Inoculation of TiO_2-Ag nanoparticles in spinach seeds

Inoculación de nanopartículas de TiO2-Ag en semillas de espinaca noculation

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Resumen

La espinaca es consumida por su valioso aporte nutricional. Sin embargo, en Colombia su baja producción en cultivos, ha sido de poco interés en el área de la investigación. Por otro lado, la nanotecnología que puede ser utilizada en la agricultura para evitar o controlar enfermedades en los cultivos a través de la aplicación de nanopartículas (NPs) se perfila como un tema de alto potencial en nuestro país. En este trabajo, se evaluó la inoculación de suspensiones de NPs de dióxido de titanio con incorporación de plata (TiO_2-Ag) en semillas de espinaca. Las NPs se sintetizaron a través del método sol-gel y se caracterizaron por medio de Difracción De Rayos X (DFR). Se obtuvieron muestras en polvo con tamaños de partícula entre 7 y 26 nm que se suspendieron en agua a diferentes concentraciones para la inoculación. En comparación con el grupo control (sin inoculación), se obtuvo un crecimiento de las plantas con NPs del tamaño más bajo y concentración del 2 %, debido a que posiblemente el TiO_2-Ag incorporado contribuye al proceso de fotosíntesis y desempeña un papel antimicrobiano. La actividad fotosintética de las plantas tratadas, se midió mediante la técnica fotoacústica y del análisis de los resultados se obtuvo que con el tratamiento hecho con la suspensión de NPs de menor tamaño, a concentración entre 0,25 y 2 %, la Razón de Evolución de Oxígeno (REO) es similar a la que se observó en las plantas del grupo control. Esto indica que el tratamiento con NPs de TiO_2-Ag entre 7 y 8 nm puede inducir el crecimiento sin alterar drásticamente la tasa fotosintética de plantas de espinaca a bajas concentraciones, lo que puede ser una alternativa eficiente para la mejora de la producción de este cultivo.

Palabras clave: espinaca; nanopartículas; fotoacústica; dióxido de titanio; plata; actividad fotosintética; inoculación de semillas.

Abstract

Spinach is consumed for its valuable nutritional contributions. However, in Colombia, this crop has been of little interest in the research area due to its low production. On the other hand, the nanotechnology that can be used in agriculture to prevent or control diseases in crops through the application of nanoparticles (NPs) is emerging as a high potential subject in our country. In this work, the inoculation of suspensions of titanium dioxide NPs with the incorporation of silver (TiO_2- Ag) in spinach seeds was evaluated. The NPS was synthesized through the Sol-gel method and characterized by means of X-ray diffraction; suspensions of these powder samples of grain size between 7 and 26 nm were prepared at different concentrations for inoculation. In comparison with the control group (without inoculation), the highest plant growth was obtained with NPs of the lowest size and

2 % concentration. It possibly due to the contribution in the photosynthesis process and antimicrobial role of incorporated TiO_2-Ag. The photosynthetic activity of the plants from control and treated groups was measured by the photoacoustic technique and it was found that the plants treated with the smallest NPs suspension, at a concentration between 0.25 and 2 %, presented a rate of oxygen evolution similar to the control group. These results indicate that the treatment with NPs of TiO_2-Ag between 7 and 8 nm at low concentration can improve the growth without drastic alteration of the photosynthetic rate of spinach plants, which can be an efficient alternative for improvement in crop production.

Keywords: spinach; nanoparticles; photoacoustic; titanium dioxide; silver; Inoculation of seeds.

Introduction

The consumption of fresh vegetables in the world has increased from 36 to 40 % in recent decades, with an annual production rate of approximately 4.1 % (881 million tons), compared to the one reported last century of 1, 7 % (324 million tons). China is the largest contributor with 50 % of world production (***Food and Agriculture*** *Organization of the United Nations* [FAO], 2018).

Likewise, the spread of spinach crops (Spinach oleraceae) has increased due to its nutritional value, low in calories and with a high water content, which provides fiber, calcium, phosphorus, iron, and vitamins A, B1, B2, and C. This plant generally develops at temperatures between 5 and 15 ^{o}C infertile soils rich in organic matter and with neutral pH (Watt, 1975; Fersini, 1976; *Centers for Disease Control and Prevention* (CDC, 2007).

In Colombia, the area planted with leafy vegetables, including spinach, is around 12,850 ha, distributed in the departments of the central region such as Cundinamarca, Boyacá, Antioquia, Santander and Norte de Santander (National Administrative Department of Statistics [DANE], 2015). However, since spinach production is low compared to total leafy vegetables (around 200 hectares grown annually), there are not enough management manuals or alternatives to solve local problems, specifically those related to disease and pest control (Sánchez, 2017).

