were excised into 1.5–2.5 cm-long nodal segments, and then for surface disinfection, the explants were agitated for 5 min in solution water and dishwashing liquid containing 5 drops of Tween-20 in 100 ml of water, finally they were washed under running tap water for 1 h to remove surface contamination. Afterward, they were soaked in 0.2 % (w/v) benomyl (fungicide) for half an hour then washed thoroughly with sterile distilled water. After the above steps, in order to internal disinfection two separate experiments were conducted: in the first experiment, the explants were pre-sterilized by immersion in 70% ethanol for 60 s followed by a rinse with sterile distilled water. These pre-sterilized explants were exposed in five concentrations of NS solution (0, 50, 100, 150 and 200 ppm) with three different immersion times (3, 5 and 7 min). Then

in order to reduce phenolic contaminants, the explants were submerged in 0.7% citric acid 2 times, each time 3 min. Finally they were cultured in Murashige and Skoog [44] (MS) medium supplemented with 0.25 mg $L^{-1}$ BAP, 30 g $L^{-1}$ sucrose and 8 g $L^{-1}$ agar.

At the same time and in another experiment, the explants were treated with 2.5% (v/v) sodium hypochlorite for 4 min. Then, the explants were washed three times with sterilized distilled water to remove all the traces of sodium hypochlorite. After this stage, explants were dipped in 0.7% citric acid 2 times, each time 3 min. Finally they were cultured in MS medium supplemented with various concentrations of NS (0, 50, 100, 150 and 200 ppm), 0.25 mg $L^{-1}$ BAP, 30 g $L^{-1}$ sucrose and 8 g $L^{-1}$ agar.

The pH of all media was adjusted to 5.8 before autoclaving and then grown in growth room with light intensity of 2500–3000 lux, photoperiod of 16/8 h light/dark, relative humidity of 45% and constant temperature of 25 ± 1 °C. McCarthy glasses were used for the establishment stage of explants. After 1 week, fungal contamination percent, bacterial contamination percent and viability percent of G × N15 buds were recorded each day. Viability index was obtained by calculating the percentage of regenerated explants without contamination (fungal or bacterial).

### 2.2. Experimental design and statistical analyses

The first experiment was conducted using a completely randomized design (CRD) with factorial arrangement consisting of fifteen treatments with 3 replications and each replication included 10 McCarthy glasses. Second experiment was performed using a completely randomized design (CRD(including 5 treatments with 5 replications and each replication included 10 McCarthy glasses. SAS version 9.1 software was used for all statistical analyses and significance ($P \leqslant 0.05$) differences among mean values were estimated using Duncan's new multiple range test.

## 3. Results

In the first experiment, the multi-factorial analysis of variance demonstrated that interaction between various concentrations of NS solution and immersion times of single node explants had a significant effect on bacterial contamination percent and viability of G × N15 buds. Results also suggest that different times of immersion and various concentrations of NS had significant effects on fungal contamination percent separately (Table 1).

**Table 1** Analysis of variance of viability of buds, fungal contamination and bacterial contamination under effect of various concentrations of NS and different immersion times.

