Hindawi
Journal of Nanomaterials
Volume 2023, Article ID 6903787, 11 pages
https://doi.org/10.1155/2023/6903787

Research Article

Toxicity of Silver Nanoparticles Synthesized from *Opuntia ficus-indica* on *Danio rerio* and *Arabidopsis thaliana* and Their Antimicrobial Activity

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Received 6 March 2023; Revised 28 August 2023; Accepted 4 September 2023; Published 5 October 2023

Academic Editor: Kaliannan Durairaj

Silver nanoparticles (AgNPs) usefulness has been widely demonstrated in fields such as agriculture, medicine, food, and pharmaceutical industries, primarily for their antibacterial properties. AgNPs synthesized from biological extracts are as effective as those obtained by chemical methods, with the advantage that in the former fewer toxic compounds are generated for the environment. In a previous work, we synthesized AgNPs from the aqueous extract of *Opuntia ficus-indica* fruit peel (OfAgNPs) and tested their activity against microorganisms from a wastewater treatment plant. OfAgNPs were proposed for use as tertiary wastewater treatment; however, more needs to be known about their antimicrobial activity and toxicological potential. In the present study, the antimicrobial effect of OfAgNPs was demonstrated against reference bacterial strains of *Escherichia coli* (DH5α), *Pseudomonas aeruginosa* (ATCC 27853), and *Staphylococcus aureus* (ATCC 29213), and against fungi isolated in the laboratory and identified as *Aspergillus* sp., *Colletotrichum* sp., and *Cladosporium* sp. In addition, in this work, we report the effects of OfAgNPs produced by exposure in two model organisms: a fish, *Danio rerio*, and a plant, *Arabidopsis thaliana*. OfAgNPs in *A. thaliana* caused negative effects on growth and photosynthetic pigment content only when the exposure was constant and greater than 6 mg/L. However, in *D. rerio*, impaired caudal fin development was observed at 0.15 mg/L from 24 hr of exposure. This is evidence of the toxicological potential of OfAgNPs, so their use should be carried out in a controlled manner.

1. Introduction

Nanoparticles (NPs) are materials that measure between 1 and 100 nm [1, 2]. Metallic NPs, such as silver NPs (AgNPs), are being widely used in agriculture, biomedical, pharmaceutical, textile, food, and water treatment industries due to their optical, electronic, antibacterial, and antifungal properties [2–5]. Moreover, due to their antimicrobial activity, they have been proposed as an alternative to antibiotics and antifungals, especially in combating infections caused by multidrug-resistant microorganisms [6, 7]. AgNPs can be synthesized by physical, chemical, and biological methods [2, 8]. Biological methods are used as reducing agents, extracts of plants, algae, fungi, or bacteria. [9, 10]. This has the benefit of decreasing the generation of release of toxic waste byproducts to the environment compared to chemical methods [2, 4, 5]. The green synthesis also has benefits in terms of costs, and in that, it improves the characteristics of nanos since they are generated with a coating of organic matter that increases their stability and antibacterial activity [5, 9].

In recent years, many publications have shown the possibility of obtaining AgNPs using extract from fruit [11], seeds [12], roots [13], leaves [14–16], or flowers [17] of various plants, which also exhibit antibacterial activity. For example, AgNPs synthesized using *Dovyalis caffra* showed inhibitory activity against *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Candida albicans*, and *Aspergillus fumigatus* [11]; AgNPs obtained from extract of *Linum usitatissimum* had activity against *E. coli*, *Klebsiella pneumoniae*, *S. aureus*, and *Streptococcus pyogenes* [12]; AgNPs obtained with extract *Rheum palmatum* root were active against *P. aeruginosa* and *S. aureus* [13]; and those produced with of pur-hea tea leaves (*Camellia sinensis*) exhibited activity against *K. pneumoniae*, *Salmonella typhimurium*, and *Salmonella enteritidis* [14]. AgNPs obtained by green synthesis have been shown to exhibit antifungal activity, such as those from *Melia azedarach* leaf extract against *Verticillium dahliae* [15], those of *Citrus limon zest* extract [16], and *Ferulago macrocarpa* flowers extract both with activity against *C. albicans* [17]. In previous work, we established a synthesis protocol to produce AgNPs taking advantage of a waste product of *Opuntia ficus-indica* fruit peel (OfAgNPs), and they showed activity against bacteria isolated from a wastewater treatment plant [18]. Based on the results of that work, we proposed that OfAgNPs can be used in tertiary wastewater treatment [18]. However, it is desirable that OfAgNPs show significant antimicrobial activity at concentrations that do not show toxic effects on other organisms. Therefore, it is necessary to evaluate the degree of inactivation produced by OfAgNPs in other microorganisms and their impact on other exposed species [19]. The toxic effect of AgNPs synthesized by other procedures has been analyzed on different organisms present in the environment: vertebrates, invertebrates, plants, and algae [20–22].

The aim of the present work was to evaluate the antimicrobial efficacy of OfAgNPs against *E. coli* (DH5α), *P. aeruginosa* (ATCC 27853), and *S. aureus* (ATCC 29213), reference strains by determining their logarithmic inactivation capacity; and against fungal isolates of *Aspergillus* sp., *Colletotrichum* sp., and *Cladosporium* sp. Also, we aimed to determine the toxicological effects of OfAgNPs in two model organisms, *Danio rerio* and *Arabidopsis thaliana*. *D. rerio* is a fish with high fecundity, rapid embryonic development, and high homology with humans [22, 23], useful in toxicology studies in aquatic environments [23–25]. *A. thaliana* is a model plant that develops in a short time, is small, requires little space, and has been used in toxicity studies in terrestrial environments [26].

2. Materials and Methods

2.1. Silver Nanoparticle Characterization. OfAgNPs were synthesized following the protocol described in Muñoz-Carrillo et al. [18] (Figure 1). The liquid nitrogen ground *O. ficus-indica* fruit peel was mixed with sterile distilled water and heated in a water bath at 80°C for 30 min to obtain an aqueous extract. The aqueous extract was mixed with $AgNO_3$ and incubated until it changed color from yellow to reddish brown. The OfAgNPs produced were recovered by centrifugation and washed with sterile distilled water and 96% alcohol. OfAgNPs were dried at 37°C for 24 hr and sonicated for 20 min for dispersion.