In relation to nanotechnology, it is applied in agriculture for the treatment of plant diseases and in the improvement of nutrient absorption. Its use can reduce the use of commercial pesticides and insecticides, which mitigates the environmental impact of this activity (Rai; Ingle, 2012). One of the materials with the greatest potential for this type of use is silver (Ag), due to its antimicrobial characteristics (Wiesner; Lowry; Dionysiou; Biswas, 2006), while titanium dioxide (TiO_2), is emerging as a candidate viable for its biocompatibility and photocatalytic activity (Osborne *et al*., 2013).

In this work, the effect of the inoculation of suspensions of TiO_2-Ag NPs in spinach seeds was evaluated, through the monitoring and monitoring of morphological measures and photosynthetic activity.

Materials and methods

Synthesis and characterization of TiO_2-Ag

TiO_2-Ag was obtained through the sol-gel method, this method is a chemical route that allows the manufacture of amorphous and polycrystalline materials at room temperature. It begins with the synthesis of a colloidal suspension of solid particles in a liquid (sol), which after a process of hydrolysis and condensation are transformed into a solid material filled with solvent (gel). This is evaporated from the gel to obtain the sample in powder form. In this case, 33 mL of methanol (Merck), 5 mL of 97 % titanium bis (acetylacetonate diisopropoxide) (Sigma-Aldrich) and 0.04 g of 99 % silver nitrate (Sigma-Aldrich) were mixed in a case Beaker with constant agitation until a complete dilution of silver nitrate is obtained. Then the mixture was poured into a Petri dish while stirring. Then, a mixture of distilled water and methanol of which 2 mL was added dropwise to the

Petri dish was added and stirring was continued for 5 min. Finally, the product was allowed to dry at room temperature to obtain a solid that was macerated and sintered at temperatures of 300, 400, 500 and 600 °C for 1 h. After each heat treatment, the samples were characterized by X-ray Diffraction (DRX).

The sol-gel method was chosen for the manufacture of the TiO_2-Ag NPS because it is relatively inexpensive, achievable in a shorter time, friendly to the environment and safety. Similarly, it allows the observation of the gel formation and the advantage that it is possible to manipulate the grain size and synthesize high purity powder material. This is one of the methods that allows producing homogeneous structures at fine scales (nm) and has been positioned within the chemical routes for the generation of advanced high-tech inorganic material (Hench; West, 1990; Morales; Moran; Quintana; Estrada, 2009).

Spinach Seed Inoculation

Spinach seeds (Spinach oleracea) were immersed in suspensions of TiO_2-Ag NPs in distilled water, previously subjected to ultrasound for a period of 3 h. The treatments were done in Petri dishes for 3 d, at an average temperature of 17 °C with alternating periods of natural light exposure, using concentrations of 0.25, 2, 4 and 6 % of NPS of sizes 7, 8, 10 and 26 nm. After this process, the seeds were left on the adsorbent paper with daily irrigation until germination and transplanted into a hydroponic substrate for cultivation under greenhouse conditions. Plant height was recorded daily for 16 days.

Photosynthetic activity

The Oxygen Evolution Reason (REO) released in the photosynthesis process, was measured with the photoacoustic technique (FA), which is part of a set of experimental methods known as photothermal, in which light pulses are periodically affected on the sample, whose energy is partially absorbed and transformed into heat, such that the temperature of the sample, and therefore, the pressure of the adjacent air, changes with the same frequency of the incident radiation. The sound that is generated can be picked up by a microphone and is known as the FA signal; taking into account that during this process photochemical phenomena such as photosynthesis can happen, the contribution is added to the FA signal. This allows the determination of photosynthetic activity using an FA cell.

In the experimental setup, the leaf of the living plant was placed by sealing the cell cavity and it was made to strike pulsed red light on its beam, while on the underside it was temporarily irradiated with a white light to saturate the process of photosynthesis every 30 s. The FA signal was acquired through an SR830 "lock-in" amplifier and was recorded as a function of time through an interface and a computer. The photoacoustic effect occurs when the modulated radiation is absorbed by the spinach leaf, but the intensity of the sound depends not only on the optical and thermal characteristics but also on the periodic generation of oxygen. Therefore, the percentage of oxygen to be generated periodically is determined from the curves obtained by saturating the photosynthesis process. Similarly, as the physiology of the plants is affected by the growing conditions, with this technique the influence of a given treatment can be assessed through the monitoring of photosynthetic activity.