| Treatments | Viability of buds (%) | Bacterial contamination (%) | Fungal contamination (%) |
|---|---|---|---|
| *Nano-silver* | | | |
| 0 ppm | 1.66 ± 0.83d | 57.22 ± 1.47a | 41.11 ± 1.38a |
| 50 ppm | 40.00 ± 2.89c | 26.11 ± 2.17b | 33.88 ± 1.11b |
| 100 ppm | 61.11 ± 1.62a | 8.88 ± 1.11c | 30.00 ± 1.44b |
| 150 ppm | 49.44 ± 1.54b | 7.22 ± 1.21c | 30.55 ± 1.00b |
| 200 ppm | 43.89 ± 1.82bc | 0.55 ± 055d | 31.11 ± 1.11b |
| *Time* | | | |
| 3 min | 36.67 ± 5.36b | 22.66 ± 5.21a | 35.66 ± 1.37a |
| 5 min | 41.00 ± 5.54a | 19.33 ± 5.62b | 31.66 ± 1.44b |
| 7 min | 40.00 ± 5.66ab | 18.00 ± 5.76b | 32.66 ± 1.28ab |
| *Nano-silver × time* | | | |
| 0 ppm × 3 min | 1.66 ± 1.66f | 55.00 ± 2.88a | 43.33 ± 1.66a |
| 0 ppm × 5 min | 1.66 ± 1.66f | 58.33 ± 1.66a | 40.00 ± 2.88ab |
| 0 ppm × 7 min | 1.66 ± 1.66f | 58.33 ± 3.33a | 40.00 ± 2.88ab |
| 50 ppm × 3 min | 30.00 ± 2.88e | 33.33 ± 3.33b | 36.66 ± 1.66abc |
| 50 ppm × 5 min | 43.33 ± 1.66d | 23.33 ± 1.66c | 33.33 ± 1.66abc |
| 50 ppm × 7 min | 46.66 ± 3.33cd | 21.66 ± 1.66c | 31.66 ± 1.66bc |
| 100 ppm × 3 min | 56.66 ± 1.66abc | 11.66 ± 1.66d | 28.33 ± 3.33bc |
| 100 ppm × 5 min | 61.67 ± 1.66ab | 10.00 ± 0.00de | 30.00 ± 1.66c |
| 100 ppm × 7 min | 65.00 ± 2.88a | 5.00 ± 0.00ef | 31.66 ± 2.88bc |
| 150 ppm × 3 min | 50.00 ± 2.88bcd | 11.66 ± 1.66d | 31.66 ± 1.66bc |
| 150 ppm × 5 min | 51.66 ± 1.66bcd | 5.00 ± 0.00def | 28.33 ± 1.66c |
| 150 ppm × 7 min | 46.66 ± 3.33cd | 5.00 ± 0.00def | 31.66 ± 1.66bc |
| 200 ppm × 3 min | 45.00 ± 5.00cd | 1.66 ± 1.66ef | 35.00 ± 0.00bc |
| 200 ppm × 5 min | 46.66 ± 1.66cd | 0.00 ± 0.00f | 28.33 ± 1.66c |
| 200 ppm × 7 min | 40.00 ± 0.00de | 0.00 ± 0.00f | 30.00 ± 0.00bc |
| *p-Value* | | | |
| Nano-silver | < 0.001 | < 0.001 | < 0.001 |
| Time | 0.027 | < 0.001 | 0.011 |
| Nano-silver × time | 0.006 | 0.007 | 0.892 |

Values in each column represent means ± SE. Different letters within columns indicate significant differences ($p < 0.05$).

