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The Study of Nano Silver (NS) Antimicrobial Activity and Evaluation of Using NS in Tissue Culture Media

Kamran Safavi
Islamic Azad University - Khorasgan Branch,
Young Researchers Club, Isfahan, Iran.
e-mail: k_safavi@ag.iut.ac.ir

Mahtab Esfahanizadeh
Islamic Azad University - Khorasgan Branch, Isfahan, Iran.
e-mail: mesfahanizadeh@yahoo.com

Forogh Mortazaeinezahad
Department of Horticulture, Islamic Azad University - Khorasgan Branch, Isfahan, Iran.
e-mail: mortazaeinezhad@khuisf.ac.ir

Hamidreza Dastjerd
payamnoor university (pnu), Isfahan, Iran.
e-mail: hdastjerd@gmail.com

***Abstract*- Nanotechnology is relevant to diverse fields of science and technology. Antimicrobial activity of silver nanoparticles is importance to control conventional antimicrobial agents. Bacterial contamination is a serious problem in plant tissue culture procedures. This research was planning to evaluate the potential of nano silver (NS) to remove bacterial contaminants. Experiment involved NS with five rates (8, 10, 20, 50, 80 mg L^{-1}) in MS medium. Explants were cultured on MS medium and evaluate at four times (1, 2, 3, 4 weeks). Adding nano silver (50 mg L^{-1}) to media and evaluate at second week was fully effective to control the microorganism infection. This research shows that NS had a good potential for removing of the bacterial contaminants in plant tissue culture procedures.**

Keywords—component: Microorganism infection, Bacterial contaminants, Nano Silver, Tissue Culture.

I. INTRODUCTION

The field of nanotechnology is one of the most active areas of research in modern materials science. Nanoparticles exhibit completely new or improved properties based on specific characteristics such as size, distribution and morphology. New applications of nanoparticles and nanomaterials are emerging rapidly. Nanocrystalline silver particles have found tremendous applications in the field of high sensitivity biomolecular detection and diagnostics, antimicrobials and therapeutics, Catalysis and micro-electronics [2].

The amount of nanoscale silver is increasing rapidly in consumer products the ability to synthesize these particles on a large scale improves. Silver nanoparticles (NPs) are toxic to bacteria, and are currently used in everything from medical devices to sport socks and washing machines to deter microbial growth [5].

The use of silver for medicine or local antibacterial agents was not recognized until the nineteenth century. Since then, the antimicrobial property of silver has been investigated and employed more extensively than any other inorganic antibacterial agent. Silver is known to attack a broad range of biological processes in microorganisms including the alteration of cell membrane structure and functions. Silver also inhibits the expression of proteins associated with ATP production, although its specific antimicrobial mechanisms are still not completely understood. Micromolar doses (1 to 10 μM) of silver ions are sufficient to kill bacteria in water, while silver can be toxic at high doses to mammals and freshwater and marine organisms, probably compromising the growth and shape of animal cells by disrupting a variety of biological functions. Such micromolar concentrations of silver have no harmful effects on humans. Therefore, silver has been widely used for the development of many biological and pharmaceutical processes, products, and appliances such as coating materials for medical devices, orthopedic or dental graft materials, topical aids for wound repair, water sanitization, textile products, and even washing machines [7].

Toxicity of silver ion and its compounds towards microbes is well established and this property was used in wound dressings, silver loaded zeolites etc. because of its higher stability, stronger antibacterial activity and broad antibacterial spectrum [1].

The use of nano-sized silver particles as antimicrobial agents has become more common as technological advances make their production more economical. One of the potential applications in which silver can be utilized is in management of plant diseases. Since silver displays multiple modes of inhibitory action to microorganisms, it may be used for controlling various plant pathogens in a relatively safer way compared to synthetic fungicides [7].

To eliminate bacterial contamination during in vitro propagation, different methods have been developed in the last few years. Antibiotics are commonly used in the medium to eliminate unwanted contaminants from plant systems. Theoretically, it might seem that all contamination problems could be overcome by the incorporation of one or more antibiotics into the culture medium. However, antibiotics are frequently phytotoxic otherwise may retard or inhibit plant tissue growth [3].

Most antibiotics have been shown inhibitory effects in the plants. Streptomycin and chloramphenicol are inhibitors of protein synthesis; rifampicin inhibits nucleic acid synthesis and penicillin inhibits cell-wall membrane synthesis. Therefore, using antibiotics without application in the medium may reduce mutation risks and inhibitory effects on them. Nano silver has antimicrobial effects at low concentrations. However, in this research we study the several article about antimicrobial activities of silver nanoparticles and then we using NS and non-NS media for regeneration some plants and evaluate the elimination of microorganisms in tissue culture procedures.

II. SEVERAL RESEARCHES:

A. *Antifungal Activity of Silver Ions and Nanoparticles on Phytopathogenic Fungi*

Antifungal activity of ionic or nanoparticle silver has a great potential for use in controlling spore-producing fungal plant pathogens. Silver may be less toxic to humans and animals than synthetic fungicides. Multiple modes of action targeting a broad range of biological pathways of microbes provide an important benefit for avoiding the development of resistance, which has been increasingly important in terms of current issues for the chemical management of many plant fungal diseases. Since the efficacy of silver is greatly influenced by application timing, preventative applications of silver ions and nanoparticles work better before spores penetrate and colonize within the plant tissue. Our follow-up research focuses on extended applicability of silver for control of *B. sorokiniana* and *M. grisea* in the field, and evaluation of the efficacy of silver on different types of pathogens such as soilborne sterile fungi that rarely produce spores. Further research should focus on the development of silver compounds, compositions, and carriers than have improved stability against chloride ions. At the same time, the environmental tracking of silver when applied in the field is important to assess the impact on environmental and human health. This information is imperative for future registration and labelling of the silver nanoparticles as fungicides for crop protection [7].