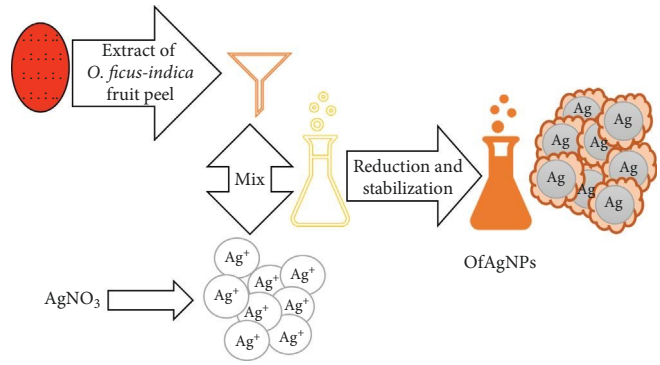

FIGURE 1: Mechanism of OfAgNPs synthesis. The aqueous extract of *O. ficus-indica* fruit peel was mixed with $AgNO_3$ and incubated until it changed color from yellow to reddish brown.

OfAgNPs were analyzed in suspension prepared at a concentration of 100 mg/L OfAgNPs in three media: deionized water, 50 mM phosphate buffer pH 7, and ISO medium, and sonicated for 20 min for dispersion (Table 1).

The dynamic light scattering technique was used to determine the hydrodynamic radius values using Litesizer equipment (Anton Paar Mexico Particle Characterization Laboratory); the dynamic and the electrophoretic light scattering (ELS) technique was used to calculate the *Z*-potential values in the different media. The particle velocity was calculated according to the Stokes–Einstein equation [27] as follows:

$$D = K_B T / 6\pi\eta R_H, \quad (1)$$

where D = translational diffusion coefficient or particle speed (m^2/s); K_B = Boltzmann constant (m^3 kg k/s); T = temperature (°K); η = viscosity (Pa s); R_H = hydrodynamic radius (m).

ELS was calculated according to the following equation:

$$\mu\varepsilon = \frac{V}{E}, \quad (2)$$

where $\mu\varepsilon$ = electrophoretic motility; V = speed; E = electric field.

2.2. Acute Toxicity Evaluation in D. rerio. The established protocol for fish embryo acute toxicity test (FET) was followed [28]. The exposure times of *D. rerio* are established in the FET protocol; these times are essential to evaluate the teratogenic effects in the embryos [28]. Sexually mature males and females of *D. rerio* were placed in a fish tank (ratio 3 : 1), and they were incubated at 25°C in photoperiod (16 hr light). After 24 hr, fertilized eggs were obtained and collected by pipetting, washed with ISO medium, and selected with the support of an Olympus Sz61 stereoscope. Twenty fertilized eggs were placed in 2 mL of ISO medium supplemented with OfAgNPs at concentrations of 0.075, 0.15, 0.3, 0.6, and 1.2 mg/L. OfAgNPS were sonicated in the suspension medium for 20 min before adding them to promote dispersion. The ISO medium was exchanged with each of the

Table 1: Composition of dispersion media for analysis of OfAgNPs.

Dispersion medium	Formulation
50 mM phosphate buffer	KH_2PO_4 reagent baker analyzed ACS, purity ≥99%, catalog number JTB-3246-01. K_2HPO_4, reagent baker analyzed ACS, purity ≥98%, catalog number JTB-3252-01, Mexico
ISO medium	0.294 g $CaCl_2-\cdot 2H_2O$ (reagent baker analyzed ACS, purity ≥74%, catalog number JTB-1332, Mexico). 0.123 g $MgSO_4 \cdot 7H_2O$ (ACS reagent, purity ≥98%, catalog number M-1144 Sigma-Aldrich, Germany). 0.6475 g $NaHCO_3$ (reagent baker analyzed, purity ≥99.7%, JTB-3506-01, Mexico). 0.0575 g KCl (reagent baker analyzed, purity ≥99%, catalog number JTB-3040-01, Mexico) for 1 L

concentrations every 24 hr, and they were maintained with continuous agitation as established by the FET protocol [28]. The sensitivity of the *D. rerio* embryos was evaluated using 3,4-dichloroaniline (LC_{50} 3.70 mg/L; 4.44–2.08 mg/L, $p \leq 0.05$). Two controls were included: one, the ISO medium without additives, and the other, the same medium with 0.874 mL/L of the extract of *O. ficus-indica* fruit peel. The extract of *O. ficus-indica* fruit peel was freeze-dried to be added to that concentration in the medium. These data were calculated considering that 24.15% of the OfAgNPs corresponds to silver and that 75.85% is organic matter from the extract, so that in 1.2 mg/L of OfAgNPs, maximum concentration evaluated, there are 0.874 mg/L of extract. Observations were made at 24, 48, 72, and 96 hr postfertilization (hpf) to determine embryo hatching percentage, sublethal effects, and lethality [28]. Analyzed sublethal effects were the absence or poor development of somites, nondetachment of the caudal fin, presence of pericardial edema, and malformations of embryos and larvae (teratogenic effects) [28]. Lethality was determined by the presence of coagulation and lack of heartbeat [28]. Observations were documented with photographs and assessed qualitatively (presence or absence) and quantitatively (as numbers of affected embryos and larvae). The lethal concentration 50 (LC_{50}) was calculated following the log-logistic model using the dose–response analysis with R Studio software (v.3.5.1) with the corresponding 95% confidence intervals [29].