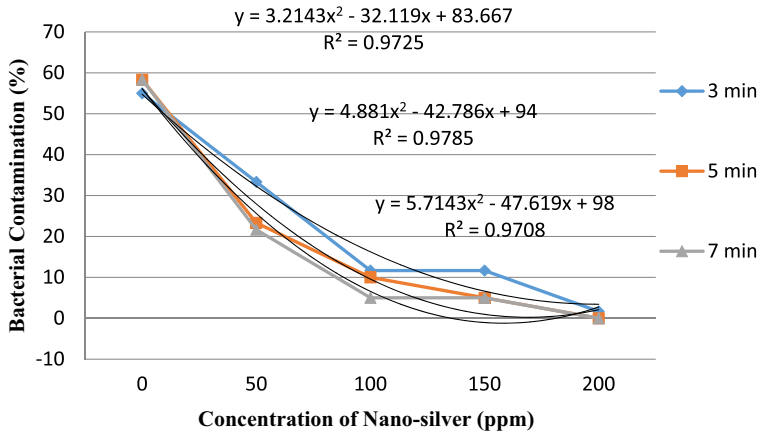


**Figure 1** Effect of different levels of Nano-silver on in vitro bacterial contamination reduction of G × N15.

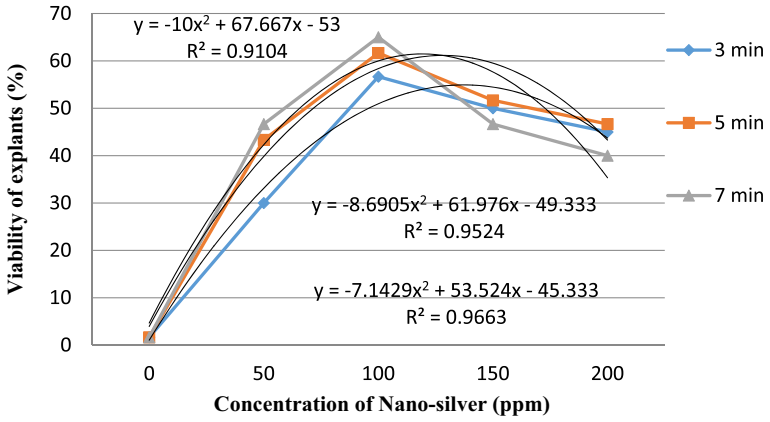


**Figure 2** Effect of different levels of Nano-silver on in vitro viability of G × N15 explants.

Summary of the regression analysis for control of bacterial contamination and viability of bud responses as affected by different times of immersion and various NS concentrations is presented in Figs. 1 and 2. The lowest rate of bacterial contamination (0%) was obtained in treatment containing 200 mg $L^{-1}$NS at 5 and 7 min immersion (Fig. 1). Treatment including 100 ppm NS for 7 min resulted in the highest viability of buds (65%), which was significantly higher than control and other treatments (Fig. 2). The lowest rate of fungal contamination was obtained on treatment including 200 ppm NS (Table 1). Interaction between different times of immersion had no remarkable effect on control of fungal. Despite, there being no remarkable difference between different concentrations of NS in controlling fungal contamination, the use of NS significantly reduced fungal contamination compared to control (Table 1).

In the second experiment, the results indicated that different concentrations of NS had a significant effect on bacterial and fungal contamination percent as well as, on viability of G × N15 buds (Table 2). The regression analyses for control of bacterial and fungal contamination, and also viability of bud responses as affected by different NS concentrations in the culture medium are summarized in Figs. 3–5. Treatment containing 200 ppm NS resulted in the lowest rate of bacterial contamination (3.75%), which was significantly lower than other treatments and control (Fig. 3). The highest rate of viability of buds (77.5%) was obtained using 150 ppm NS into medium which was not also significantly better compared with that of using 200 and 100 ppm NS into media (Fig. 4). The lowest rate of fungal contamination was obtained in medium containing 200 ppm NS. Despite, there was no remarkable different between various concentrations of NS in culture medium on controlling fungal contamination but these treatment were significantly more efficient compared to control (Fig. 5). Using up to 200 ppm NS solution for immersion method and direct addition to the culture medium reduced bacterial contamination from 58% to 0% and 43.75% to 3.75%, respectively (Tables 1 and 2).

Although increasing concentrations of NS significantly reduced bacterial contamination in both of the methods, higher concentrations of NS had an adverse effect on thez viability of G × N15 single nodes, it is also notable that immersion explants in NS solution had a more adverse effect than adding NS directly to the culture medium on viability of buds and regeneration of plantlets, because the buds are susceptible to surface sterilization, such effect may be due to the adverse

**Table 2** Analysis of variance of viability of buds, fungal contamination and bacterial contamination under effect of various concentrations of NS into culture medium.

