

Research Article

Biosynthesis of silver nanoparticles by using *Fusarium oxysporum* and their therapeutic applications

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Abstract

Biological method has evolved to become an important field of nano-biotechnology due to its harmless nature, to be fast and cost-effective. Silver nanoparticles (SNPs) have been the research topic for their unique properties such as diagnosing, treating, and preventing various diseases in all aspects of human life. This work aimed to establish an extracellular and intracellular synthesis of SNPs from *Fusarium oxysporum*, focusing on evaluating their technological potential. The SNPs thus synthesized were visually characterized by the change of colour then confirmed by Ultra-violet (UV) Visible spectroscopy. The evaluation of the antimicrobial activity against *Proteus mirabilis*; *Streptococcus bovis*; *Staphylococcus epidermidis*; *S. aureus*; *Salmonella typhi*; *Escherichia coli* and *Candida albicans* showed a very effective inhibitory with 18 ± 0.66 mm as a highest value. The antioxidant activity was tested using the DPPH method, and the synthesized nanoparticles recorded a remarkable percentage of free radical scavenges at $82,12 \pm 0,42\%$, $70,46 \pm 1,53\%$ and $72,65 \pm 1,33\%$ for aqueous fungal extract, cell filtrate and biomass, respectively. The ability of the SNPs to detect hydrogen peroxide was illustrated by discoloration of the synthetic mixture, then confirmed by decreasing towards the disappearance of the characteristic peak. Finally, the photocatalytic performance was studied by the degradation of methylene blue. This activity showed a very interesting decrease in the peak intensity characteristic of this dye. In conclusion, synthesizing SNPs using *F. oxysporum* has proved their important technological property for the biological activities investigated.

Keywords: AgNPs, Biological activities, Fungus, Green synthesis, Nanobiotechnology

INTRODUCTION

Nanotechnology is an area of the most promising leading science in modern key technology development (Chen *et al.*, 2019), involving synthesis and applications of nanomaterials with significant applications in various fields. Bio-nanotechnology is concerned in particular biological machines and the application of biological building blocks to solve engineering challenges and

create new areas of technological development. Learning about the structure and function of the inner workings of biological systems such as cells, bacteria and viruses has improved existing nanotechnology applications and developed entirely new applications (Agrawal *et al.*, 2022).

One of the most important aspects of nanotechnology is the synthesis of nanoparticles (NPs) from different metals (silver, gold, copper, zinc, etc.) at the nanoscale

level (ranging from 1 to 100nm). Nanoparticles can be synthesized using various methods of traditional physical and chemical processes, such as vapour condensation, sol-gel process, and solvent evaporation process. However, they are characterized by low stability; they are toxic and can present risks to health and the environment. These methods are considered unfavourable due to high capital cost, high energy requirements and involve the use of toxic and hazardous chemicals (Li *et al.*, 2011). Recently, biogenic synthesis of nanoparticles has emerged as an alternative to conventional method, using organisms such as bacteria, fungi, algae and plants (Mohd Yusof *et al.*, 2019) (Guilger-Casagrande & Lima, 2019). These methods are cheap, non-toxic and eco-friendly (Mohd Yusof *et al.*, 2019). The microbes act as a tiny nano-factory in reducing the metal ions into metal NPs with the involvement of enzymes and other biomolecules compounds secreted by the microbes (Mohd Yusof *et al.*, 2019).

Out of diverse microorganisms used, it was found that fungi have excellent potential for the secretion of many metabolites as proteins and enzymes, which can be used as reducing and stabilizing agents for synthesis of various metal nanoparticles of different shapes and size (Guilger-Casagrande & Lima, 2019) (Rai *et al.*, 2021). Among the different types of metallic nanoparticles, silver nanoparticles (AgNPs) are extensively applied in various fields such as pharmaceuticals, food, agriculture, textile industries, water treatment, and antimicrobial, anti-cancer.

The present study suggests an eco-friendly method for intracellular and extracellular synthesis of silver nanoparticles using *Fusarium oxysporum*. The synthesized nanoparticles were checked by visual observation as well as by measuring its UV-visible absorption and were tested for their potential application as an antimicrobial agent, antioxidant ability, detection of H₂O₂ and photocatalytic performance.

MATERIALS AND METHODS

Microorganisms

The fungus *Fusarium oxysporum* was obtained from the Applied Microbiology Laboratory of Oran University 1 Ahmed Ben Bella (Plant pathology laboratory). Potato Dextrose Agar medium (PDA) and nutrient broth medium (NB) were microbiological media for culturing fungus.