Results and Discussion

Characterization with X-Ray Diffraction (DRX)

The structure of the TiO_2-Ag NPs was analyzed using DRX in the range of 2θ between 20 and 80 °. It is one of the most used configurations for polycrystalline samples in dust form, thin films or multilayer, called Bragg-Brentano geometry (Quiroz, 2014). In the diffractograms it was observed that the Bragg diffraction peaks agree with those reported by Hernández (2010), indicating the presence of TiO_2 in anatase phase, which is favorable for photocatalysis (Macías; García; De La Torre; Chávez, 2000) and variations in the width of the peaks that depend on the average crystal size were observed (Macak *et al*., 2007), which was estimated using the Scherrer formula (Villar; Bonilla, 2015). On the other hand, the grain sizes of the sintered samples at 600, 500, 400 and 300 °C were 26.0 ± 0.6, 10.9 ± 0.4, 8.3 ± 0.4 and 7, 3 ± 0.1 nm, respectively. This behavior with the increase in sintering temperature corresponds to a coalescence phenomenon (Mosquera; Rosas; Debut; Guerrero, 2015).

Plant growth

The results of measuring the height of the 10 spinach plants of each treatment are shown in Figure 1. It was observed that the plants treated with NPs of 7 nm, at a concentration of 2 %, reached 89 % higher than those of control. This result is consistent with the study conducted by Martínez (2015), who found that the application of low concentrations of NPs of TiO_2 influences the growth and development of corn plants, having an effect on biomass and micromorphology, depending on the type of NPs and the concentration applied. On the other hand, Yang *et al*., (2007), provided NPs of TiO_2 in anatase phase in the soil, with concentrations in the range 2.5 - 40 g kg-1, and obtained an average yield of spinach in a 95 % higher than the control, which related to an optimization effect on nitrogen fixation. It has been shown that TiO_2 increases the activity of several enzymes responsible for metabolic reactions (Capaldi; Diniz; Moretto; Antunes; Zizzi, 2015) and facilitates the absorption of nutrients, which improves germination speed because it favors formation of indole acetic acid (IAA) in the roots or shoots that can increase the vigor of the seed and the growth of the seedlings (Krishnaraj; Ji; Harper; Yun, 2016).

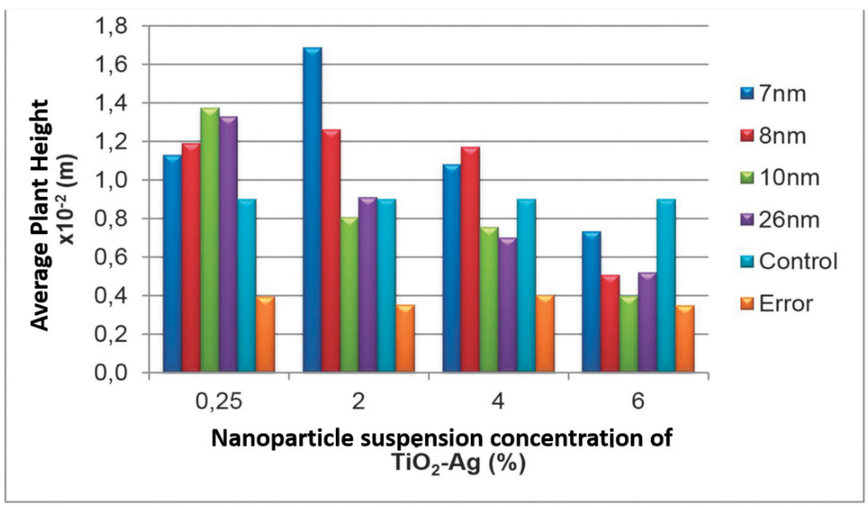

Figure 1. The average height of spinach plants, 16 days after being sown, after inoculation with suspensions of nanoparticles of titanium dioxide incorporated with a silver (NPS TiO2-Ag) with different particle sizes and concentration.
Source: self-made.

Although in this work the silver content in the inoculated suspensions was low, it has been shown that this element has antibacterial properties, which inhibit the growth of s*taphylococcus aureus, pseudomonas aeruginosa* and *escherichia coli* (Stockmann; Sherwin; Ampofo; Spigarelli, 2014), which affect the growth of plants and are reported as one of the main microorganisms that cause foodborne illness (Figueroa; Navarrete; Caro; Troncoso; Faúndez, 2002; Luján, 2014; Ocaña de Jesús *et al*., 2017).

