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Research Article

Green Synthesis of Silver Nanoparticles from *Abronia villosa* as an Alternative to Control of Pathogenic Microorganisms

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Abstract: The aim of this study was to evaluate the antibacterial and antifungal activities of eco-friendly synthesized silver nanoparticles. The silver nanoparticles were synthesized by biological method using aqueous extract of *Abronia villosa*. Synthesis of silver nanoparticles was confirmed by color change and characterized using UV-visible spectroscopy, scanning electron microscope (SEM), energy dispersive X-ray spectroscopy (EDX), dynamic light scattering (DLS), and zeta potential analysis. The SEM analysis revealed the presence of spherical silver nanoparticles of the size range 21 to 33 nm. Synthesized silver nanoparticles were used to evaluate their antibacterial effects at different concentrations (25, 50, 75 and 100 µg/ml) on gram negative and gram positive bacteria. The biggest halo zone was observed at 75 and 100 µg/ml concentrations of silver nanoparticles against both gram positive and gram negative bacteria. Antifungal activity of biosynthesized silver nanoparticles was evaluated against seven different phytopathogenic fungi. AgNPs showed high inhibition of radial growth toward all tested fungi. The highest inhibition of fungal growth by AgNPs was recorded against *Macrophomina phaseolina* (86.06 ± 0.92%). Biosynthesized AgNPs using plant extract are a promising to use safety for various biomedical and agricultural applications.

Keywords: Antibacterial activity; antifungal activity; green synthesis

1 Introduction

Nowadays, nanotechnology is one of the most promising researches rapidly developing in many fields such as medicine, industry, agriculture and others. The rapid emergence of drug-resistance to available antibiotics as a result of extensively use of antibiotics led to look for natural sources of effective drugs or development of antimicrobial compounds and alternative processes such as nanoparticles [1]. The nanotechnology considered as particle with at least one dimension less than 100 nm. Recently, nanotechnology is growing rapidly on various industries and biomedical fields due to large surface area to volume ratio and their unique physical, chemical and biological properties as compared to the bulk material [1]. Among the various metal nanoparticles there is more interest in silver nanoparticles (AgNPs) due to it is a nontoxic, safe

inorganic and widespread applicable as antibacterial and antifungal agents [2], in addition to their unique properties such as anti-viral, anti-inflammatory, chemical stability, good conductivity and catalytic [3]. Colloidal silver has special features such as chemical stability, good conductivity, catalytic and an antibacterial activity which enables silver nanoparticles to play the major role in the nanotechnology and nanomedicine fields [4]. Antimicrobial properties of (AgNPs) against pathogenic fungi and bacteria have been reported previously by many researchers [1, 2, 5]. The application nanoparticles (NPs) in the field of plant pathology were focused on disease control. AgNPs can eliminates microorganisms through various mechanisms of action such as change in permeability of cell wall, formation of pores, inhibition of respiration process and their accumulation on the membrane of microorganisms. As well as inhibition of DNA replication, cell division and cellular respiration which lead to cell death in the finally [6]. There are many traditionally approaches for nanoparticles synthesis such as physical and chemical processes. Chemical methods are not suitable for usage in health-related areas due to the presence of hazardous chemicals on their surface which causing negative effects on the environment and human health. As that physical method for synthesis of nanoparticles are expensive and need to high energy [7]. Therefore, there is a strong demand for developing of alternative procedures much safer and eco-friendlier for synthesis of nanoparticles to reduce the toxicity and harmful effects of chemical methods. The use plant extracts, bacteria and fungi for preparation of nanomaterial is an eco-friendly, safety, easily, simplicity, rapidly and cheaply approaches [8]. By comparison with other biosynthesis methods of nanoparticles the extracts of plant have various advantages and human application due to it eliminates the elaborate process of maintaining cell cultures and suitable for large-scale synthesis [9]. It is well known, nanoparticles biosynthesis is an extremely complicated process in which they usually starts with the reduction of Ag^{+} ions to Ag^{0} in the first step. The reduced silver ions start to grow through the self-assembly of atoms to new nuclei, which propagate into nano-size molecules. Finally, the capping agent is broadly used on the grown molecules for nanoparticles stabilization process [10]. The plant extract contain various active compounds such as antioxidant biomolecules and metabolites can act as reducing and capping agents for nanoparticles synthesis and to avoid the agglomeration of nanoparticles. [11]. Recently, plant extracts are one of the most promising natural sources because they containing many antioxidant and antimicrobial compounds, which used as reducing and stabilizing agents for synthesis of nanoparticles [12]. In this sense, *Abronia villosa* is one of the well-studied plants, that has a positive effect against degenerative diseases [13]. *Abronia villosa* is a member of Nyctaginaceae family and it is well known with other names like; hairy sand-verbena. This plant is originally from southwestern United States and northern Mexico and it is usually grown in southern California and Baja coast between February and May [14].