2.3. Toxicity Evaluation in A. thaliana. A. thaliana ecotype Columbia Col-0 seeds were disinfected in a solution of 2% (V/V) commercial bleach (Cloralex® with 5% sodium hypochlorite, Mexico) and 0.15% Tween 20 (purity GC >40%, catalog number P1379, Sigma-Aldrich, Germany) for 8 min. Seeds were sown on Murashige–Skoog (MS) solid culture medium [30] medium supplemented with OfAgNPs at concentrations of 0, 6.25, 12.5, 25, 50, and 100 mg/L. They were incubated at 25°C in the darkness for 2 days and were later transferred to 16 hr light photoperiod for the following 17 days. Germination and development of 30 seeds per treatment were examined. Root elongation was calculated with the Image J2 software [31]. In addition, the chlorophyll and carotenoid content of the seedlings was analyzed.

2.4. Chlorophyll and Carotenoid Quantification. Pigment extraction from seedlings was performed following the protocol of Wellburn [32] with slight modifications: 10 mg crushed plant tissue was mixed with 5 mL of 90% methanol (Baker analyzed ACS, purity ≥99.8%, catalog number JTB-9070-03, Mexico), boiled for 5 min, and incubated at 4°C in the dark overnight. The absorbance of the extract was measured at 665, 649, and 470 nm wavelengths (A_{665}, A_{649}, and A_{470}) with a UV–visible spectrophotometer (Thermo Scientific Genesys 10s). The quantification was performed in triplicate. The pigment content was calculated with the following equations, where the result was expressed in μg pigment/g of tissue [32].

$$\text{Chlorophyll a (Cla)} = \frac{(12.19\,A_{665} - 3.45\,A_{649})}{1,000}P, \tag{3}$$

$$\text{Chlorophyll b (Clb)} = (21.99\,A_{649} - 5.32\,A_{665})/1,000\,P, \tag{4}$$

$$\text{Carotenoid} = (1,000\,A_{470} - 2.86\,\text{Cla} - 129.2\,\text{Clb}/221)/1,000\,P, \tag{5}$$

where A = absorbance; P = sample mass (g).

2.5. Antibacterial Activity Evaluation. The antibacterial activity of OfAgNPs was evaluated in *E. coli* (DH5α), *P. aeruginosa* (ATCC 27853), and *S. aureus* (ATCC 29213). The sensitivity of the bacteria was evaluated using ampicillin (0.5 mg/L) as a reference substance. A single colony was taken from axenic cultures of each strain, inoculated in 5 mL of liquid LB (Luria–Bertani) medium prepared with 10 g/L of casein peptone (catalog number BX-252606, BD Bioxon, Mexico), 5 g/L of yeast extract (catalog number BX-230900, BD Bioxon, Mexico), and 5 g/L of NaCl (Baker analyzed ACS, purity ≥99%, catalog number JTB-3624-01, Mexico), and incubated at 37°C under agitation for 16 hr. A sample of 1 mL of this culture was centrifuged at 14,000 *g* for 2 min, the supernatant was removed, and the bacterial pellet was resuspended in 1 mL of sterile 50 mM phosphate buffer pH 7. Microbial suspension was adjusted to a titer of 10^{11} colony-forming units per milliliter (CFU/mL) for *E. coli* and *S. aureus* and 10^9 CFU/mL for *P. aeruginosa*. OfAgNPs were added to 1 mL of these bacterial suspensions to concentrations of 0.25, 0.5, and 1 mg/L, and they were incubated with constant agitation to favor their dispersion. A bacterial suspension without OfAgNPs was used as a negative control. Aliquots of 10 μL were inoculated in solid LB at baseline and after 5, 10, 15, 30, 45, and 60 min of exposure. The exposure time of the OfAgNPs in bacteria and fungi was carried out the same as in *D. rerio*; however, it had to be modified to determine from what time the antimicrobial activity began. The cultures were incubated at 37°C for 18 hr.

Table 2: Physicochemical properties of OfAgNPs.

Dilution medium	pH	Hydrodynamic radius (nm)	Zeta potential (mV)	Conductivity (mS/cm)	Electroforetic mobility (μm cm/Vs)
Deionized water	7	137.33 ± 4.42	-23 ± 0.2	0.011	−1.805
Phosphate buffer	7	149.1 ± 2.66	-21.82 ± 1.09	13.167	−1.729
ISO medium	8	317.36 ± 205.10	-9.72 ± 0.26	2.203	−0.7501

Colony development was counted, and the number of CFU/mL was calculated. The experiments were performed in triplicate. Log inactivation was calculated according to Garcidueñas-Piña et al. [33] as follows:

$$\log10\,(N/N_0), \tag{6}$$

where N_0 represents initial CFU/mL, and N the CFU/mL after treatment.

2.6. Evaluation of Antifungal Activity. The antifungal activity of OfAgNPs was evaluated on *Aspergillus* sp., *Colletotrichum* sp., and *Cladosporium* sp. The fungal strains were isolated, identified, and donated by the Microbiology Laboratory of the Autonomous University of Aguascalientes. The fungi were grown on potato dextrose agar (PDA) medium (catalog number BX-211900, BD Bioxon, Mexico) for 1 week. Spores were recovered in 50 mM phosphate buffer pH 7, and concentration (spores/mL) was calculated with a Neubauer camera (Marienfeld). A suspension of 10^5 spores/mL was incubated in 50 mM phosphate buffer pH 7 with OfAgNPs at concentrations of 0.25, 0.5, and 1 mg/L for 24 hr. Spore suspension without OfAgNPs was used as a negative control. Subsequently, a 10 μL aliquot of this spore suspension was inoculated into the center of the petri dish with PDA medium, allowed to be absorbed, and incubated at 25°C for 4 days. Three replicates were performed. The diameter of the colony of the fungus developed in each treatment was measured. The percentage of radial inhibition (% RI) was calculated, which considers the diameter of the fungus without OfAgNPs (D) and the diameter of the fungus exposed to OfAgNPs (d) in the following formula [33]:

$$(\%)\,\mathrm{IR} = (D - d)\,100/D. \tag{7}$$

2.7. Statistical Analysis. The results of the different tests were expressed as mean ± standard error. The difference between treatments was determined by a one-way analysis of variance (ANOVA). When significance was detected, Tukey's multiple comparison test was applied using the Statistic software (v.10.0). Values $p < 0.05$ were considered significant. Before applying ANOVA, a Levene test was performed to verify the homoscedasticity of variances.