On the other hand, in relation to the ecotoxicity that can be generated in the soil by the use of NPS in agriculture, some authors have reported repercussions on the microbial composition, such as the generation of ecological imbalance and affectation to the population of bacteria that promote growth, fundamental in agricultural production. NPS can cause cell lysis, production of reactive oxygen species in intracellular location, production of oxidative stress, lesions of deoxyribonucleic acid (DNA), alteration of transport mechanisms across membranes and denaturation of biomacromolecules (Shah; Belozerova, 2009; Fang; Shan; Wen; Lin; Owens, 2009; Nowack, 2009; Somasundaran; Fang; Ponnurangam; Li, 2010; Gutarowska; Skora; Zduniak; Rembisz, 2012). However, it has been concluded in most studies that the ecotoxicity of NPS depends on the concentrations used (Zhang; Niu Yan; Cai, 2011; Gutarowska *et al*., 2012). Therefore, in this work, the inoculation of the NPS was made directly in the seeds and the deposition of NPs in the soil was avoided and although there are reports that confirm that both the NPS of TiO_2 and of silver, at low concentrations and particle sizes are not toxic to humans (Weir; Westerhoff; Fabricius; Hristovski; Von Goetz, 2012; Vladimir, 2012), it is necessary to make corresponding studies with the treated plants before defining the use of the product in the field.

However, it is necessary to highlight that the TiO_2 has been approved for human consumption since 1960 by the Food and Drug Administration of the United States, and later in Europe by the Scientific Committee on Foods under the nomenclature E171 (FAO; World Health Organization [WHO], 2011). The National Institute of Safety and Health at Work (INSHT) of the United States currently recommends exposure limits in relation to extra fine particles of TiO2: 0,3 mg/m3 for NPs of TiO_2 (< 100 nm), front a 2,4 mg/m3 for fine particles (> 100 nm) (The National Institute of Safety and Health at Work [NIOSH], 2011).

Oxygen Evolution Reason (REO)

The behavior of the amplitude of the FA signal from the leaves of the plants is shown in Figure 2. These curves are the average of the measurements of 10 plants, which were chosen at random from the groups of each treatment and were taken 20 d after sowing. In them, the photothermal component of the signal was observed, added to the corresponding oxygen release (photo basic component). This last one stopped contributing when continuous white light was affected since it saturated the photosynthetic process. Therefore, it was evidenced that when this light is turned on, the signal drops abruptly and is restored when it is turned off. The REO is calculated as the percentage of decrease or increase in this voltage.

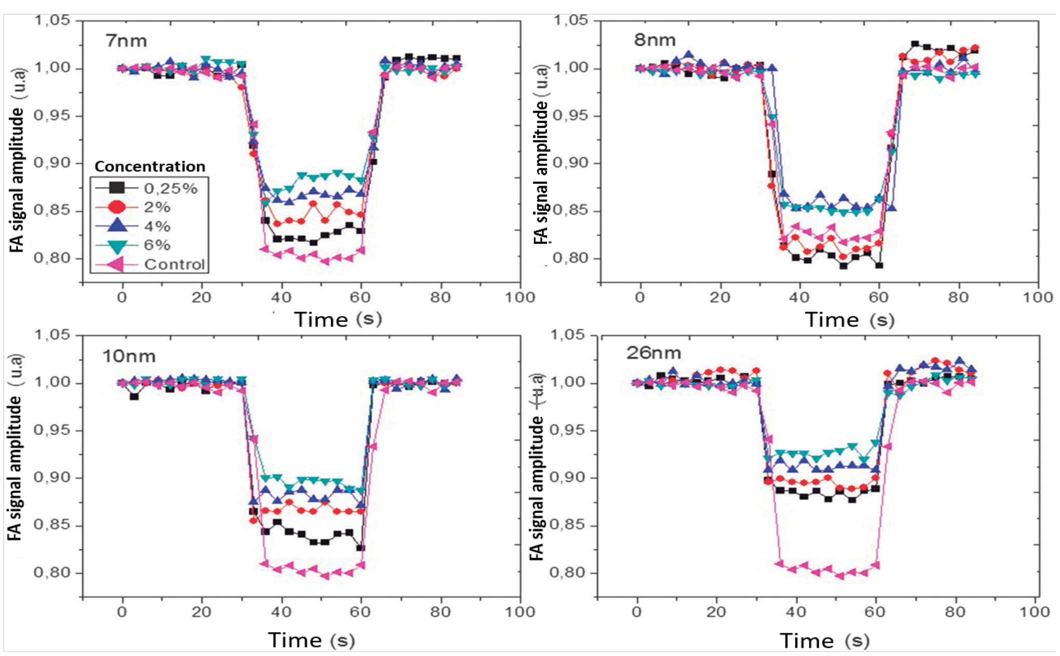

Figure 2. The amplitude of the photoacoustic signal as a function of time, corresponding to the treatment plants with nanoparticles TiO2-Ag and of the control group.
Source: self-made.