In the present study, we report the synthesis of AgNPs by an environmentally friendly procedure involving the in situ reduction of Ag by *Abronia villosa* extracts and the evaluation of their antimicrobial activity against different bacterial and fungal strains.

2 Experimental

2.1 Materials

The interested plant was collected from the available sites in Mexicali valley, Mexico. The aerial parts of the plant material washed thoroughly with tap water to remove contamination and surface sterilized with double distilled water and air dried at room temperature. The plant material cut into very small pieces and about 30 g were boiled in 300 mL distilled water for 20 min. The solid residues were removed by centrifugation at 4000 rpm/10 min. The crude aqueous extract was filtered through Whatman filter paper No.1. The obtained clear extract was stored at 4°C for AgNPs preparation. For AgNPs synthesis, solution of silver nitrate (10 mM) was prepared and mixed with aqueous extract of *Abronia villosa* at a ratio of 8:2. The pH of the mixture was of 3 and the solution was incubated at 40 ± 2°C with stirred continuously (160 rpm) for 30 min. The reduction process Ag^{+} to Ag^{0} nanoparticles was followed by the color change of the solution pale yellow to brown, which indicates the formation of AgNPs [15]. The purification process of the silver nanoparticles starts with the centrifugation of the colloidal solution for 10 min at 10000 rpm (Thermo Scientific Sorvall Legend Micro17 with dual Row 24×1.5/2.0 ml Rotor). The pellet,

which contains the nanoparticles were washed with sterile distilled water to remove any impurities. The washed pellet was freeze-dried to get the dried powder that was used for further studies [15].

2.2 Characterization of Biosynthesized Silver Nanoparticles

The synthesized silver nanoparticles (AgNPs) in colloidal solution were primarily monitored by UV-visible spectrophotometric analysis (DR6000TM UV VIS Spectrophotometer, USA) in the wavelength range of 300-700 nm. Silver nitrate (10 mM) was used to adjust the baseline as a blank.

2.3 Scanning Electron Microscopy (SEM) and Energy Dispersive Spectroscopy (EDS)

The colloids containing silver nanoparticles were centrifuged at 10,000 rpm for 10 min and the precipitate obtained was completely dried at room temperature and analyzed by scanning electron microscopy (SEM) (JEOL 6010L; JEOL, Tokyo, Japan) equipped with energy dispersive spectroscopy (EDS) at 10 kV to determine elemental composition of the particles [15].

2.4 Zeta Potential and Dynamic Light Scattering (DLS)

The colloidal solution of synthesized AgNPs was sampled in dynamic light scattering (DLS) cuvettes and examined for size distribution and zeta potential by Nanotrac wave instrument particle size analyzer (Microtrac, USA) [6]. The particle diameters were assessed at 25°C at a scattering angle of 90° in the range of 0.1-1000μm.

