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Using Silver Nano- Particles to Increase Efficiency Of Sterile Solution for *in vitro* Techniques

Nabeel K.Al-Ani *

* *Biotechnology Dept/ College of science/ University of Al-Nahrain*

Abstract :

This work was done by using explants cultured in MS medium supplemented with 3mg/l of NAA plus BAP, 30g/l sucrose beside different concentrations of silver nano particles 4,8,12 mg/l. to assess the efficiency of using these particles in control the infection percent.

The results indicate that healthy plants percent were increase from 45% to 100% in all treatments. However, in 4 mg/l treatment the explants were continued to grow better even after 28 days in culture. This is the first report on using silver particles *in vitro* technique especially in Iraq .

Key words: *tissue culture, Blood leaf plant, sterilizing solution, MS medium*

Introduction:

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 control these contaminations, but the efficiency of these chemical are either limited or toxic.

Antibiotics are also used in controlling internal bacterial contaminations (1).

However, they may affect the growth and response of explants and may induce resistance in bacteria.

Therefore, they are not suggested for using in plant tissue culture techniques (1). Mercury chloride (HgCL2) has been widely used to control infections in explants. HgCL2 is very toxic and should be used with high cautions (2).

Such chemicals are not only toxic for the explants and peoples working in this field but they may affect the environment. Therefore, finding an effective and safe substance for decontaminated of explants is very important, and that is what this paper aim to.

The term Nano-technology was first used by Japanese scientist "Taniguchi" at the university of Japan (3).

Nano-agriculture involves the employment of nano-particles in agriculture with the ambition that these particles will impart some beneficial effects to the crops.

Recently, confocal fluorescence image studies have revealed the capacity of single walled carbon nanotubes (SWNTs) to traverse across both the plant cell wall and cell membrane(4).

The study also pointed out that SWNTs can serve as effective nanotransporters to deliver DNA and small dye molecules into intact plant cells. There are also some reports on other nanoparticles as smart treatment- delivery systems in plants(4).

Nano – silver is new and non – toxic material which shows high capabilities in decontamination of microorganisms, e.g. fungus, bacteria, and viruses.

The detrimental effects of this chemical have been shown more than 600 microorganisms (5).

This capability of nano-silver is due to release of tiny particles of silver so it is able to destroy not only bacteria, fungus but also the viruses (6).

Corresponding Address:
Nabeel K.Al-Ani
Biotechnology Dept/ College of science/ University of Al-Nahrain
Email: nkalani54@yahoo.com

Materials and Methods:

Tissue culture techniques were flow according to (7).

Types and media preparation:

MS medium were prepared by dissolving 4.1g/l from a readymade sachets MS medium. After dissolving, this medium was supplemented with 3m/l NAA and BAP as growth regulators plus 30g/l sugar.

The medium was divided into three groups each with different concentration of silver nano particles bought from local market. 4, 8, and 12mg/l of silver nano particles were supplemented to each media.

The nano particles were dissolved in drops of distilled water for half an hour before used (8). The whole media were sterilized in autoclave at 121 °C for 15 minutes (9).

Explants sterilization:

5mm Stem cutting of Blood leaf plant Iresine herbstii Family Araceae were sterilized in 3% hypochloride for 15 minute.

Then, washed three times in sterilized distilled water 5 minutes each time. Some explants were soaked in the above nano - particles solution, the other were cultured directly in MS medium. (negative control).

Results and discussion:

Micro-propagation can offer great advantages over traditional methods. Callus from explants can give a rise to hundred explants within few months of callus initiation (10)

In experiment 1 figure one below the positive control treatment(which means culturing explants without Nano treatment) produced only 45% clean plants, however, the negative control (which cultured without autoclave or explants sterilized with nano particles only without hypochloride) the clean plant percent were about 5%.

This gives us indications that sterilize explants with nano- particles only will not be helpful for our experiment. Nevertheless, the contaminated vials noticed mostly in fungus without any bacterial contamination.

This may be because that Ag+ collapses the proton motive force on the bacterial membrane (11).