The pathogenic strains used as indicator for antimicrobial activity study were *Proteus mirabilis* (ATCC 35659); *Streptococcus bovis* (ATCC 33317); *Staphylococcus epidermidis* (ATCC 25923); *Staphylococcus aureus* (ATCC 259292); *Salmonella typhi* (ATCC 19430); *E. coli* (ATCC 25922); *Candida albicans* (ATCC 10231) obtained from the Laboratory of Applied Microbiology of the University of Oran -1-. All bacteria strains were stored at -80°C in Nutrient broth, supplemented with

20% of sterilized glycerol.

Synthesis of silver nanoparticles

Following Gupta *et al.*, (2021) protocol; a fragment of *F. oxysporum* was inoculated in 200 ml Erlenmeyer flasks containing nutrient broth medium (NB) and incubated at 30°C with continuous stirring for 3 days. Later, filtration was carried through Whatman filter paper no.1. to separate biomass from medium, followed by substantial washing with distilled deionized water in order to remove any nutrient medium from fungal biomass that might interact with metal ions. The medium was preserved at 4°C to study the extracellular reduction of silver ions.

The washed mycelia were resuspended into 100 mL sterilized distilled water and incubated at 30°C for 24 hrs. followed by a second filtration. Again mycelia were harvested by filtration using Whatman filter paper no.1. The cell filtrate was another sample for extracellular synthesis compared with nutrient medium. At the same time, fungal biomass was taken for intracellular synthesis. For this, medium and cell filtrate were treated with 1 mM silver nitrate solution in 1:2 ratio, and biomass was resuspended into 100 ml of 1 mM silver nitrate solution. Each mixture was agitated and heated at 60°C for 3hrs in the dark.

Determination of total proteins

In order to detect and confirm the presence of reducing agents such as proteins resulting from mycelial metabolism, cell filtrate and medium were analysed for their total protein content using Qubit 3.0 assays by Invitrogen.

Analysis and characterisation of AgNPs

The detection of AgNPs was done primarily by visual observation of colour change in different samples after treatment with silver nitrate. The appearance of the dark brown colour of the mixture indicates the formation of AgNPs. Finally, they were confirmed by optical absorption using the UV-Visible spectrophotometer (Optizen 2120 UV) in the range of 200 – 900 nm.

Synthetic kinetics of silver nanoparticles

During the synthesis, samples were taken from each mixture every 10 to 15 mn for UV-Visible spectrophotometer analysis (Optizen 2120 UV) at different wavelengths ranging from 200 to 900 nm in order to follow the formation of silver nanoparticles over time.

Antimicrobial activity

The antimicrobial potentials of silver nanoparticles synthesis by *F. oxysporum* (*intracellular and extracellular synthesis*) were studied against pathogenic microorganisms, using three measurement methods (Disc diffusion assay, suspended method and agar method) Karthika *et al.*, (2017) and Sumitha *et al.*, (2020). Syn-

thesized AgNPS plus commercial antibiotics and antibiotics alone was tested for their potential synergistic antimicrobial activity.

Cell culture

The indicator microbial strains were stored at -20 °C. Precultures was carried out by inoculating an aliquot of the microbial strain in 10 ml of Mueller Hinton medium (MH). Then the mixture was incubated for 24 hrs at 37 °C. Later, the strains were centrifuged at 3500 rpm for 15 min. The microbial pellet was taken in 10 ml sterile distilled water and adjusted using 0.5 McFarland turbidity (1.5×10^8 CFU/ml).

Disc diffusion assay

For disc diffusion method, 100 µL bacterial inoculate were spread on Mueller Hinton agar plate (MH) using sterile swabs. Sterile paper discs of 6 mm were soaked in 50 µl of each biosynthesized solutions to be analysed were deposited aseptically on the agar plate surface, and then incubated at 37 °C for 24 h. Subsequently, after the incubation, inhibition zone diameter was measured (mm).

Suspended method

A suspended assay was used for determination of MIC (minimum inhibitory concentration) and MLC (minimal lethal concentration) of each sample. Serial concentrations ranging from 5 to 50 µg/mL of the AgNPs were prepared and introduced directly into test tubes containing different cultures of pathogenic strains which were then incubated at 37 °C for 24 hrs. The minimum inhibitory concentration was determined by measuring the absorbance (A) at 600 nm.