2.5 Antibacterial Analysis

The disc diffusion technique was used to evaluate antibacterial activity of biosynthesized AgNPs and plant extract against 3 bacterial strains including *Bacillus cereus*, *Klebsiella pneumoniae* and *Enterobacter aerogenes*. The bacterial strains were cultivated separately in flasks containing Nutrient broth media (NB) at 37°C with shaking at 120 rpm, and diluted to 10^7 CFU/mL based on the OD at 600 nm after overnight of incubation. 1 ml of each bacterial culture was added separately to sterile Nutrient agar media (previously cooled to 45°C) and well homogenized in the media then poured into the Petri dishes. Sterile paper discs with 6 mm diameter were placed on the agar plates and allowed to dry. To evaluate the antibacterial activities 20 μL of synthesized AgNPs with different concentrations, 25, 50, 75 and 100 μg/mL. Plant extract, sterile distilled water (as negative control) and $AgNO_3$ (10 mM) were aggregated separately onto sterile paper discs in triplicate. All plates were incubated at 37°C for 24 hrs. The result was recorded as the average diameter (mm) of the zone of inhibition around the discs.

2.6 In Vitro Inhibitory Effect of Synthesized AgNPs against Phytopathogenic Fungi

Antifungal activity of the synthesized AgNPs was evaluated by poisoned food method proposed by Mishra [16] against seven different phytopathogenic fungi (*Macrophomina phaseolina*, *Botrytis cinerea*, *Alternaria alternate*, *Colletotrichum gloeosporioides*, *Fusarium solani*, *Fusarium equiseti* and *Fusarium oxysporum*). The PDA media was poured into petri dishes and allowed to solidify. PDA media was divided into two halves in the same petri dish then the PDA was removed from one of them and replaced with PDA containing 25% colloidal solution of 25 μg/mL AgNPs. Five day-old mycelial discs (6 mm) of each tested fungus were placed in the center of the plate containing AgNPs, individually. All plates were incubated at 28°C for 10 days. The fungal growth in both directions (PDA containing AgNPs and PDA alone) of petri dish was observed and expressed in increase of colony radius in mm and the inhibition percentage of fungal growth was calculated by the following equation:

$$\% \text{ inhibition} = \left(\frac{\text{control radius} - \text{treatment radius}}{\text{control radius}} \right) \times 100$$

2.7 Statistical Analysis

SPSS version 21 software (Statistical Package for the Social Sciences, Inc., Chicago, USA) was used for statistical analysis and the results compared using a one-way ANOVA and a Tukey test. All tests were performed at a confidence level of 95%.

3 Results and Discussion

3.1 UV-VIS Spectrometry

Biosynthesized silver nanoparticles using plant extract of *Abronia villosa* was visually observed by color change from light yellow to reddish brown within 30 minutes after the addition of leaf extract to silver nitrate (10 mM) solution (Fig. 1a). Previous studies noted that *Abronia villosa* contains bio-molecules such as abronione, boeravinone, triterpene lupeol and rotenoids [13]. Thus, the reduction of silver Ag^{+} into silver nanoparticles Ag^{0} via the active molecules present in the plant extract formation could be due to presence one or more of this bioactive compounds in the aqueous extract [17, 18]. In this regard, previous study reported that the presence of many of metabolites and reductive biomolecules such as terpenoids, ketones, carbohydrates, proteins, amides, carboxylic acids, and vitamins are responsible for the reduction of metal ions [1]. As shown in Fig. 1b, a characteristic and well-defined surface plasmon resonance band for silver nanoparticles was obtained at around λ 450 nm indicating that the synthesis of silver nanoparticles was realized without problem [19].