| Treatments | Fungal contamination (%) | Bacterial Contamination (%) | Viability of buds (%) |
|---|---|---|---|
| *Concentration of Nano-silver* | | | |
| 0 ppm | 21.25 ± 1.25a | 43.75 ± 2.39a | 35.00 ± 2.04d |
| 50 ppm | 17.5 ± 1.44ab | 31.25 ± 1.45b | 51.25 ± 2.39c |
| 100 ppm | 13.75 ± 1.25bc | 21.25 ± 1.25c | 63.75 ± 1.25b |
| 150 ppm | 12.5 ± 1.44bc | 11.25 ± 1.50d | 77.5 ± 1.44a |
| 200 ppm | 11.25 ± 1.25c | 3.75 ± 1.25e | 70.00 ± 2.04ab |
| *p-value* | | | |
| Different concentration of Nano-silver | 0.002 | < 0.001 | < 0.001 |

Values in each column represent means ± SE. Different letters within columns indicate significant differences ($p < 0.05$).

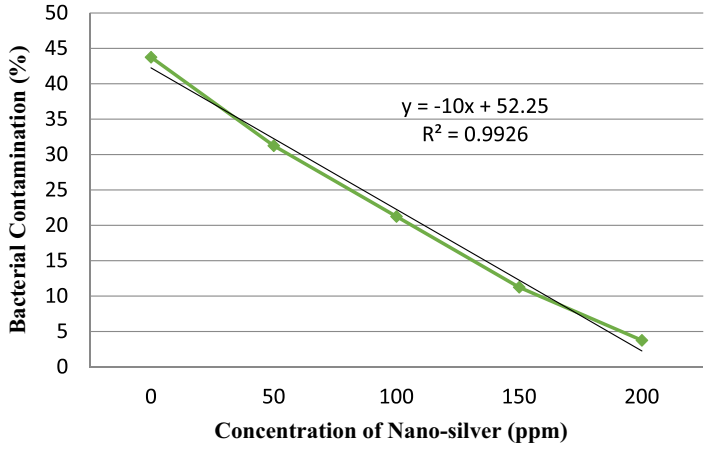


**Figure 3** Effect of different levels of Nano-silver into the culture medium on in vitro bacterial contamination control of G × N15.

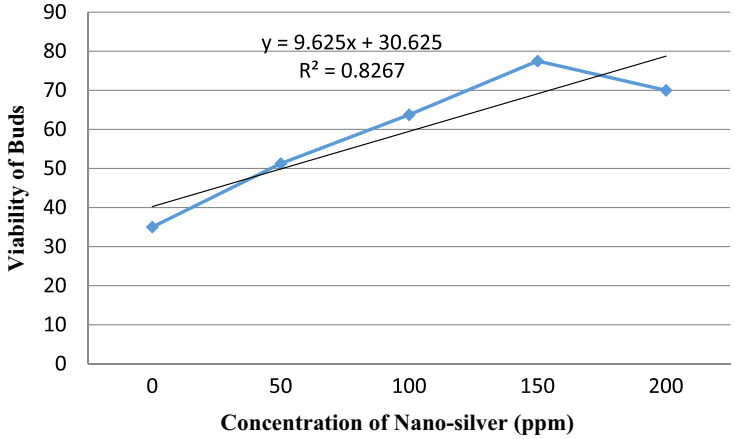


**Figure 4** Effect of different levels of Nano-silver into the culture medium on in vitro viability of G × N15 buds.

effect of high concentration of Ag ions on cell membrane of explants. Comparison of the means indicated that the best treatment for decreasing the indigenous bacterial contamination and having most viability of G × N15 single nodes were 100 and 150 ppm as immersion treatment and as added directly to the culture medium, respectively, and it was demonstrated that these treatments had a significant difference with other treatments because they showed the highest viability of G × N15 buds and an appropriate level of contamination control.