B. Assessment of antibacterial activity of silver *nanoparticles* on Pseudomonas aeruginosa and its mechanism of action

Antimicrobial activity of silver nanoparticles is gaining importance due its broad spectrum of targets in cell compared to conventional antimicrobial agents. In this context, a UV photo-reduction method was used for the synthesis and the nanoparticles were characterized by UV–Visible spectroscopy, transmission electron microscopy, and atomic force microscopy and thermogravimetric analysis techniques. The antibacterial activity of the synthesized silver nanoparticles was evaluated both in liquid and solid growth media employing various susceptibility assays on Pseudomonas aeruginosa, a ubiquitous bacterium. The dose dependent growth suppression by nanoparticles was studied with well diffusion method. By broth dilution method, the minimum inhibitory concentration (MIC) was found to be 2 lg/ml. It was observed that the bactericidal effect depends both on nanoparticle concentration and number of bacteria present. In this study, they could demonstrate the complete antibiofilm activity of silver nanoparticles at a concentration as low as 1 lg/ml. Our observations substantiated the association of reactive oxygen species and cell membrane damage in the antibacterial mechanism of silver nanoparticles. This article suggested that these nanoparticles can be exploited towards the development of potential antibacterial coatings for various biomedical and environmental applications [1].

C. Impregnation of silver nanoparticles into bacterial cellulose for antimicrobial wound dressing

The researchers succeeded in the chemical reduction of silver nanoparticles in the three dimensional non-woven networks of bacterial cellulose nanofibrils. The size and size distribution are controllable by adjusting the molar ratio of NaBH4:AgNO3. Under the optimized conditions, well dispersed and regular spherical silver nanoparticles were obtained. The unique structure and the high oxygen (ether and hydroxyl) density of bacterial cellulose fibers constitute an effective nanoreactor for in situ synthesis of silver nanoparticles. These properties are essential for introduction of silver ion and reduction into bacterial cellulose fibers and removal of the excess chemical from bacterial cellulose fibers. The ether oxygen and the hydroxyl group not only anchor silver ions tightly onto bacterial cellulose fibers via ion–dipole interactions, but also stabilize silver nanoparticles by strong interaction with their surface metal atoms. The preparative procedure is surprisingly simple. It can provide a facile approach toward manufacturing of metallic nanocomposites, antimicrobial materials, low-temperature catalysts and other useful materials. The freeze-dried silver nanoparticle-impregnated bacterial cellulose exhibited a strong antimicrobial activity against both S. aureus (Gram-positive bacteria) and *E. Coli* (Gram-negative bacteria), which are general bacteria that found on the contaminated wound. A recent study showed that impregnation, instead of coating the wound dressing with silver nanoparticle or nanocrystal improved the antimicrobial activity of the wound dressing and lowered possibility of the normal human tissue damage. This is probably due to the slow and continual release of silver nanoparticles and then was slowly changed to silver ions under our physiological system and interact with bacterial cells, thus silver ions will not be so high enough to cause the normal human cells damage and can prolonged the antimicrobial effect [6].

III. USING NANO SILVER IN TISSUE CULTURE MEDIA

Plant tissue culture techniques are essential to many types of academic inquiry, as well as to many applied aspects of plant science. In the past, plant tissue culture techniques have been used in academic investigations of totipotency and the roles of hormones in cytodifferentiation and organogenesis.

Currently, tissue-cultured plants that have been genetically engineered provide insight into plant molecular biology and gene regulation. Plant tissue culture techniques are also central to innovative areas of applied plant science, including plant biotechnology and agriculture. For example, select plants can be cloned and cultured as suspended cells from which plant products can be harvested. In addition, the management of genetically engineered cells to form transgenic whole plants requires tissue culture procedures; tissue culture methods are also required in the formation of somatic haploid embryos from which homozygous plants can be generated. Thus, tissue culture techniques have been, and still are, prominent in academic and applied plant science [4]. Successful tissue culture of all plants depends on the removal of exogenous and endogenous contaminating microorganisms. Fungi and bacteria are the most common microorganisms to be found on or in plant tissues. Nano silver is a new and non toxic which shows high capabilities in eliminating microorganisms, fungus, bacteria and viruses. In this research we electing some plants and their explants were sterilized with 70% ethanol for 1 min and 10% Clorox (containing 5.25% sodium hypochlorite) for 1 min and then rinsed four times with sterilized distilled water. Then we added different amount of nano silver (8, 10, 20, 50, 80 mg L^{-1}) to tissue culture media and the explants were placed on Murashige and skoog (MS) media. We evaluate the explants after 1, 2, 3 and 4 weeks. Our results showed that presence of 50 mg L^{-1} NS in media and at second week can control the microorganism infection in tissue culture conditions.

IV. CONCLUSION

Researches have been intensive on antibacterial materials containing various natural and inorganic substances. Among them, silver or silver ions have long been known to have strong inhibitory and bactericidal effects as well as a broad spectrum of antimicrobial activities. It is surprising that these applications were used without understanding the mechanism of action of silver ion. In this research we study the antimicrobial activity of nano silver and after confirm the ability of nano silver to reduce the microorganism, we decide to using and adding NS to MS tissue culture media. The results show that NS can reduce and remove microorganisms in MS media and then the explants can growth very well.

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