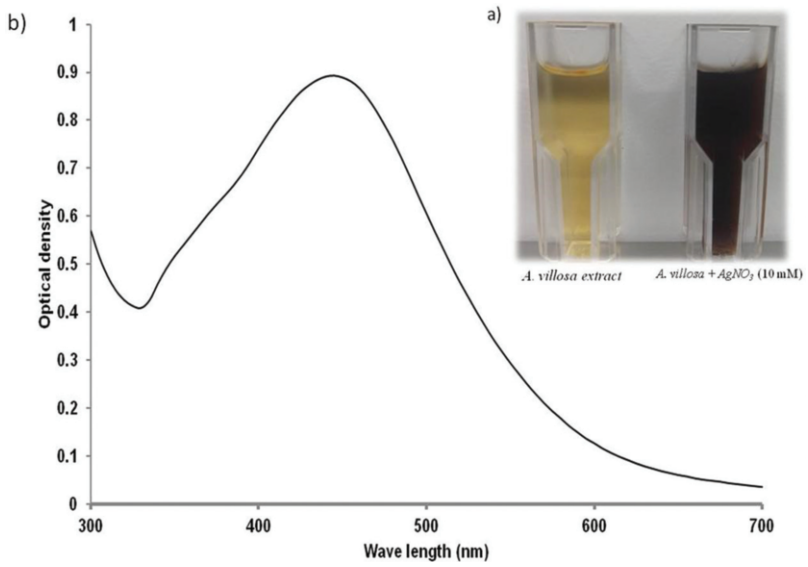

Figure 1: Synthesis of AgNPs from reaction between $AgNO_3$ and *Abronia villosa* extract (a) and UV–Vis spectra of AgNPs synthesized using *A. villosa* extract (b)

3.2 SEM and EDS

As shown in Fig. 2a, the elemental compositional analysis using EDS of AgNPs revealed the presence of carbon (56.37%), chloride (5.90%) and silver (37.73%). The elemental profile of synthesized AgNPs show higher counts at 3 keV due to silver, confirming the formation of silver nanoparticles [20]. On the other hand, the image obtained by SEM revealed that synthesized silver nanoparticles showed a nanoparticle size between 21 and 33 nm with spherical morphology (Fig. 2b).

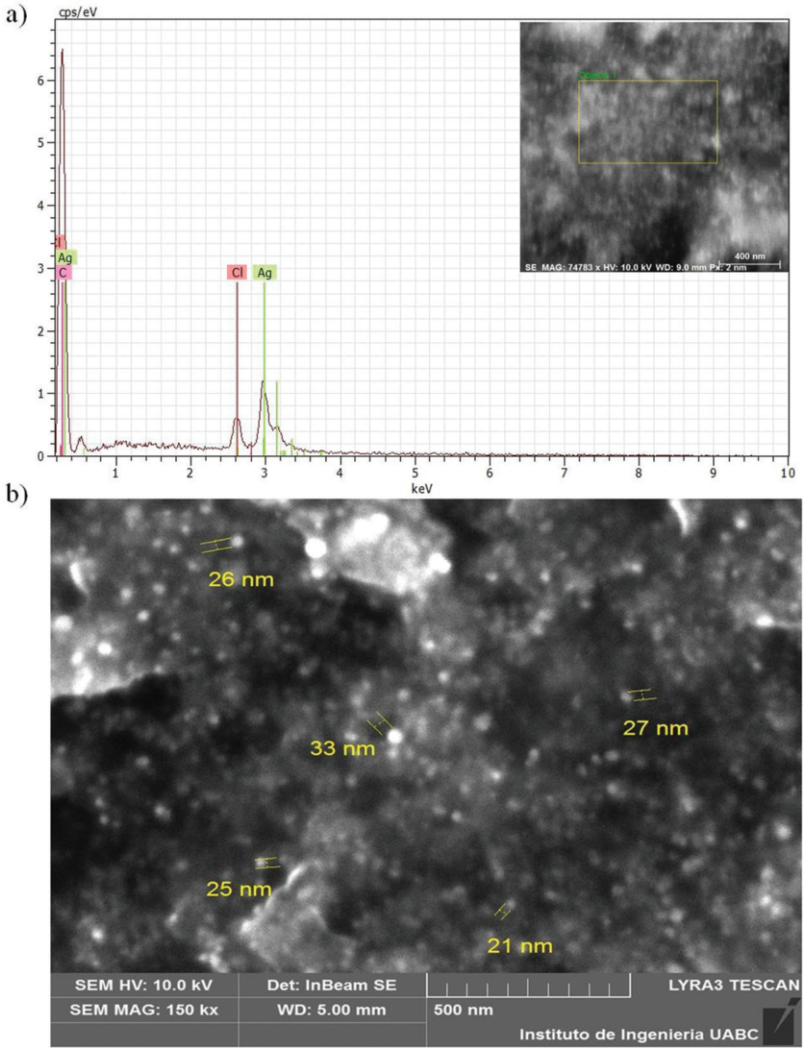

Figure 2: Energy dispersive X-ray spectrometer (a) and scanning electron microscopy (b) image of silver nanoparticles produced from *A. villosa*