The minimum lethal concentration was determined by transferring 10 µl of the tubes with no visible growth into a new medium free of nanoparticles. MLC represents the lowest concentration which gives no growth after incubation. To measure the minimum lethal concentration (MLC), 10 µL of cultures was taken from wells of the tubes of MIC, with no visible turbidity, inoculated into a new medium without nanoparticles, and incubated for 48 hours, at 37 °C. MLC was defined to be the lowest concentration of nanoparticles that killed 99.9% of the microorganisms in culture on the agar plate after the incubation time.

Agar method

This method consists at identifying silver nanoparticles concentration that inhibit microbial growth. 25 µL of each tube containing increasing concentrations were spread over the MH Petri plates containing reference strains. After overnight incubation at 37°C, the number of colonies forming units was calculated.

The experiences were repeated in triplicate in order to confirm the results.

H₂O₂ detection

Hydrogen peroxide was widely used in various industries, it is highly toxic and can pose various health and environmental hazards. In order to detect the ability of Ag NPs to catalyse reduction of H₂O₂, the protocol developed by Raj *et al.* (2013) was used. The mixt was thoroughly prepared by blending H₂O₂ and AgNPs in a ratio 1:2. Once the colour change was visualized, the absorbance values were measured at wavelengths ranging from 200 to 900 nm.

Photocatalytic activity

In order to study the photocatalytic performance of the biosynthesized AgNPs, the reduction of methylene blue (MB) under UV light was used as the model reaction. Following protocol of Atout *et al.* (2018), 100 mg of each photocatalyst was added to 100 ml aqueous solution of MB (10 mg/l) under constant stirring for 30 to 60 min in the dark to attain the adsorption equilibrium. Then, the mixture was exposed to UV lamp at 365 nm for a few minutes. A control experiment was carried out under the same condition without the addition of nanoparticles.

Meanwhile, the catalytic performance of biosynthesized AgNPs was monitored by recording the UV-vis spectra (Optizen 2120 UV) in 30 min until the end of the reaction 150 min.

Antioxidant activity

Antioxidant activity was estimated using DPPH method. For this analysis, protocols of Adebayo *et al.*, (2019) and Jahan *et al.* (2021) were adopted with slight modifications. Methanolic solution of DPPH at 0.1 mM was mixed with biosynthesized AgNPs solution at 160 µg/ml concentration in 1:1 ratio. A negative control was prepared by mixing methanol with a methanolic solution of DPPH in the same ratio. The mixtures were incubated in the dark for 30 min at room temperature. Absorbances were read at 517 nm using methanol as blank.

The positive control was the ascorbic acid solution which the absorbance was measured in the same conditions as the tested samples. The obtained results for each extract were compared with the positive control.

The antioxidant capacity was expressed as inhibition percentage of the DPPH (%) calculated (Lateef, et al., 2018) with the following formula:

$$\% I = \left[\frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \right] \times 100 \quad \dots \text{Eq.1}$$

RESULTS AND DISCUSSION

Synthesis and characterization of AgNPs

The fungal system has been found to be a versatile biological system with the ability to synthesize metal nanoparticles intracellularly as well as extracellularly

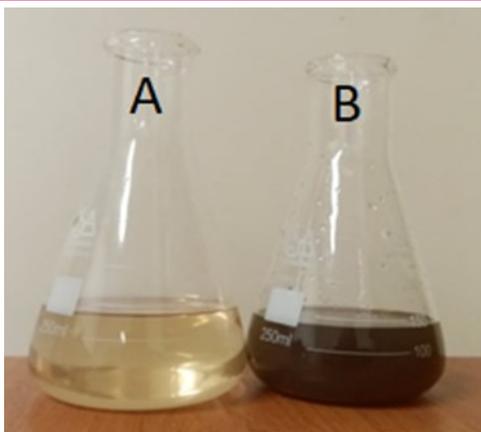


Fig. 1. Biosynthesis of AgNPs by *F. oxysporum* **A:** Aqueous fungal extract. **B:** AgNO₃ solution with aqueous fungal extract after incubation