The current study showed that using NS solution as immersion and as added directly to the culture medium significantly reduces internal and external contamination compared with the control and using NS in the culture medium was more

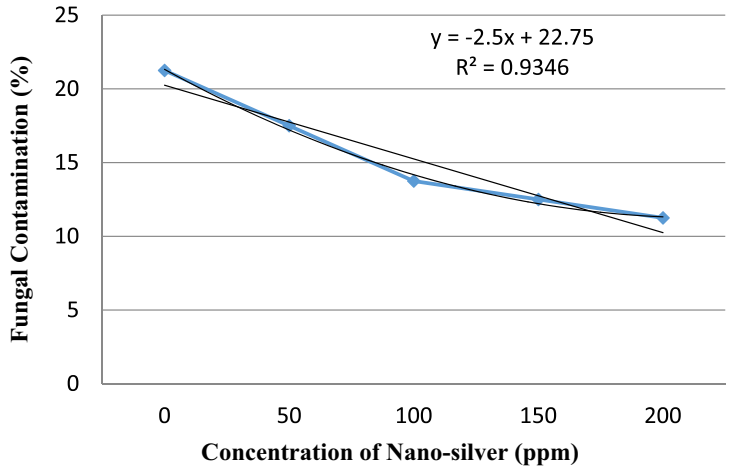


**Figure 5** Effect of different levels of Nano-silver into the culture medium on in vitro fungal contamination control of G × N15.

efficient to reduce fungal and bacterial contamination as well as had it less undesired effects on regeneration of plantlets (Tables 1 and 2).

## 4. Discussion

The most important problems in the expansion of fruit orchards are the lack of suitable and uniform rootstocks [13]. Successful micro-propagation of all plants depends on the condition of plant material at the time of collection of the explant from grown trees in the field and removal of exogenous and endogenous contaminating microorganisms. Fungi and bacteria are the most common microorganisms to be found on or in plant tissues [22]. Establishing a sterile in vitro culture is the first and most important step to success in commercial micro-propagation of fruit trees [36,2].

One of the major problems associated with the micro-propagation of G × N15 is microbial contamination; this problem has been observed in its single node culture establishment. There are novel approaches for controlling the contamination in micro-propagation. In previous studies have been used various substances such as alcoholic, sodium hypochlorite, $HgCl_2$ and antibiotic solutions with advantages and disadvantages to eradicate bacterial and fungal contamination [40]. Because of the importance of contamination controlling for achieving the goals of micro-propagation and the side effects of frequently used very high toxic chemical, $HgCl_2$ [17] the necessity to consider new antimicrobial agents is obvious [25] and it would be beneficial to provide additional chemical agents that could be directly added to plant tissue culture media, which would reduce or prevent bacterial and fungal contamination for the duration of the culture period, and which would allow for substantially normal germination of seeds or the development of plant cultured seeds, organs, cells or tissues. Various types of antimicrobial chemical agents have been tested in plant tissue cultures. Antibiotics have been extensively tested for their ability to inhibit or prevent the growth of bacteria in plant in vitro cultures. However, the use of antibiotics has certain limitations. For example, antibiotics are expensive; their range of efficacy against types of bacteria is often narrow, usually are heat-labile, phytotoxic and only effective against bacteria and not fungi or otherwise capable of altering the behavior of cultured plant tissues by retardation and inhibition of plant growth. Meanwhile, prolonged exposure of cells or tissues to antibiotics can cause mutation in cytoplasmic organelles genes [42].

Silver offers a valuable alternative that is relatively free of adverse effects of antibiotics and disinfectants with low toxicity profile and excellent tissue tolerance. The free silver ion has two distinctive antimicrobial mechanisms. The first involves denaturation of disulfide bonds of bacterial proteins, which are essential elements of bacterial structure, become disjointed due to the catalytic effect of silver ion. The second involves oxidization, ionized silver helps generate reactive oxygen in the air, which in turn attach around the cell prevents it from reproduction, and cause cell death [45]. Synthesized silver nanoparticles retain these properties of silver and are produced using various colloidal processes that involve surfactant-stabilization [23]. Strong antibacterial and disinfectant characteristics of NS can cause the cytotoxic effect on cells. High concentration of NS (200 mg $L^{-1}$) was found to be cytotoxic in the present study, and at safe concentration levels (100 and 150 mg $L^{-1}$) it was found to decrease significantly the in vitro contamination.