The amplitude of the signal is shown in Figure 3, where it is evidenced that its behavior was close to that of the control group with the suspension of average size of 8 nm and concentration of 2 %, while with the largest particle size (26 nm), the behavior of the FA signal moved away from the control, proportionally with the increase in concentration. In other words, the plants reacted positively to the treatment with the NPS of size less than 10 nm and concentrations less than 4 %, since the normal metabolism of the plant is not affected. This corresponds to what was found by Bradfield (2015), who in his study showed that the foliar application of 0.25 % of TiO_2 (2500 mg L^{-1}) increased spinach biomass, which was attributed to the increase in the photosynthetic rate or to its maintenance in the presence of the treatment.

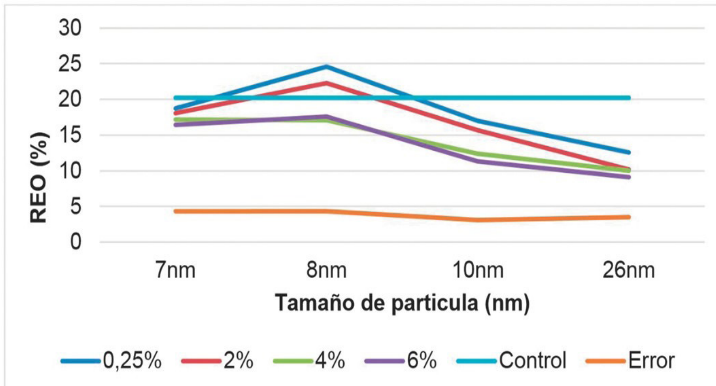

Figure 3. Oxygen Evolution Ratio of the control group plants and those treated with the nanoparticles of TiO_2-Ag.
Source: self-made.

For its part, Ze; Liu; Wang; Hong; Kong (2010), determined the mechanisms by which the NPS of TiO_2 can favor photosynthesis, for this they used *Arabidopsis thaliana* as the object of study. The plants were sprayed with 0.25 % of NPS TiO_2 and concluded that this compound can induce a significant increase in the expression of the genes encoding the *light-harvesting complex II* (LHCII) since NPs could be accelerating the transport speed of the electrons of the entire chain and the transformation of light energy into electronic energy. They could also promote the absorption of light from chloroplasts.

Therefore, it can be assumed that the photosynthesis of spinach plants may also be influenced by the inoculation of this photocatalyst (TiO_2), taking into account that when joining the plant it can eventually absorb sunlight, which excites electrons until they gain enough energy to jump from the Valencia band (VB) to the Conduction Band (CB) and the generated charge carriers can migrate to the surface to react with adjacent molecules. In aqueous solutions, the holes generated in the VB of the TiO_2 form OH radicals, while electrons in CB reduce dissolved oxygen (Kazuya; Tsuyoshi; Taketoshi; Akira, 2012; Quiroz, 2014). These species, particularly OH radicals, have a high oxidation potential that acts on carbon dioxide and water (Ollis; Ekabi, 1993), which can accelerate the photosynthetic process. Similarly, Hong *et al*., (2005) and Zheng *et al*., (2008), indicated that the properties of NPS de TiO_2 to increase photosynthetic capacity, they come from their ability to decrease Reactive Oxygen Species (ERO) in spinach chloroplasts.

Also, it has been found that after 24 h of exposure to 4 mM (319 mg L^{-1}) of TiO_2 NPs, the levels of Malondialdehyde (MDA) in onion roots are increased. MDA is an indicator of lipid peroxidation because it is the product of the breakdown of polyunsaturated and fatty acids found in biomembranes. This indicates that TiO_2 NPs at these concentrations increase the generation of ROS that leads to increased lipid peroxidation and oxidative stress (Ghosh; Bandyopadhyay; Mukherjee, 2010).

Likewise, it has been determined that the content of MDA in algae that grows in contact with NPS de TiO2 is significantly greater than the control. This shows that the NPS of TiO_2 could impose oxidative stress on algal cells, depending on the concentration (Lin; Ji; Long; Yang; Wu, 2012). Therefore, it is considered that the TiO_2 in high concentrations of greater than 1 g/L that can induce stress in plants, changing the structure and constitution of the wall and cell membrane (Liu; Lin; Zhao, 2013), which alters photosynthetic efficiency, due to the interactions of these NPs with photosystems, since chlorophyll can transfer energy to the NPS (Olejnik *et al*., 2013; Rico; Peralta-Video; Gardea-Torresdey, 2015).