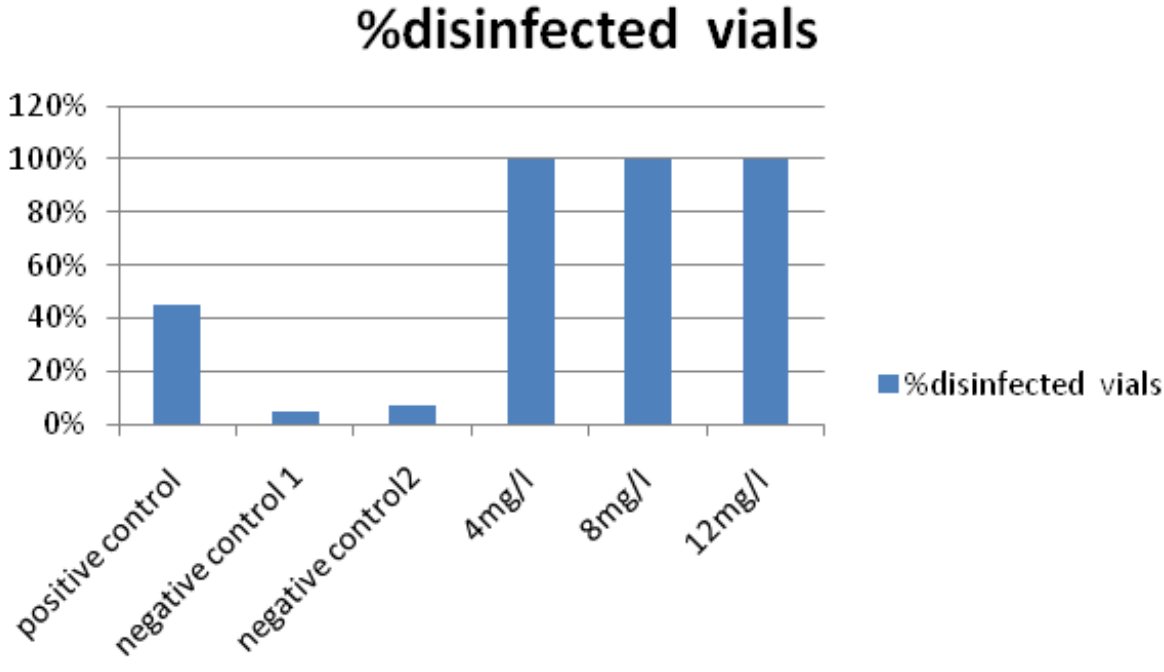

Figure1: % disinfected plants grown on MS medium supplemented with different concentrations of silver Nano- particles and positive and negative control. n=12.

In Figure 2 and picture 1 below the growth of explants continued after 28 days in culture without any infections or dead explants. This gives us indication that plants stay healthy for long time in culture better than control treatment and gives high rate of biomass specially in 4mg/l (pic.1 below).

This may be due to the effect of Ag+ (12).The results indicate that the explants are completely clean even from internal infections.

There is only one more note that in negative control, vials there are some fungi infections with no bacterial symptoms. This may be due to a spherical assembly needles found on the surface of bacteria use to inject poison into their targets (12). These finding may give us new challenge to introduce new genes in simple and fast way.

This paper referred for the first time to use silver nano particles in helping to decrease bacterial infections at least in ornamental plants.