Knowledge of the effect of different NS concentrations on both microbes and plants is crucial for the elimination of contaminants and the recovery of healthy plants as what is conducted for the effect of different antibiotics [7].

Our observation is in concurrence with earlier investigators in that they suggested silver and its compounds had efficient effects on the control of bacterial contamination [21,12,26,22]. The results obtained from current study indicated that using NS in culture medium after surface sterilization by sodium hypochlorite compared with immersion explants in alcohol following submerge in NS solution was more efficient to reduce both fungal and bacterial contaminations as well as had less adverse effects on viability and regeneration of explants. These results agreed with those obtained by Safavi et al. [22] that reported using NS in culture medium after surface sterilization displayed a more noticeable effect on removing fungal and bacterial contaminations. Using 200 ppm NS in both of the methods (immersion and into medium culture) had an adverse effect on regeneration and viability of buds, high concentration of NS in the culture medium

compared to immersion system had less destructive effects. Findings of the present study are in agreement with those of Rostami and Shahsavar [1] that reported using low concentrations of nano silver as a disinfecting agent in plants tissue culture media is recommended.

Strong antibacterial and disinfectant characteristics of NS can cause the cytotoxic effect on cells. Phytotoxicity varies greatly with plant type, so preliminary testing with plant culture is important for successful treatment. NS concentrations effective on isolated organisms may be ineffective in treating contaminated plants due to phytotoxicity or poor penetration into plant tissues as for antibiotics [5].

Several reasons can be involved high efficiency of NS in medium culture compared with soaking explants in NS solution. One of the main reasons could be that pre-sterilized explants with fungicides benomyl and sodium hypochlorite in combination with NS, has increased its efficiency, and also destructive effects of NS on viability and regeneration may be explained by the fact that immersion explants in NS due to direct contact with high concentration of Ag ion particles with cell membranes of explants.

The results of this study are in concurrence with those of Abdi et al. [8], Fakhrfeshani et al. [25] that indicate, using NS solution after surface sterilization had acceptable influence on the bacterial contaminants control, but, in contrast to our results, they reported that NS had no adverse effects on growth characters in micro-propagation of valerian, While, High concentrations of nano silver (200 ppm) in both methods revealed undesirable effects. In the present study, high R2 coefficient (82–99%) demonstrated that regression is an appropriate method to describe the relationship between the treatment levels and also the response variable. Although the mechanism of action of NS to eradicate internal and external contamination is not obvious, various investigators have expressed different reports about mode of action of NS; Dibrov et al. [32] suggest that Ag ion affects the phospholipids and destroys the cell membrane of microorganism, Tang et al. [10] reported that the destructive effect of Ag is associated with the production of active silver including organic compound and these compounds have a destructive effect on microorganism. Lubick [30] demonstrated that NS particles release Ag ions and these ions can destroy the cell structure of microorganism. The interaction of silver ions with sulfhydryl (–SH) groups of proteins that cause the DNA unwinding and inhibition of respiratory processes has been reported in former literatures. Cell division inhibition and contact with hydrogen bonding processes are also been demonstrated [43]. Furthermore, abiotic factors such as pH, concentration, and natural organic matter have also been shown to influence the antibacterial properties of NS [14]. Natural organic matter was observed to mitigate the toxicity of nanoparticles due to their sorption on the NS surfaces, preventing the interaction of nanoparticles with the bacteria [20].