(Rai *et al.*, 2021). In the present study, AgNPs were successfully synthesized from aqueous solution of AgNO₃ using medium culture, cell filtrate and fungal biomass, by noticing visually the color change after time incubation from slight yellow to dark brown (Fig.1). In this regard, El Domany *et al.* (2017) and Ahmed *et al.* (2018) reported the same observation, as they stated that primary confirmation of AgNPs biosynthesis from *F. oxysporum* can be noticed visually by the colour changing, which indicates the reduction of Ag⁺ to Ag⁰ by reducing agent present in the aqueous fungal extract as metabolites and proteins (Costa Silva *et al.*, 2017). Based on the results obtained using the Qubit 3.0 assays by Invitrogen, total protein concentration in the medium was estimated at 400 µg/ml, compared with fungus filtrate, which was in the order of 101 µg/ml. As far as the mechanism of extracellular synthesis, it was reported that proteins are one of the metabolites secreted by microorganisms involved in the reduction of metallic agents (Mohd Yusof *et al.*, 2019) (Rai *et al.*, 2021) and Khan *et al.*, (2018).

UV-Visible spectra (Optizen 2120 UV) of the samples were recorded (Fig. 2). Synthesis of AgNPs showed a maximum peak around 420 nm. It is well known that there is a very close relationship between the UV-Vis absorbance spectrum and size and shape of SNPs. With the increase in the particle size, the optical absorption spectra of metal nanoparticles that are dominated by surface plasmon resonances (SPR) shift towards longer wavelengths (redshift). Small blueshift or red shift in the wavelength of the absorbance peak could be related to obtaining SNPs in different shape and size (Birlawad *et al.*, 2013 and Mostafa, 2017). It is reported that the size of silver nanoparticles synthesis using *F. oxysporum* range between 5 and 50 nm with a spherical shape (Nasreen *et al.*, 2014, Mohd Yusof *et al.*, 2019); furthermore, size and shape of metal nanoparticles are influenced by a number of factors including pH, reductant concentration, incubation time, tem-

perature as well as the method of preparation (Umoren *et al.*, 2014, Mohammed *et al.*, 2018).

Synthetic kinetics of silver nanoparticles

In order to study the influence of the time factor on the formation of silver nanoparticles, a study was carried out keeping the same volumes and concentrations of AgNO₃ (1mM) and samples used previously.

The present results showed that the formation of nanoparticles occurs progressively, proportional to the incubation time and/or reaction time. By mixing the samples with the synthesis precursor (silver nitrate), the colour was clear at the beginning, but after a few minutes of interaction, the colour became darker, which indicates the reduction of aqueous silver ions Ag⁺ to silver nanoparticles Ag⁰. It was observed that the considerable intensity of surface plasmon resonance (SPR) bands of AgNPs appeared almost at 40 and 80 min of reaction for aqueous fungal extract and the cell filtrate, respectively, as it has been reported that *F. oxysporum* f. sp. *cutense* JT1 could synthesised gold nanoparticles (AuNPs) in 60 min (Rai *et al.*, 2021). Then it increased as the reaction time progressed, which indicated the continued reduction of the silver ions (Fig.3). The rapid synthesis obtained for the aqueous fungal extract compared to filtrate, may be due to the presence of high protein concentration in the aqueous fungal extract, and hence enhanced the synthesis compared to the cell filtrate.

Concerning the biomass, the contact of a metal salt solution, which is considered an antimicrobial agent, with the mycelium, causes the activation of the fungal metabolism to produce metabolites and enzymes to defend itself from metal stress. Therefore, this defensive behaviour and these possible agents affect the reduction of silver ions to solid non-toxic nanoparticles (Zielonka and Klimek Ochab, 2017) which explains the colour change from pale-yellow to brown after 40 min of reaction.

After 160 min, the UV-vis spectroscopy showed maximum absorption between 400-420 nm, indicating the complete reduction of silver ions. This may be due to

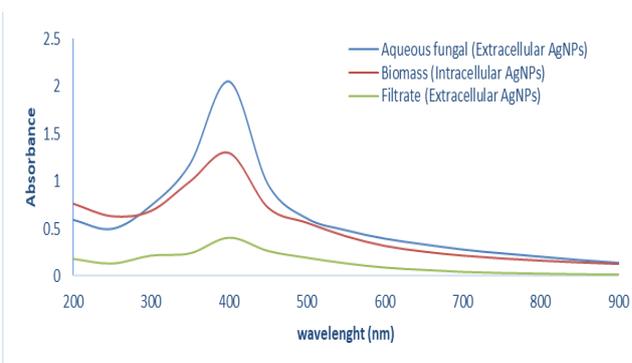


Fig. 2. UV-Vis Spectrophotometer peak absorbance for synthesis of silver nanoparticles of *Fusarium oxysporum*