Conclusion

The nanoparticle treatment of TiO_2-Ag in powder with particle sizes between 7 and 8 nm and at concentrations between 0.25 and 2 % have a positive effect on the growth of spinach plants in greenhouse conditions, without drastically altering the natural metabolism of the plant since the oxygen evolution rate of the treated plants is very similar to that of the individuals in the control group.

According to the results obtained, it can be affirmed that both the treatment described and the method of measuring this photosynthetic parameter have the potential to become tools for crop improvement and evaluation of new products.

References

Bradfield, S. (2015). *Influence of TiO_2 engineered nanoparticles on photosynthetic efficiency and contaminant uptake* (tesis doctoral). Southern Illinois University Carbondale, Illinois.

Capaldi, S.; Diniz, A.; Moretto, R.; Antunes, R.; Zezzi, M. (2015). Nanoparticles applied to plant science. A review. *Talanta. 131*, 693-705. https://doi.org/10.1016/j.talanta.2014.08.050

Centers for Disease Control and Prevention. (2007). Multistate outbreaks of Salmonella infections associated with raw tomatoes eaten in restaurants. The United States, 2005-2006. *MMWR. Morbidity and mortality weekly report*, *56*(35), 909-911.

Departamento Administrativo Nacional de Estadística (2015). *Boletín técnico. Encuesta Nacional Agropecuaria*. (ENA 2015). Recuperado de: https://www.dane.gov.co/files/investigaciones/agropecuario/enda/ena/2015/boletin_ena_2015.pdf

Fang, J.; Shan, X.; Wen, B.; Lin, J.; Owens, G. (2009). Stability of titania nanoparticles in soil suspensions and transport in saturated homogeneous soil columns. *Environmental pollution*, *157*(4), 1101-1109. https://doi.org/10.1016/j.envpol.2008.11.006

Food and Agriculture Organization of the United Nations (2018). *World Food and Agriculture, Statistical Pocketbook*. Rome, 254pp.

Food and Agriculture Organization of the United Nations y Organización Mundial de la Salud (2011). *Reunión conjunto de FAO/OMS de Expertos acerca de la aplicación de la nanotecnología en los sectores alimentario y agropecuario: posibles consecuencias para la inocuidad de los alimentos.* (Informe de la reunión 2011). Recuperado de: http://www.fao.org/3/i1434s/i1434s00.pdf

Fersini, A. (1976). *Horticultura práctica*. Edit. Diana (2ª ed.). México

Figueroa, G.; Navarrete, P.; Caro, M.; Troncoso, H.; Faúndez, G. (2002). Portacion de Staphylococcus aereus enterotoxigénicos en manipulación de alimentos. *Revista médica de Chile, 130*(8), 859-864. https://doi.org/10.4067/S0034-98872002000800003

Ghosh, M.; Bandyopadhyay, M.; Mukherjee, A. (2010). Genotoxicity of titanium dioxide (TiO_2) nanoparticles at two trophic levels: plant and human lymphocytes. *Chemosphere*, *81*(10), 1253-1262. https://doi.org/10.1016/j.chemosphere.2010.09.022

Gutarowska, B.; Skora, J.; Zduniak, K.; Rembisz, D. (2012). Analysis of the sensitivity of microorganisms contaminating museums and archives to silver nanoparticles. *International Biodeterioration & Biodegradation*, *68*, 7-17. https://doi.org/10.1016/j.ibiod.2011.12.002

Hench, L. L.; West, J. K. (1990). The sol-gel process. *Chemical Reviews*, *90*(1), 33-72. https://doi.org/10.1021/cr00099a003

Hernández, C. (2010). *Estudio de la actividad catalítica del dióxido de titanio soportado en alúmina en la síntesis de ozone a partir de oxígeno por el método de descarga silenciosa.* (Tesis de maestría). Universidad Nacional De Colombia, Bogotá, Colombia.

Hong, F.; Zhou, J.; Liu, C.; Yang, F.; Wu, C.; Zheng, L.; Yang, P. (2005). Effect of Nono-TiO_2 on the photochemical reaction of chloroplasts of spinach. *Biological trace element research*, *105*(1-3), 269-279. https://doi.org/10.1385/BTER:105:1-3:269

Kazuya, N.; Tsuyoshi, O.; Taketoshi, M.; Akira, F. (2012). Photoenergy conversion with TiO_2 photocatalysis: New materials and recent applications. *Electrochimica Acta*, *84*, 103-111. https://doi.org/10.1016/j.electacta.2012.03.035

Krishnaraj, C.; Ji, B. J.; Harper, S. L.; Yun, S. I. (2016). Plant extract-mediated biogenic synthesis of silver, manganese dioxide, silver doped manganese dioxide nanoparticles and their antibacterial activity against food and waterborne pathogens. *Bioprocess and biosystems engineering*, *39*(5), 759-772. https://doi.org/10.1007/s00449-016-1556-2

Lin, D.; Ji, J.; Long, Z.; Yang, K.; Wu, F. (2012). The influence of dissolved and surface-bound humic acid on the toxicity of TiO_2 nanoparticles to Chlorella sp. *Water research*, *46*(14), 4477-4487. https://doi.org/10.1016/j.watres.2012.05.035

Liu, K.; Lin, X.; Zhao, J. (2013). Toxic effects of the interaction of titanium dioxide nanoparticles with chemicals or physical factors. *International journal of nanomedicine*, *8*, 2509-2520.