3. Results and Discussion

3.1. Characteristics of Biosynthesized Nanoparticles. The biological activity of AgNPs, their functionality, safety, and potential applications are directly related to their physicochemical properties, especially their stability [34]. The Z potential and the hydrodynamic diameter were determined in the different media used for the evaluation of toxicity and antimicrobial activity. The values of potential Z obtained show that the OfAgNPs are negatively charged in the media analyzed and that they are stable in deionized water and phosphate buffer, while they are unstable in iso medium (Table 2). Higher stability of the NPs in the medium indicates longer persistence in colloidal suspension and more bioavailable to biological systems. Thus, the stabilizing agents influence the interaction of NPs with the exposed organism [35]. The Z potential results obtained show that OfAgNPs are more bioavailable in water than in the other media analyzed (Table 2).

On the other hand, the values of the hydrodynamic radius of OfAgNPs found in deionized water, phosphate buffer, and ISO medium (Table 2) exceed the nanometric scale. However, previously, by SEM, it was determined that OfAgNPs have a size of 64.28 ± 11.82 nm [18]. This difference is because the charge, size, and stability of the particles are affected by their dispersion medium [35, 36]. In addition, medium conditions such as pH, ionic strength, and electrolyte composition affect not only the physicochemical properties of AgNPs but also their toxicity [4, 34]. Factors such as the size, shape, composition, and stability of the AgNPs play an important role in their interactions with the environment; they can influence their mobility, dispersion, and surface adsorption capacity, as well as their potential to enter the food chain [4, 31]. The chemical components present in the *O. ficus-indica* fruit peel, such as phenolic compounds, vitamins, and sterols, act as reducing agents during the synthesis process and coat the AgNPs to give them stability [18, 37].

The results of the Z potential and hydrodynamic radius obtained show that the OfAgNPs are less stable and have higher values in the ISO medium than in the other media analyzed (Table 2). As this medium was used to evaluate the activity of OfAgNPS in *D. rerio*, their toxicity could be underestimated for these fish under natural conditions.

3.2. Toxicological Effects of OfAgNPs in D. rerio. FET results in *D. rerio* showed a direct relationship between OfAgNPs concentration, exposure time, and toxicological effects. A decrease in hatching percentage was observed at the highest tested concentrations (Figure 2(a)). *D. rerio* embryos from the control group, plant extract, and those exposed to concentrations of 0.075, 0.15, and 0.3 mg/L OfAgNPs started to hatch at 48 hpf (Figure 2(a)). Hatching in the presence of the plant extract was significantly higher than in the other conditions at 48 hr (Figure 2(a)). This suggests that components of the extract from the peel of the fruit of *O. ficus-indica* used

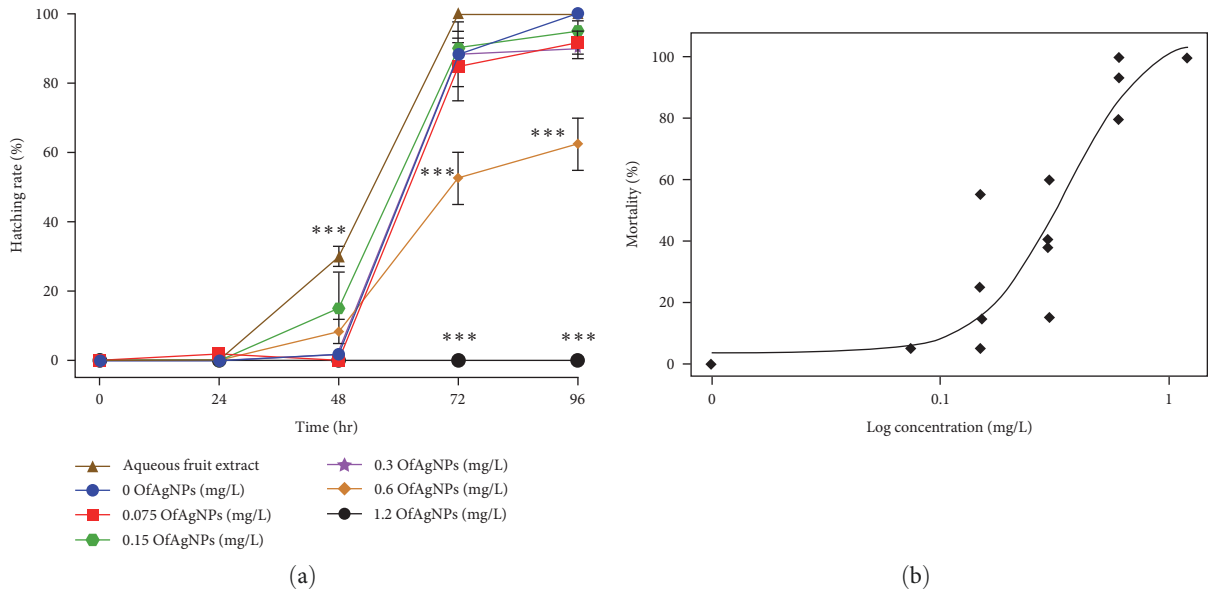

FIGURE 2: Toxicity in *D. rerio* by exposure to OfAgNPs. (a) Hatching of *D. rerio* embryos in different concentrations of OfAgNPs. Average values and the standard error bars are shown. The asterisk (*) indicates a significant difference with respect to the other treatments ($p = 0.00027$) assessed by Tukey's post hoc test. (b) Dose–response curve of OfAgNPs in *D. rerio*, representing embryos dead at 96 hr ($p \leq 0.05$).

in the synthesis of OfAgNPs have beneficial properties for these fish. In all the above conditions, hatching was from 100% to 72 hpf. In all the above conditions, hatching was from 100% to 72 hpf. However, hatching was only 60% at 0.6 and 0% at 1.2 mg/L at 72 hpf. (Figure 2(a)), which shows the toxicity of OfAgNPs only at high concentrations. In the lethality analysis, LC_{50} was determined to be 1.02 mg/L at 24 hr, 0.85 mg/L at 48 hr, 0.63 mg/L at 72, and 0.53 mg/L at 96 hr (Figure 2(b)). As might be expected, the concentration of OfAgNPs required to produce lethality in *D. rerio* is lower as exposure time increases. The lowest value of LC_{50} obtained is higher than that reported for AgNPs synthesized by chemical methods (0.08 mg/L) and biosynthesized by other protocols (0.4 mg/L) [38].