3.3 DLS and Zeta Potential

For particle size distribution analysis, the Dynamic light scattering (DLS) was used. DLS and zeta potential were used to determine the mean nanoparticle size (hydrodynamic diameter) and existing surface charge on the nanoparticles surface, which associated to their stability. Fig. 3 shows the DLS analysis of *Abronia villosa* extract-mediated synthesis of AgNPs; the average size (1.28 nm) and is very similar to reported by Tuoriniemi et al. [21] who observed that hydrodynamic diameter determined by DLS were smaller than the SEM diameter. This can probably be attributed to the fact that the SEM image represents only a very small fraction of the sample [22]. Therefore our results from SEM can be more reliable than DLS values. In this way, Eaton et al. [23] found that in general scanning electron microscopy (SEM) is suitable for to metallic particles (above 20 nm in diameter), while DLS reveals details about the particles' solution dynamics, but is inappropriate for polydisperse samples, or mixtures of differently sized samples. Another hand, Cotreras-Trigo et al. [24] mention that particles size values might be affected by the pH in the medium. According this author the particle size values are lower for an acidic medium and higher for a basic medium. Similar results have been reported previously by

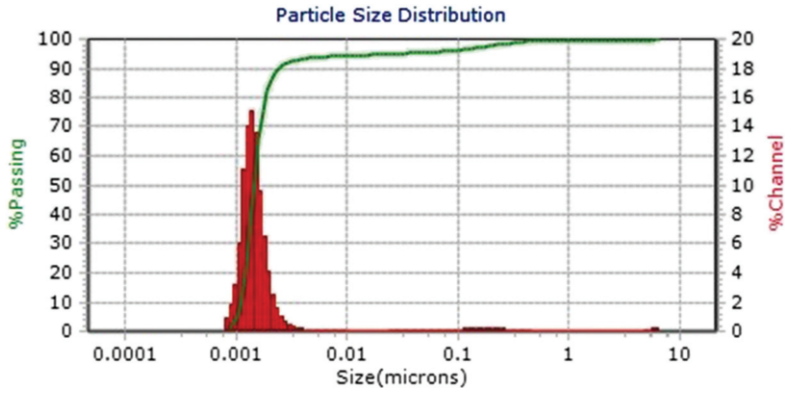

Figure 3: Particle size distribution of silver nanoparticles from dynamic light scattering measurements

Traiwatcharanon et al. [25] who reported that the size of Ag-NPs in acidic medium (pH 4) was lower than basic medium (pH 8). In this study, the zeta potential value of AgNPs was -66.9 mV; where high negative zeta potential value of the compound is an indication of the stability of the AgNPs [20,26]. The synthesized AgNPs can be considered stable with a less tendency for aggregation due to the high negative value of zeta potential. This high negative value can be attributed to functional groups (from *Abronia villosa*) distribution on the surface of nanoparticles which carry sufficient surface charge to be electrostatically stabilized and resistant to spontaneous aggregation [27].

