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ORIGINAL ARTICLE

Effect of Titanium Dioxide Nanoparticles in Plant Tissue Culture Media for Enhance Resistance to Bacterial Activity

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ABSTRACT
The titanium dioxide nanoparticles TiO_2 have been investigated extensively for the killing or growth inhibition of bacteria. Bacterial contamination is a serious problem in plant tissue culture procedures. This research was planning to evaluate the potential of titanium dioxide nanoparticles (TiO_2) to remove bacterial contaminants. In this stage, we added different amounts of TiO_2 NPs to tissue culture media. The percentages of nano materials antimicrobial activity used in tissue culture media were recorded four weeks after culture. This research shows that TiO_2 had a good potential for removing bacterial contaminants in plant tissue culture procedures.
Keywords: Titanium, Nanoparticles, Bacterial activity

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INTRODUCTION

The field of nanotechnology is one of the most active areas of research in modern materials science. The essence of nanotechnology is the production of nanoparticles (NPs) with unique physicochemical properties allowing worldwide application in new structures, materials, and devices. The consequently increasing human exposure to NPs has raised concerns regarding their health and safety profiles [1]. 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. 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 [2]. Nanostructured materials are attracting a great deal of attention because of their potential of achieving specific processes and selectivity, especially in biological and pharmaceutical applications [3, 4]. TiO_2 is the most commonly used semiconductor photocatalyst. Among the different nanomaterials, it is the most studied. Activated by UV-A irradiation, its photocatalytic properties have been utilized in various environmental applications to remove contaminants from both water and air. A wealth of information on TiO_2photocatalytic inactivation of bacteria has been acquired over the last 20 years. The concentration of TiO_2 usually required to kill bacteria varies and depending on the size of the particles and the intensity and wavelength of the light used [2]. The antibacterial activity of TiO_2 has been found to be due to a reaction of the TiO_2 surface with water. On exposure to ultraviolet (UV) irradiation, TiO2 releases free radicals such as OH, HO_2 and H_2O_2. This potent oxidizing power characteristically results in case of bacteria and other organic substances [5,6]. Plant tissue culture techniques involve the growing and multiplication of totipotent cells, tissues and organs of plants on defined solid or liquid media comprising nutrients under an aseptic and controlled environment. Plant tissue culture has an important role in the production of agricultural or ornamental plants and in the manipulation of plants for improved agronomic performance (7). *In vitro* propagation methods using meristem tips, nodal cuttings and micro tubers are more reliable for maintaining genetic integrity of the multiplied clones since de-differentiation and the subsequent organogenesis/embryo genesis with the accompanying genetic changes have been reported [8]. The success of plant biotechnology relies on several factors which include an efficient tissue culture system for regeneration of plants from cultured cells and tissues [9]. Potato is one of the world's

most economically important tuber crops, belonging to the family Solanaceae. Million tonnes are produced annually in the world for a wide range of applications, including cooking, processing, and use as a material for starch [10]. Potato (*Solanumtuberosum*L.) is a crop of high biological value for its protein and a substantial amount of vitamins, minerals and trace elements [9]. It is most productive horticultural vegetable crop. Micropropagation is the alternative to conventional propagation of potatoes [8]. Until now, there have been low reports of using nanomaterial in plant tissue culture for removal of bacteria [2, 11]. But we have not any report of using Titanium dioxide (TiO_2) nanoparticles (NPs) in plant tissue culture. This is the first report of using Titanium dioxide (TiO_2) nanoparticles (NPs) in plant tissue culture. In this research, we used the MS tissue culture media with TiO_2 NPs (with different concentration) and without-TiO_2 NPs to evaluate the elimination of bacteria in potato tissue culture procedures.

MATERIALS AND METHODS

Plant Materials

In this research, we elected potato (*Solanum tuberosum* L.) and propagated in Murashige and Skoog (MS) medium through bud cultivating. Plant grew in the temperature of 22°C, under a 16/8 h (light/darkness) photoperiod and re-cultivated in MS medium every three weeks. When we wanted to re-cultivate their explants in modified media they 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.

Nano Materials Preparation

The APS of TiO_2 NPs used in this research was 10 nm. The base working fluid was pure water.

Modification of Plant Tissue Culture Media by Nanomaterial

In this stage, we added different amounts of TiO_2 NPs to tissue culture media. Experiment involved TiO_2 NPs with 2 rates (1 and 2 w/w) in MS medium. Explants were cultured on MS medium and evaluate at four times (1, 2, 3 and 4 weeks).

Data Collection

The percentages of nano-materials antimicrobial activity used in tissue culture media were recorded four weeks after culture. The experiment was conducted as a completely randomized design in a factorial arrangement with three replications.

RESULTS AND DISCUSSION

Nanotechnology is regarded as a key technology which will have economic, social and ecological implication. 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. Nanotechnology is currently employed as a tool to explore the darkest avenues of antibacterials [12]. Nanobiotechnology, an extended term, can be defined as the Science and Engineering involved in the design, synthesis and characterization of non-toxic bioactive nanomaterials and devices which interact with cells and tissues at a molecular level with a high degree of specificity [13].Titanium dioxide (TiO_2) is an example of a fine, white, crystalline, odourless, low-solubility powder which was considered to exhibit relatively low toxicity [14, 15]. It is a natural, thermally stable and nonflammable, nonsilicate mineral oxide found primarily in the form of the minerals rutile, anatase, brookite, and as the iron-containing mineral *Ilmenite* [16]. It has excellent physicochemical properties, such as good fatigue strength, resistance to corrosion, machine ability, biocompatibility, whitening and photocatalysis, as well as excellent optical performance and electrical properties. With regard to its potential adverse health effects, several studies have defined TiO_2, at least under non overload conditions, as biologically inactive and physiologically inert in both humans and animals and thus as little risk to humans [17]. Pulmonary inflammation, fibrosis, epithelial hyperplasia, and tumorigenesis were reported in animals under conditions of substantial TiO2particle lung burden due to sufficiently high dose and/or duration of exposure [18, 21]. Tissue culture is a technique of growing plant cells by culturing explant aseptically on a suitable nutrient medium. Tissue culture techniques have been, and still are, prominent in academic and applied plant science [22].Technique of Tissue culture is using for gene transfer and the successful of this process depend on the removal of exogenous and endogenous contaminating micro-organisms [23 ,24]. Bacteria are the most common micro-organisms to be found in plant tissue culture.In this research, we study the antimicrobial activity of TiO_2 NPs in potato tissue culture media. After confirming the ability of TiO_2 NPs to reduce the micro-organism, we decided to use and add TiO_2 NPs to MS tissue culture media. Potato tissue culture was done in 2 rates of TiO_2 NPs 1 and 2 w/w. When we added 1 and 2 w/w TiO_2 NPs in tissue culture media, the potato was growth very well and it can to enhance resistance to bacterial activity in each four weeks that we evaluated.

Fig 1: Potato plant growing in MS media with 1 w/w TiO_2 NPs

CONCLUSION

The results show that TiO_2 NPs can reduce and remove micro-organisms in MS media and the best results can be achieved by using 1 w/w TiO_2 NPs in potato tissue culture media. On the basis of this study, it can be suggested that the TiO_2 NPs may be a useful material for removing the bacterial contaminants in plant tissue culture.

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