In the analysis of the sublethal effects, the embryos and larvae of both the control group and those exposed to 0.075 mg/L of OfAgNPs showed normal development at all times analyzed (Figure 3(a)–3(c)). Larvae showed poor caudal fin development with 0.15, 0.3, and 0.6 mg/L OfAgNPs (Figure 3(d)–3(f)). All embryos exposed to 1.2 mg/L reported adverse effects: nondevelopment of somites, pericardial edema, and coagulation (Figure 3(g)). Figure 4 shows the percentage of the population analyzed that presented each of the teratogenic effects. AgNPs penetrate *D. rerio* eggs by passive diffusion through pores found in the chorion, NPs accumulate in tissues, and Ag^+ ions are released to cause oxidative stress and an increase in the expression of genes involved in cell apoptosis [39, 40].

3.3. Toxicological Effects of OfAgNPs in A. thaliana. Exposure of *A. thaliana* seeds to OfAgNPs under the conditions evaluated did not affect germination. This has been previously reported with seeds of *Phaseolus vulgaris*, in which only in concentration of 120 mg/L of AgNPs germination was diminished [41]. Possibly, at evaluated concentrations, OfAgNPs

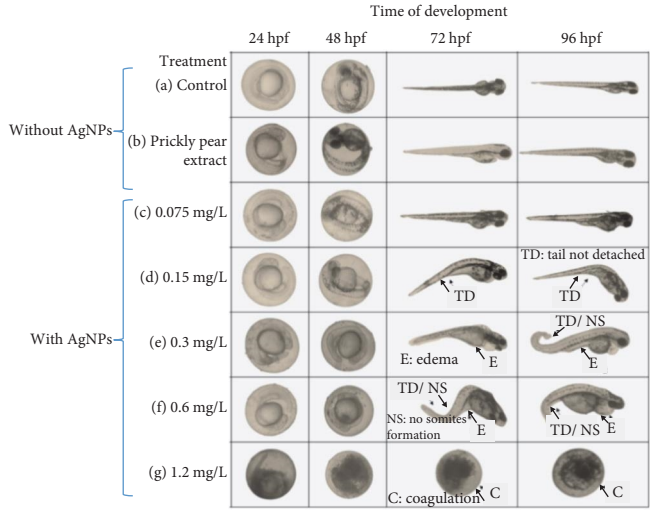

FIGURE 3: Teratogenic effects of OfAgNPs in *D. rerio*. Embryonic and larval development of *D. rerio* in different media: (a) control group, (b) with extract of *O. ficus-indica* fruit peel, (c) with 0.075, (d) 0.15, (e) 0.3, (f) 0.6, and (g) 1.2 mg/L of OfAgNPs. Where TD, tail not detached; NS, no somites formation; E, edema; and C, coagulation.

do not impede the flow of water into the seeds, which is necessary for germination, and neither NPs nor silver ions enter in sufficient amounts to affect it. However, those grown in a medium supplemented with OfAgNPs showed significantly lower growth than those in the control group (Figures 5 and 6(a)). The main root of seedlings grown with 25 mg/L OfAgNPs had an elongation of 50% less than that of the control (Figures 5 and 6(a)). There were also differences in the root morphology of the seedlings since the control seedlings only presented primary roots, while in

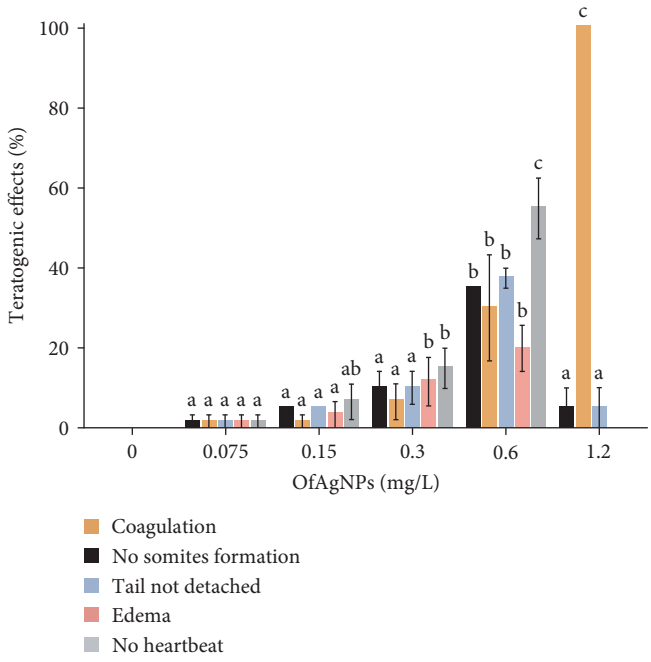

Figure 4: Teratogenic effects of OfAgNPs in *D. rerio*. Effects include coagulation, nonformation of somites, maldevelopment of the caudal fin, edema, and absence of heartbeat. Bars represent the mean values ± standard error. Different letters indicate significant differences between effects ($p = 0.0045$) after Tukey's pairwise comparisons.

those exposed to the OfAgNPs showed secondary roots (Figure 5).