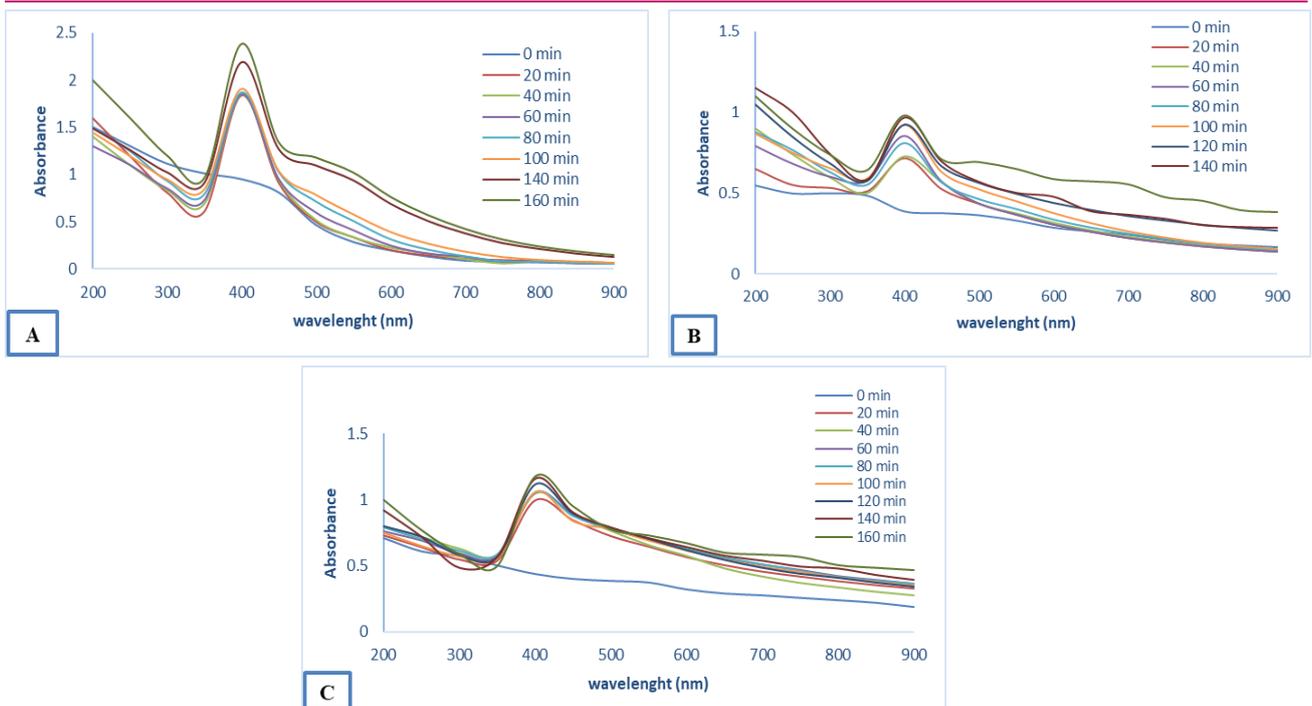


Fig. 3. Synthetic kinetics of silver nanoparticles by *F. oxysporum* Aqueous fungal extract, B. cell filtrate, C. Biomass



Fig. 4. Antimicrobial activities of biosynthesized silver nanoparticles against various pathogenic microorganisms.

the high protein concentration of the aqueous fungal extract compared to the cell filtrate, which allowed rapid reduction of silver nitrate.

Antimicrobial activity

The effect of antimicrobial agents in the aqueous fungal extract and in cell filtrate used during the synthesis of silver nanoparticles against various pathogenic microorganisms was first used as negative control to determine the action of the synthesised nanoparticles (Table 1). Then, silver nitrate solution was tested as a positive

control for its antibacterial and antiviral properties (Pal et al., 2007).

Fig. 4 showed zone of growth inhibition ranging from 8-18 mm. The highest inhibitory zone ($18 \pm 0,66$ mm) against *S. aureus* was given by the synthesised AgNPs from the cell filtrate, whereas the lowest inhibitory zone (8 ± 0 mm) was recorded against *P. mirabilis* from the same extract. This difference may be due to the properties of the nanoparticles, such as size, shape, surface charge, dose and state of dispersion of the particles (Liao and Tjong, 2019).