Luján, D. (2014). Pseudomonas aeruginosa: un adversario peligroso. *Acta Bioquímica Clínica Latinoamericana*, *48*(4), 465-474.

Macak, J. M.; Tsuchiya, H.; Ghicov, A.; Yasuda, K.; Hahn, R.; Bauer, S.; Schmuki, P. (2007). TiO2 nanotubes: Self-organized electrochemical formation, properties and applications. *Current Opinion in Solid State and Materials Science*, *11*(1-2), 3-18. https://doi.org/10.1016/j.cossms.2007.08.004

Macías, L.; García, M.; De La Torre, I.; Chávez, A. (2000). Cuantificación de fases en óxidos de titanio por medio de difracción de rayos X. En *Congreso Técnico Científico ININ-SUTIN*. Instituto Nacional de Investigaciones Nucleares, Ocoyoacac, México. Recuperado de: https://inis.iaea.org/collection/NCLCollectionStore/_Public/33/013/33013257.pdf?r=1&r=1

Martínez, L. A. (2015). *Micromorfología de tallo y raíz de plantas de maíz y frijol tratadas con nano partículas de titanio, hierro y zinc, bajo invernadero* (Tesis de grado). Universidad Autónoma Agraria Antonio Narro, Saltillo, México. Recuperado de: http://repositorio.uaaan.mx:8080/xmlui/bitstream/handle/123456789/6844/T20520%20MARTINEZ%20RODRIGUEZ%2c%20LUIS%20ALBERTO%20%2063436.pdf?sequence=1&isAllowed=y

Morales, J.; Moran, J.; Quintana, M.; Estrada, W. (2009). Síntesis y caracterización de nanopartículas de plata por la ruta Sol-Gel a partir de Nitrato de plata. *Revista de la Sociedad Química del Perú*, *75*(2), 177-184

Mosquera, E.; Rosas, N.; Debut, A.; Guerrero, V.H. (2015). Síntesis y caracterización de nano partículas de dióxido de titanio obtenidas por el método Sol-Gel. *Revista Politécnica*, *36*(3), 7.

National Institute for Occupational Safety and Health (2011). *Occupational exposure to titanium dioxide.* Current Intelligence. (Bulletin 63, 2011). Recuperado de: https://www.cdc.gov/niosh/docs/2011-160/pdfs/2011-160.pdf

Nowack, B. (2009). The behavior and effects of nanoparticles in the environment. *Environmental pollution*, *157*(4), 1063-1185. https://doi.org/10.1016/j.envpol.2008.12.019

Ocaña de Jesús, R.; Gutiérrez, I.; Sánchez, P.; Barsain, M.; Campos, C.; Laguna, A. (2017). Persistencia, internalización y translocación de Escherichia coli O157: H7, O157: H16 y O105ab en planta y frutos de tomate (Solanum lycopersium L). *Revista Argentina de Microbiología, 50*(4), 408-416. https://doi.org/10.1016/j.ram.2017.12.001

Olejnik, M.; Krajnik, B.; Kowalska, D.; Twardowska, M.; Czechowski, N.; Hofmann, E.; Mackowski, S. (2013). Imaging of fluorescence enhancement in photosynthetic complexes coupled to silver nanowires. *Applied Physics Letters*, *102*(8), 083703. https://doi.org/10.1063/1.4794171

Ollis, D.F.; Ekabi, H. (1993). *Photocatalytic purification and treatment of water and air*. Elsevier: Amsterdam.

Osborne, O.J.; Johnston, B. D.; Moger, J.; Balousha, M.; Lead, J. R.; Kudoh, T.; Tyler, C. R. (2013). Effects of particle size and coating on nanoscale Ag and TiO_2 exposure in zebrafish (Danio rerio) embryos. *Nanotoxicology*, *7*(8), 1315-1324. https://doi.org/10.3109/17435390.2012.737484

Quiroz, H. (2014). *Preparación y estudio de las propíedades estructurales, ópticas y morfológicas de nanotubos de* TiO_2 *para su aplicación en sensores ópticos* (tesis de maestría). Universidad Nacional de Colombia. Bogotá, Colombia.