The content of chlorophylls and carotenoids in *A. thaliana* seedlings was also evaluated, as these pigments are biomarkers that show the physiological state of a plant [42]. Significantly lower chlorophyll content was observed in seedlings exposed to 6.25, 12.5, and 25 mg/L of OfAgNPs than in the control group (Figure 6(b)). The carotenoid content found in all groups exposed to OfAgNPs was significantly lower than that of the control group too (Figure 6(b)). AgNP can accumulate in the leaves and alter the structure of the thylakoid, decreasing the chlorophyll content, which causes less photosynthesis and an affectation in the development of the plant [43]. AgNPs produce oxidative stress and lipid peroxidation damage in chloroplast membranes [43]. However, at concentrations of 50 and 100 mg/L, there was a higher chlorophyll concentration, although in neither case was it like that found in the control (Figure 6(b)). In other works, using *Stevia rebaudiana*, increases in chlorophyll, carotenoids, and biomass have been observed [44]. It has been suggested that biosynthesized AgNPs may contain nitrogen and magnesium, which are elements associated with chlorophyll synthesis [45].

3.4. OfAgNPs Antibacterial Activity. In previous work, the activity of OfAgNPs was evaluated in water from the effluent of a treatment plant, and the antimicrobial activity of OfAgNPs significantly higher than that obtained with chlorination was observed [18]. In that work, the activity against some isolated bacteria from the treatment plant was demonstrated [18]. In the present work, the activity of OfAgNPs against bacteria of recognized identity was analyzed. OfAgNPs showed activity against *P. aeruginosa*, *E. coli*, and *S. aureus* at concentrations of 0.25, 0.5, and 1 mg/L (Figure 7). OfAgNPs at concentrations of 0.5 and 1 mg/L showed an inhibition of *E. coli* of 11 U log after 10 min of exposure, while at 0.25 mg/L, the same inhibition was achieved until 60 min (Figure 7(a)). The activity of AgNPs against *E. coli*, such as those synthesized from extracts of *Cestrum nocturnum* [45] or *Acacia cyanophylla* [46], has been reported. At concentrations of 0.5 and 1 mg/L of OfAgNPs, an inhibition of *P. aeruginosa* of 9 U log was observed at 10 min of exposure, while at the concentration of 0.25 mg/L, the same inhibition was achieved until 45 min (Figure 7(b)). This antibacterial effect has been observed even against antibiotic-resistant *P. aeuroginosa*, where AgNPs could enter bacteria, affect their morphology and structure, and induce oxidative stress and cell apoptosis [47]. AgNPs cause damage to both the cell wall and the cell membrane of *P. aeruginosa*, so a loss of cellular components occurs [47]. Also, Ag^+ ions and AgNPs generate oxidative stress that cannot be adequately counteracted because, in addition, AgNPs inactivate antioxidant enzymes (catalase, superoxide dismutase, peroxidase) [47]. In *S. aureus*, an inhibition of 11 U log was observed with 1 mg/L of the OfAgNPs after 5 min of exposure, while at concentrations of 0.25 and 0.5 mg/L, the same inhibition was observed until 30 min (Figure 7(c)). The bactericidal activity of AgNPs against *S. aureus* is mainly caused by those of silver ions from AgNPs [45]. Ag^+ inhibits bacterial DNA replication, they cause degradation of bacterial cytoplasmic membranes, and alter intracellular levels of adenosine-5′-triphosphate (ATP) [48]. A greater sensitivity of *E. coli* than *S. aureus* to the activity of OfAgNPs was observed at 0.5 mg/L (Figure 7). This may be because *E. coli* is Gram-negative bacteria, and *S. aureus* is Gram-positive [49], and there are differences in their cell wall structure, which could facilitate or hinder the access of AgNPs to cells. The cell wall of Gram-negative bacteria consists of a thin peptidoglycan layer and a lipopolysaccharide membrane, whereas the wall of Gram-positive bacteria is a thick peptidoglycan layer [10]. However, the bactericidal mechanisms of action of AgNPs could be similar in both types of bacteria; they release Ag^+ that generates free radicals and the inactivation of proteins in the cell by silver ions [49]. In addition to the type of bacteria, antibacterial activity depends on pH, temperature, and biosynthesis protocol [49].

3.5. OfAgNPs Antifungal Activity. OfAgNPs showed antifungal activity by inhibiting the radial expansion of the fungi *Colletotrichum* sp., *Cladosporium* sp., and *Aspergillus* sp. (Figure 8, Table 3). A 100% IR was observed with 0.25 mg/L of OfAgNPs for *Colletotrichum* sp. and *Cladosporium* sp., as well as with 0.5 mg/L for *Aspergillus* sp. The results indicate that *Aspergillus* sp. is slightly more resistant than the other evaluated fungi. The antifungal activity has been