3.4 Antibacterial Activities of Synthesized AgNPs

Antibacterial activity of synthesized AgNPs at various concentrations was evaluated using disc diffusion method against both gram positive and gram negative bacteria. The growth inhibition of bacterial cells was measured as the clear area around the discs after 24 h of growth. The highest inhibition zone was observed at 100 µg/ml compared with other concentrations of synthesized AgNPs and $AgNO_3$ (10 mM) against all tested bacteria. Generally, as the concentration of AgNPs increases, the inhibition zone around the discs increases too, which indicates that a positive relationship between the concentration of nanoparticles and the rate of cell inhibition (Fig. 4a). At most concentrations of AgNPs, the inhibitory effect of silver nanoparticles was more

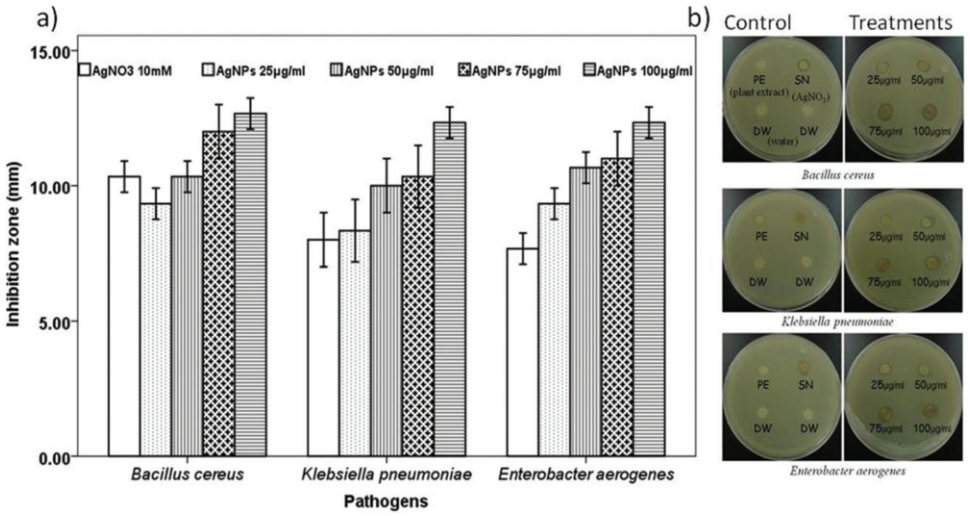

Figure 4: Antimicrobial activity of AgNPs from *Abronia villosa* (a); inhibition of microorganisms with with *A. villosa* extract, $AgNO_3$, water and different doses of Ag-NPs (b)

active on gram positive bacteria than gram negative bacteria. Our data reveals that the Gram-positive bacteria are more sensitive than Gram-negative bacteria. Similar results were reported by Anandalakshmi and Venugobal [28], who indicated that the AgNPs can cause the alteration of the bacterial membrane permeability resulting in cell death. These results are in conflict with those obtained by Rautela et al. [29], who reported that, the *E. coli* which classified as Gram-negative bacteria exhibit a larger inhibition zone than that of the Gram-positive bacteria (*B. cereus* and *S. aureus*) at the same concentration of the used nanoparticles. On the other hand, Escárcega-Gonzalez et al. [12] investigated antibacterial activity of AgNPs using *Acacia rigidula*, against Gram-negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*, and a multidrug-resistant *P. aeruginosa* strain) and Gram-positive bacteria (*Bacillus subtilis*) and their results showed that the AgNPs exhibit a strong antimicrobial activity against both Gram-positive and Gram-negative bacteria. Although, the exact mechanisms of antibacterial activity of green silver nanoparticles is not completely clear; previous studies demonstrated that the antibacterial activity of Ag-NPs may be due to their size and specific surface area which allows efficient binding with the bacterial surface (smaller particles show the better antibacterial activities) [30]. On the other hand, among all tested bacteria, *Klebsiella pneumoniae* was inhibited by the plant extract (Fig. 4b).