According to aforesaid reports, efficient effects of NS in the present study may be explained by one of the mechanisms that are mentioned. Nevertheless, NS can prevent bacterial and fungal in different ways, depending on the using method, given the limited studies on the application methods, advantages and disadvantages of NS through the process of plant tissue culture, its specific antimicrobial mechanisms are still not completely understood, it seems extensive research is needed to identify and understand the molecular and cellular mechanisms of the action of NS toxicity so it would be interesting to study the activity of NS as culture media component or their effects on the other explant and pathogen species along the establishment and proliferation phases.

On the basis of the obtained results it could be noticed that the substitution of NS as a new generation of antimicrobial agent in plant tissue culture can be proposed, because the low concentrations of silver have no harmful effects on plant and humans and is of low cost. Therefore, silver has been widely used for the development of many tissue culture processes. All these reports clearly indicated that NS can be used as a replacement for toxic substances and chemical compounds for the disinfestations of internal and external contaminations in tissue culture of plant. Further researches are suggested to investigate the toxicity of NS concentrations on plant cells.

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## References

[1] A. Rostami, A. Shahsavar, Asian J. Plant Sci. 8 (2009) 505–509.
[2] A. Rostami, A. Shahsavar, J. Biol. Environ. Sci. 6 (2012) 155–159.
[3] A.C. Cassells, in: P.C. Debe rgh, R.H. Zimmerman (Eds.), Micropropagation Technology and Application, Kluwer Academic Publishers, Netherlands, 1991, pp. 31–44.
[4] A.J. Felipe, Hortscience 44 (2009) 196–197.
[5] B.M. Reed, P.M. Buckley, T.N. DeWilde, In Vitro Cell. Dev. Biol.-Plant 31 (1995) 53–57.
[6] C. Leifert, A.C. Casselles, In Vitro Cell. Dev. Biol.-Plant 37 (2001) 133–139.
[7] D. Cornu, M.F. Michel, Acta Hortic. 212 (1987) 83–86.
[8] G.R. Abdi, H. Salehi, M. Khosh-Khui, Acta Physiol. Plant. 30 (2008) 709–714.
[9] G.R. Rout, A. Mohapatra, S. Mohan Jain, Biotechnol. Adv. 24 (2006) 531–560.
[10] H.J. Tang, H. Feng, J. Zheng, J. Zhao, Surf. Coat. Technol. 201 (2007) 5633–5636.
[11] H.S. Shin, M.K. Ye, H.S. Kim, H.S. Kang, Int. Immunopharmacol. 7 (2007) 1813–1818.
[12] I. Sondi, B. Salopek-Sondi, J. Colloid Interface Sci. 275 (2004) 177–182.
[13] J. Dejampour, V. Grigorian, E. Majidi, N. AliAsgarzadeh, Iran J. Hortic. Sci. Technol. 8 (2007) 43–54.
[14] J. Fabrega, S.R. Fawcett, J.C. Rensha, J.R. Lead, Environ. Sci. Technol. 43 (2009) 7285–7290.
[15] J. Gómez Aparisi, M. Carrera, A.J. Felipe, R. Socias i Company, Inf. Técn. Econ. Agrar. 97V (2002) 282–288.
[16] J. Houng, H.N. Murty, K.Y. Peak, Plant Cell Tissue Organ Cult. 65 (2001) 123–128.
[17] J. Mutter, J. Naumann, R. Schneider, H. Walach, B. Haley, Neuroendocrinol. Lett. 26 (2005) 439–446.
[18] J. Pinochet, C. Calvet, A. Hernández-Dorrego, A. Bonet, A.J. Felipe, M.A. Moreno, Hortscience 34 (1999) 1259–1262.
[19] J.J. Castellano, S.M. Shafii, F. Ko, G. Donate, T.E. Wright, R.J. Mannari, W.G. Payne, D.J. Smith, M.C. Robson, Int. Wound J. 4 (2007) 114–122.
[20] K.H. Cho, J.E. Park, T. Osaka, S.G. Park, Electrochim. Acta 51 (2005) 956–960.
[21] K. Nomiya, A. Yoshizawa, K. Tsukagoshi, N.C. Kasuga, S. Hirakava, J. Watanabe, J. Inorg. Biochem. 98 (2004) 46–60.