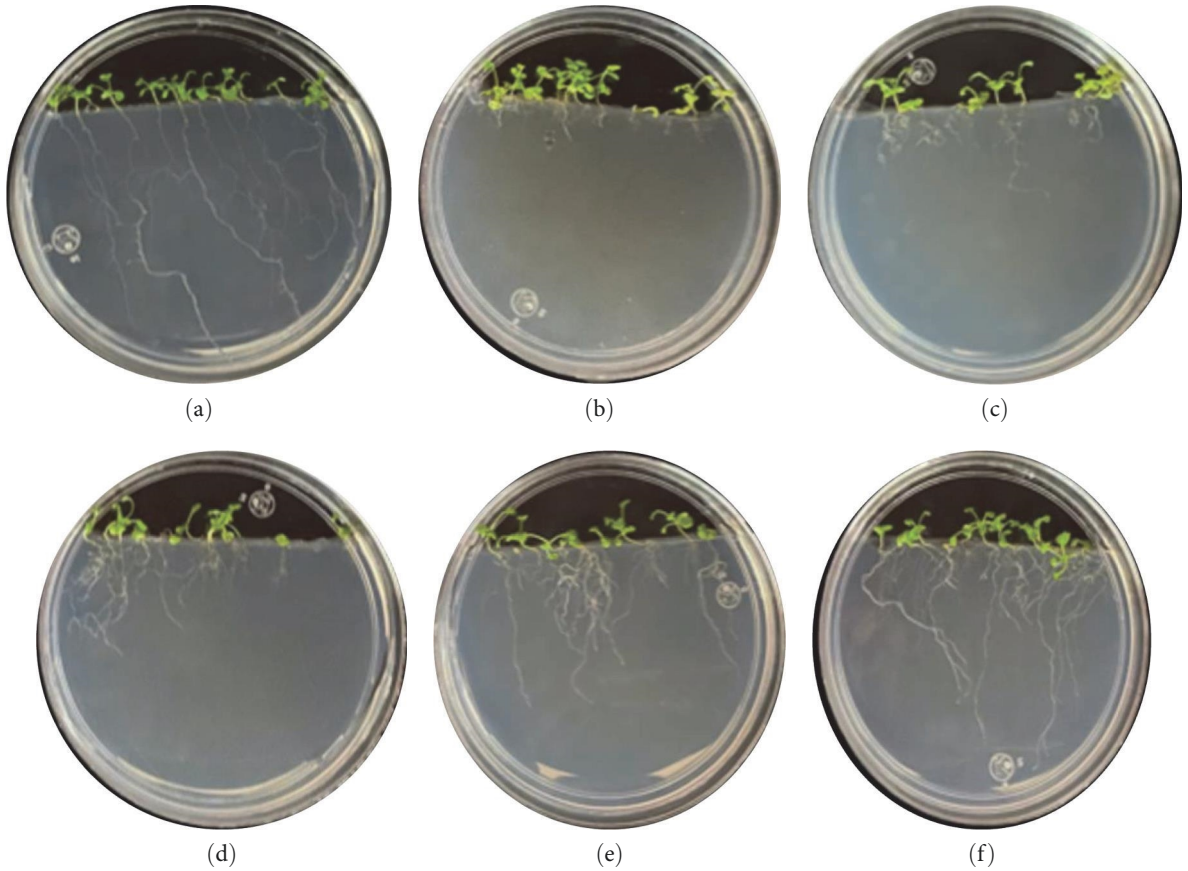

Figure 5: Growth of *A. thaliana* in MS medium supplemented with different concentrations of OfAgNPs: (a) Control, (b) 100, (c) 50, (d) 25, (e) 12.5, and (f) 6.25 mg/L.

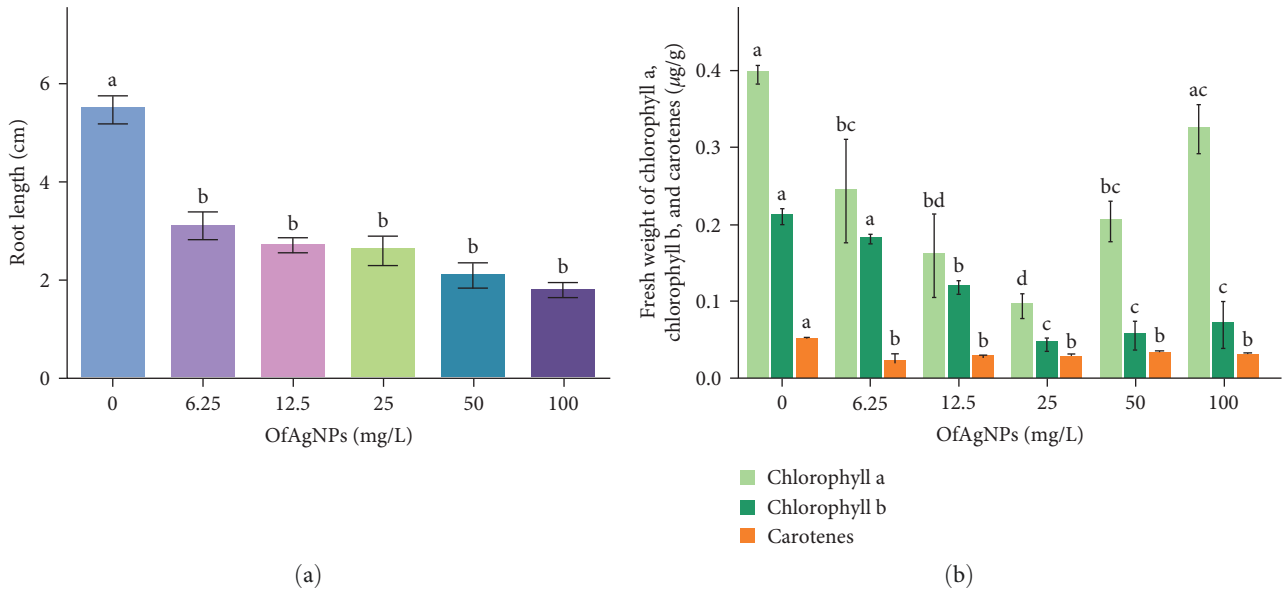

Figure 6: Growth and pigment content in *A. thaliana* exposed to OfAgNPs. (a) Elongation of the main root. (b) Chlorophyll and carotenoid content. The bars represent the mean ± the standard error.