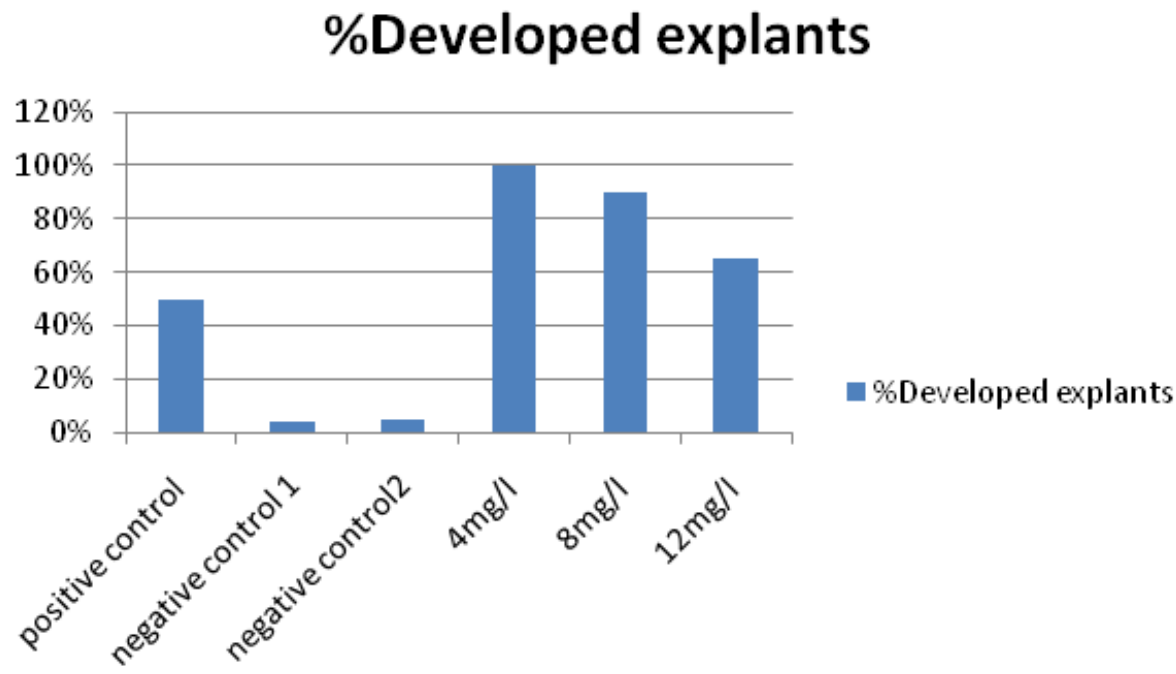

Figure 2: Healthy explants in MS medium supplemented with 4, 8, and 12 mg/l silver nano particles after 28 days in culture. N=12 .

4mg/l **8mg/l** **12mg/l**

Picture 1: different growth of explants in MS medium supplemented with 4,8,12mg/l silver nano particles after 28 days in culture

References:

1. Dabai y.u.,Arzai A.H.,Muhammad S.,2007.*In vitro* antibacterial activity of single and combined antibiotics on bone bacterial isolate. Pak.J.Bio.sci.6:1-3
2. Leifert C. and weedward S., 1997.Laboratory contamination Management ; The requirement for microbial contamination management in Micropropagation. Cassells A.C.(ed) .Kluwer Academic publisher ,Dordrecht ,pp: 237-244.
3. Taniguchi N.1974. "On the basic concept of Nano-technology" Proc.Intl.con.prod.engng Tokyo Part 2. (Japan society precision Engng)
4. Liu Q.Chen B. Wang Q. Shi x. Xiao Z. Lin J.and Fang x., 2009. Nano let.9,1007.
5. Abdi G.Salehi H.and Khosh-khuri M., 2008.Nano silver : Anovel nanomaterial for removal of Bacterial contamination in Valerian (V.officinalis) tissue culture .Acta physiol.plant.30:709-714
6. Sondi I.and Salopek- Sondi B., 2004. Silver nano particles as antimicrobial agent : Case study on E.coli as a model for gram – negative bacteria . J.colloid interference sci., 275: 177-182
7. Toby M.Horn 1989:Plant tissue culture . Fralin Biotechnology Center Virginia Tech Blacksburg, VA 24061
8. Roslami A.A and Shahsavar A. 2009. Nano-silver particles eliminate the *in vitro* contaminations of Olive "Mission" explants . Asian J.plant science 8(7): 505-509.
9. Stanley, D.: 1995 Tissue-Culturing Plants on Cornstarch Agricultural Research, July , p.11,
10. Tony storr.1985. plant tissue culture .the association for science and education,Herts,AL10 9AA.
11. Pavel Dibrov. Judith Dzioba. Khooshek K.Gosink. and Claudia C.Hase ., 2002 . Chemiosmotic mechanisms of antibacterial activity of Ag+ in vibro cholera. Antimicrobial Agents and chemotherapy Pp:2668-2670.
12. Plant tissue culture methodology , sterile technique. 2004. Internet site A\kpp\375\nutrient\nutrient.ppt.
13. Ramsden J.2005. What is nanotechnology? .Nanotechnology perceptions 1:3-17.

استعمال Sliver Nano- Particles لزيادة كفاءة التعقيم في تقنية *in vitro*

نبيل خلف ابراهيم العاني *

* كلية العلوم/ جامعة النهرين.

الخلاصة:

تم انجاز العمل باستعمال اجزاء نباتية زرعت على وسط MS جهز بـــ 3ملغم/لتر NAAوBAP بالاضافة الى 30غم/لتر سكروز وتراكيز مختلفة من مادة Silver Nano particle 4,8,12 ملغم/لتر . وذلك لتقدير فعالية هذه المواد في التقليل من التلوث .

اظهرت النتائج زيادة ملحوظة في نسبة النبتات غير المصابة من 45% الى 100% في كافة المعاملات . بالاضافة الى ذلك فان النمو في النبيتات ازدات بالنمو لفترة 28 يوم بعد المعاملة خاصة في المعاملة 4 ملغم/لتر .

وهذا البحث يعتبر الاول الذي يشير الى امكانية استعمال silver particles خارج الجسم الحي خاصة بالعراق .