[22] K. Safavi, M. Esfahanizadeh, D.H. Mortazaeinezahad, H. Dastjerd, International Conference on Life Science and Technology IPCBEE, 2011.
[23] K.J. Lee, Y.I. Lee, I.K. Shim, J. Joung, Y.S. Oh, J. Colloids Interface Sci. 304 (2006) 92–97.
[24] M. Erbenova, F. Paprstein, J. Sedlak, Acta Hortic. 560 (2001), ISHS.
[25] M. Fakhrfeshani, A. Bagheri, A. Sharifi, J. Biol. Environ. Sci. 6 (2012) 121–127.
[26] M. Ip, S.L. Lui, V.K. Poon, I. Lung, A. Burd, J. Med. Microbiol. 55 (2006) 59–63.
[27] M. Izadpanah, Pajouhesh-Va-Sazandegi (2001).
[28] M. Rai, A. Yadav, A. Gade, Biotechnol. Adv. 27 (2009) 76–83.
[29] M. Sarmast, H. Salehi, M. Khosh-Khui, Acta Biol. Hung. 62 (2011) 477–484.
[30] N. Lubick, Environ. Sci. Technol. 42 (2008) 8617.
[31] P. Andreu, J.A. Marín, Sci. Horticul. 106 (2005) 258–267.
[32] P. Dibrov, J. Dzioba, K.K.K.K. Gosink, C.C. anHäse, Antimicrob. Agents Chemother. 46 (2002) 2668–2670.
[33] P. Li, J. Li, C. Wu, Q. Wu, J. Li, Nanotechnology 16 (2005) 1912.
[34] P.R. Viss, E.M. Brooks, J.A. Driver, In Vitro Cel1. Dev. Biol. 27P (1991) 42.
[35] R. Nazary Moghaddam Aghaye, A. Yadollahi, A. Moeini, S. Sepahvand, J. Biol. Environ. Sci. 7 (2013) 57–64.
[36] R. Nazary Moghaddam Aghaye, A. Yadollahi, J. Agric. Sci. 4 (2012) 1916–9752.
[37] R. Socias i Company, M.J. Rubio, J.M. Alonso, O. Kodad, J. Gómez Aparisi, Options Mediterraneennes 94 (2010) 205–214.
[38] R.M. Slawson, H. Lee, J.T. Trevors, J. Colloid Interface Sci. 275 (1990) 177–182.
[39] S. Gholamhoseinpour Anvari, J. Carapetian, J. Dejampour, Int. J. Agric. Sci. 2 (2012) 457–465.
[40] S.A. Counter, L.H. Buchanan, Toxicol. Appl. Pharmacol. 198 (2004) 209–230.
[41] S.L. Percival, P.G. Bowler, J. Dolman, Int. Wound J. 4 (2007) 186–191.
[42] S.S. Bhojwani, M.K. Razdan, Plant Tissue Culture: Theory and Practice, Revised ed., Elsevier Science, Netherlands, 1996.
[43] T. Davod, Z. Reza, V.A. Ali, C. Mehrdad, Int. J. Agric. Biol. 13 (2011) 986–990.
[44] T. Murashige, F. SKoog, Physiol. Plant. 15 (1962) 473–497.
[45] T. Spies, Infection 27 (1999) 34–S37.
[46] T.G. Beckman, G.A. Lang, Acta Hortic. 622 (2003) 531–551.
[47] T.M. Gradziel, HortScience 43 (2008) 1321–1343.
[48] V. Alt, T. Bechert, P. Steinrucke, M. Wagener, P. Seidel, E. Dingeldein, E. Domann, R. Schnettler, Biomaterials 25 (2004) 4383–4391.