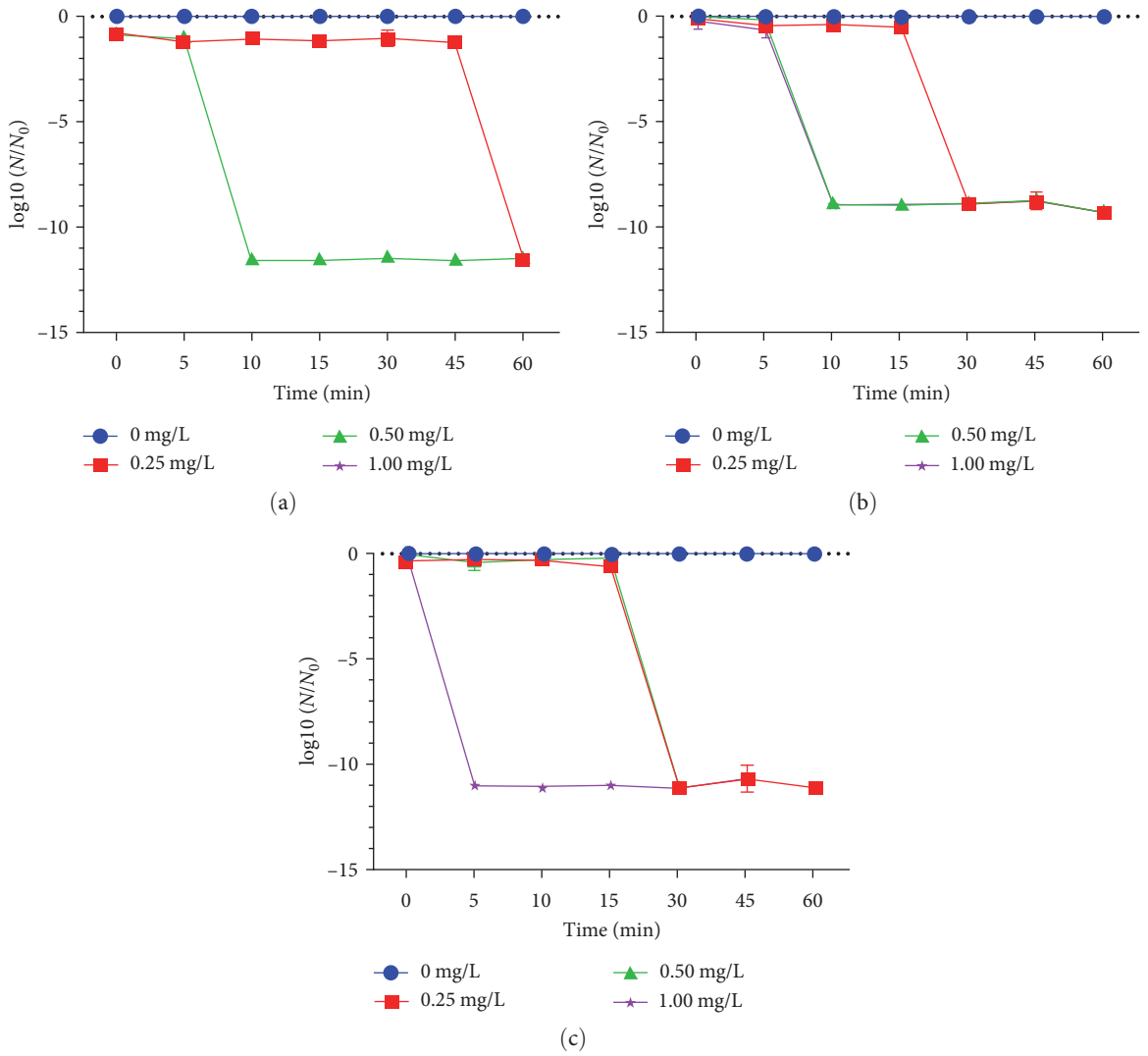

Figure 7: Logarithmic inactivation of microorganisms with different concentrations of OfAgNPs. (a) *E. coli* DH5α, (b) *P. aeruginosa* ATCC 27853, and (c) *S. aureus* ATCC 29213. Each point on the graphs represents the mean ± standard error.

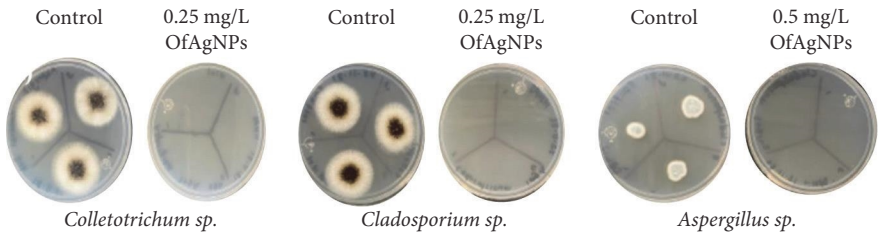

Figure 8: Antifungal activity of OfAgNPs. Radial inhibition was observed at 24 hr exposure to OfAgNPs.

Table 3: Percentage of radial inhibition of fungi exposed to OfAgNPs.

	OfAgNPs concentration		
Fungus species	0.25 mg/L	0.5 mg/L	1 mg/L
Colletotrichum sp.	100	100	100
Cladosporium sp.	100	100	100
Aspergillus sp.	71.43	100	100

related to the action of Ag^+ ions that can bind to mycelial proteins or enzymes, damaging cell structures, generating reactive oxygen species, or interfering with fungal cellular pathways [50]. AgNPs increase cell membrane permeability, causing protein and sugar leakage; they also inhibit cellular respiration, which stops mycelial growth and sporulation [51, 52].

4. Conclusions

The current study involved nanoparticles synthesized from the waste product of *O. ficus-indica* fruit peel (OfAgNPs). We observed that OfAgNPs produce toxicological effects on *D. rerio* and *A. thaliana* only at exposure times longer and/or higher concentrations than those necessary to achieve antibacterial activity. It was observed in *D. rerio*: (1) a reduction in embryonic hatching, (2) an alteration of the development of the caudal fin of the larvae, and (3) a value of LC_{50} of 0.53 mg/L at 96 hr of exposure. In the culture of *A. thaliana* in a medium supplemented with OfAgNPs, it was found that (1) germination was not affected yet at the maximum concentration evaluated, but (2) the plants showed a smaller size and content of photosynthetic pigment from the lowest concentration evaluated after 3 weeks of incubation. OfAgNPs produced an inhibition against *P. aeruginosa* and *E. coli* after 10 min of exposure and against *S. aureus* after 30 min. OfAgNPs can be used as antimicrobials, but dispersal in the environment should be avoided as they can cause adverse effects in animals and plants.

Data Availability

The data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

The authors are thankfull to the Consejo Nacional de Ciencia y Tecnología (CONACYT) and the Department of Chemistry of the Autonomous University of Aguascalientes; Dra. Gabriela Beatriz Alvarado Suárez and M. C. Daniel Alejandro Díaz García, from the Autonomous University of Aguascalientes; Antor Pear company for characterizing the nanoparticles (hydrodynamic diameter and Z potential) with the Litesizer Series equipment. This project was financially supported by the Autonomous University of Aguascalientes through grant number PBIT21-4.

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