Fig. 5. Effect of antibiotic alone and associate with AgNPs

In present study, antibacterial activity against *S. bovis*, *S. epidermidis*, *S. aureus* and *S. typhi* presented good results showing approximately the same inhibitory zone of $16 \pm 0,66$ mm, from both extract AgNPs synthesized by the filtrate (extracellular synthesis) and the biomass of *F. oxysporum* (intracellular synthesis).

Antifungal activity against *C. albicans* showed a moderate inhibition zone (10 ± 0 mm) from both aqueous fungal extract and biomass but showed higher inhibition from the filtrate (12 ± 0 mm). The present results are similar to Ishida et al. (2013), who showed a high antifungal activity biosynthesized AgNPs from the *F. oxysporum* against *Candida*. Materials with antifungal potential (such as biogenic silver nanoparticles) that are obtained from sustainable sources can be inexpensive and safe options for treating systemic and surface fungal infections, enabling the control of resistant fungi

(Ashajyothi et al., 2016). Silver nanoparticles have also been used in combination with antibiotics, suggesting as a novel and potential alternative to standard antibiotic drugs since they have a great potential against the increasing multidrug resistance in pathogenic bacteria and fungi. (Guilger-Casagrande and Lima, 2019).

According to Bhat et al. (2015), the activity of the antibiotic improved when combined with nanoparticles. This applies to our results, in which the use of Erythromycin ($15 \mu\text{g/ml}$) and chloramphenicol ($30 \mu\text{g/ml}$) alone did not give an inhibition zone of inhibition. On the other hand, the association of the latter with AgNPs gave a stronger antimicrobial activity (Fig.5), El Domany et al., 2017 noticed the same increase in the antimicrobial activity. Thus, enhanced antimicrobial activity of extract mediated nanoparticles is due to the biomolecules attached on the surface of nanoparticles (Roy et al., 2019).

The minimum inhibitory concentration (MIC) and minimum lethal concentration (MLC) tested only against *Staphylococcus aureus*, *E. coli*, and *Candida albicans* showed a decrease in absorbance, indicating the change in the percentage of microbial inhibition as a function of the increasing concentration of AgNPs.

As represented in (Fig.6) the MIC of the biosynthesized AgNPs against the three strains tested was estimated around $5 \mu\text{g/ml}$. At the same time, the minimum lethal concentration (MLC) was estimated at $35 \mu\text{g/ml}$. These results are consistent with the work of Roy et al., 2019 and also with the work of Ishida et al., 2013; Jain et al.,