3.5 Antifungal Activities of Abronia villosa Synthesized Silver Nanoparticles

Figure 5 showed the results of growth inhibition (a) and antifungal activity (b) of synthesized AgNPs from plant extract against all tested fungi. The maximum inhibition of fungal growth was (86.06 ±

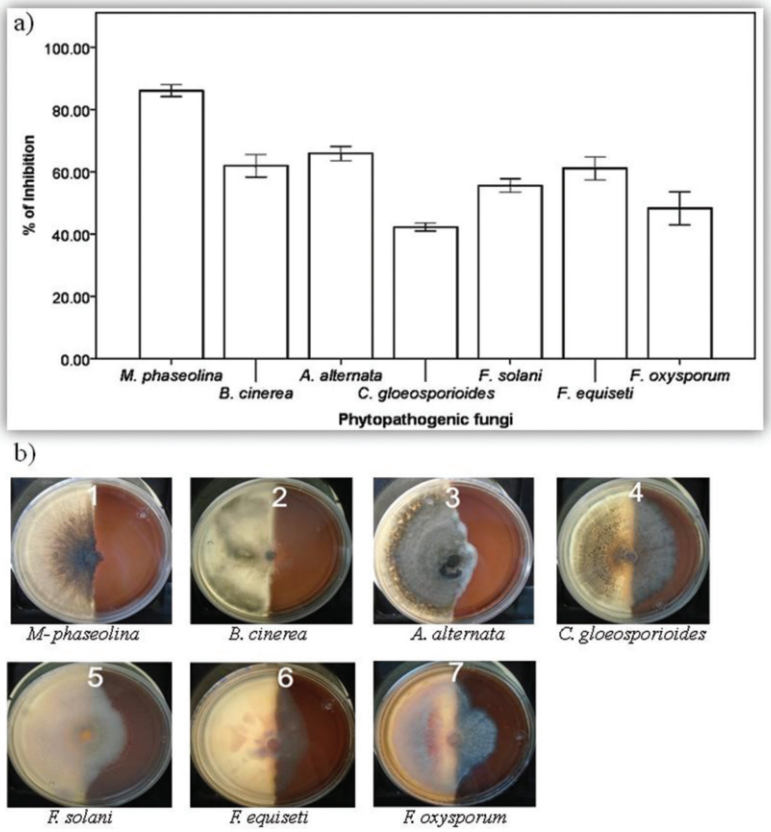

Figure 5: Mycelial growth inhibition by AgNP (25 µg/mL) from *Abronia villosa* (a) and antifungal activity of AgNPs in the growth of phytopathogenic fungi (b)

0.92%) which recorded against *M. phaseolina* compared with others ($p < 0.001$), followed by *A. alternate* (65.86 ± 1.15%). On the other hand, the minimum inhibition of radial growth was observed against *C. gloeosporioides* (42.26 ± 0.66%) ($p < 0.001$). Similar results were reported by Ruiz-Romero et al. [15] and Bernardo-Mazariegos [16] who observed that silver nanoparticles obtain by green synthesis, against different phytopathogenic fungi caused damage on fungal hyphae and conidia. Previous studies also showed that *in vitro* evaluation of silver nanoparticles had antimicrobial effects (inhibition of colony formation of spores) against different plant pathogenic fungi [31,32]. The exact mechanisms of AgNPs in the inhibition of phytopathogenic fungi are not yet fully explored. However, recent studies showed that specific physicochemical properties of nanoparticles such as size, zeta potential and shape are important factor in the inhibition of phytopathogenic fungi [11,26]. In this sense, previous studies showed that nanoparticles of smaller sizes had in general a more pronounced influence on the inhibition of growth and production of mycotoxin production of *Penicillium verrucosum* in comparison to particles that exhibiting larger sizes [33].

4 Conclusion

In the present study our results confirmed the ability of *A. villosa* extract as a reducing agent for the green synthesis of silver nanoparticles. Moreover, biological activity assessment of AgNPs showed their appreciable antimicrobial activity against bacterial and fungal pathogens strains tested, especially *Bacillus cereus*, *Enterobacter aerogenes*, *Macrophomina phaseolina* and *Alternaria alternate*. Further studies about the effect of physical parameter such as pH, temperature on the green synthesis and antimicrobial activities of silver nanoparticles from *A. villosa* are necessary for their utilization to treat infections caused by fungal pathogens and multidrug resistant bacteria.

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Conflicts of Interest: The authors declare that they have no conflicts of interest to report regarding the present study.

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