Rai, M.; Ingle, A. (2012). Role of nanotechnology in agriculture with special reference to the management of insect pests. *Applied Microbiology and Biotechnology*, *94*(2), 287-293. https://doi.org/10.1007/s00253-012-3969-4

Rico, C. M.; Peralta-Videa, J. R.; Gardea-Torresdey, J. L. (2015). Chemistry, the biochemistry of nanoparticles, and their role in antioxidant defense system in plants. In Siddiqui M., Al-Whaibi M., Mohammad F. (Eds.) *Nanotechnology and Plant Sciences* (pp. 1-17). Springer, Cham. https://doi.org/10.1007/978-3-319-14502-0_1

Sánchez, J. (2017). Mercado de productos agrícolas ecológicos en Colombia. *Suma de negocios*, 8(18), 156-163. https://doi.org/10.1016/j.sumneg.2017.10.001

Shah, V.; Belozerova, I. (2009). Influence of metal nanoparticles on the soil microbial community and germination of lettuce seeds. *Water, Air, and Soil Pollution*, *197*(1-4), 143-148. https://doi.org/10.1007/s11270-008-9797-6

Somasundaran, P.; Fang, X.; Ponnurangam, S.; Li, B. (2010). Nanoparticles: characteristics, mechanisms, and modulation of biotoxicity. *KONA powder and particle journal*, *28*, 38-49. https://doi.org/10.14356/kona.2010007

Stockmann, C.; Sherwin, C. M.; Ampofo, K.; Spigarelli, M. G. (2014). Development of levofloxacin inhalation solution to treat Pseudomonas aeruginosa in patients with cystic fibrosis. *Therapeutic advances in respiratory disease*, *8*(1), 13-21. https://doi.org/10.1177/1753465813508445

Villar, M.; Bonilla, M. A. (2015). *Obtención y caracterización de nanopartículas de* $LiMn_2O_4$, $LiMn_{1.5}Fe_{0.5}O_4$ *y* $LiMn_{1.25}Fe_{0.75}O_4$ (Tesis de maestría). Universidad Tecnológica de Puebla. Puebla, México.

Vladimir, M. (9 de mayo de 2011). Titanium Dioxide: A Changing Paradigm in Occupational Risk Management. (Blog). Recuperado de: http://blogs.cdc.gov/niosh-science-blog/2011/05/09/TiO2

Watt, B. K. (1975). *Composition of Foods. Agricultural hanbook*. Washington DC, U. S. Dep of Agric.

Weir, A.; Westerhoff, P.; Fabricius, L.; Hristovski, K.; Von Goetz, N. (2012). Titanium dioxide nanoparticles in food and personal care products. *Environmental science & technology*, *46*(4), 2242-2250. https://doi.org/10.1021/es204168d

Wiesner, M. R.; Lowry, G. V.; Dionysiou, P.; Biswas, P. (2006). Assessing the risk of manufactured nanomaterials. *Environmental Science & Technology*, *40*(14),4336-45. https://doi.org/10.1021/es062726m

Yang, F.; Liu, C.; Gao, F.; Su, M.; Wu, X.; Zheng, L.; Hong, F.; Yang, P. (2007). The improvement of spinach growth by nano-anatase TiO_2 treatment is related to nitrogen photoreduction. *Biological trace element research*, *119*(1), 77-88. https://doi.org/10.1007/s12011-007-0046-4

Ze, Y.; Liu, C.; Wang, L.; Hong, M.; Hong, F. (2010). The regulation of TiO_2 nanoparticles on the expression of light-harvesting complex II and photosynthesis of chloroplasts of Arabidopsis thaliana. *Biological trace element research*, *143*(2), 1131-1141. https://doi.org/10.1007/s12011-010-8901-0

Zhang, X.; Niu, H.; Yan, J.; Cai, Y. (2011). Immobilizing silver nanoparticles onto the surface of the magnetic silica composite to prepare magnetic disinfectant with enhanced stability and antibacterial activity. Colloids and Surfaces A: *Physicochemical and Engineering Aspects*, *375*(1-3), 186-192. https://doi.org/10.1016/j.colsurfa.2010.12.009

Zheng, L.; Mingyu, S.; Xiao, W.; Chao, L.; Chunxiang, Q.; Liang, C.; Hao, H.; Xiaoqing, L.; Fashui, H. (2008). Antioxidant Stress is promoted by nano-anatase in spinach chloroplasts under UV-B radiation. *Biological Trace Element Research*, *121*(1), 69-79. https://doi.org/10.1007/s12011-007-8028-0