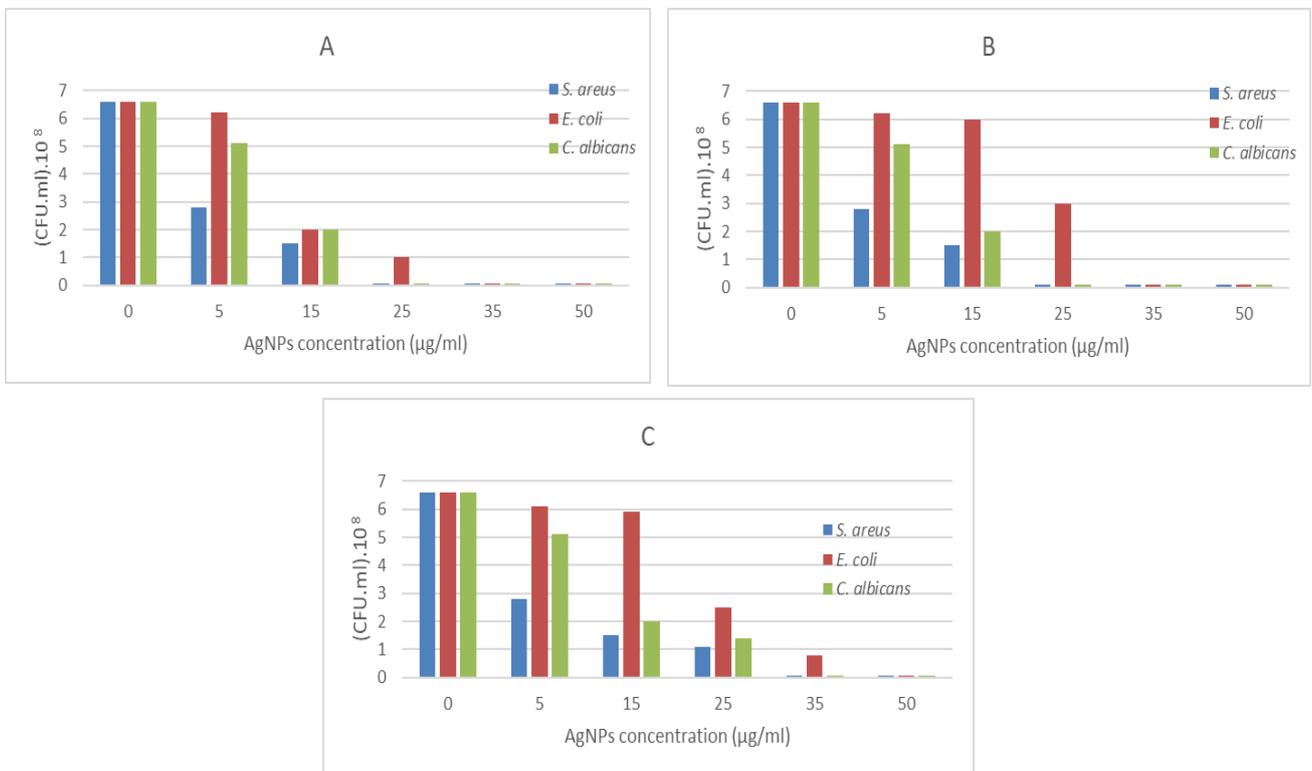


Fig. 6. Antimicrobial activity of the mycosynthesized AgNPs against *St. aureus*, *E. coli*, and *C. albicans*. A. Aqueous fungal extract, B. filtrate, C. Biomass

2015; Salari *et al.*, 2016 who tested the inhibition potential against *S. aureus*, *C. albicans*, *E. coli* respectively and confirmed the phenomenon of inhibition by MIC measurement.

On the agar method (Fig. 7), after 24 hours of incubation, a decrease of 25% was recorded for *C. albicans* and *E. coli*, and of 50% for *S. aureus* at a concentration of 5 µg/ml of AgNPs, on the other hand an inhibition of 98% from 25 µg/ml for *S. aureus* and *C. albicans* and from 35µg/ml for *E. coli*. The MIC and MLC were estimated around 5 µg/ml and 50 µg/ml, respectively, for the three strains tested. These results are similar to those obtained previously by the suspension method. Increasing concentrations of AgNPs reduce microbial growth, which has been shown by the study of Hamed *et al.* (2017).

Comparing the study conducted by Kim *et al.* (2007) on antibacterial activity against *E. coli* and *Staphylococcus aureus* with our results, the MIC is different. This difference may be related to the synthesis origin and size of silver nanoparticles, as reported by (Mtimet, 2011), because the size of nanoparticles determines their antimicrobial potential since smaller nanoparticles have greater effects (Lu *et al.*, 2013).

According to Javaid *et al.* (2018), the nanoscale size of AgNPs gives it the ability to penetrate into bacterial and fungal cell. Due to the high affinity of AgNPs towards sulfur, phosphorous, proteins and DNA, these molecules are susceptible to being attacked by nanoparticles.

Based on this evaluation and different studies (Sherif Moussa *et al.*, 2015) (Mohd Yusof *et al.*, 2019), the study concluded that gram-positive bacteria are more susceptible to AgNPs than gram-negative. It may be related to the presence of the outer membrane in the gram-negative wall, which prevents the penetration of nanoparticles.

H₂O₂ detection

In order to evaluate the ability of silver nanoparticles to catalyse the reduction of hydrogen peroxide (H₂O₂), AgNPs synthesised from different samples and H₂O₂ was thoroughly mixed in 1:1 proportion (to get 1ml). After a few minutes of reaction, a discolouration of the solution towards the transparent was noticed (Fig.8). H₂O₂ mediated degradation of AgNPs was confirmed by UV-vis absorption spectra in different wavelengths from 200 to 900 nm at time 0 and after 10 minutes of reaction (Fig.9). The peak intensity was found to decrease as a function of time due to decrease in concentration of AgNPs, who was observed at the wavelength of 450 nm with respect to time. As H₂O₂ is a strong oxidizing agent, it oxidizes AgNPs to convert Ag₀ to Ag⁺ form (Tagad *et al.*, 2013, Mohan *et al.*, 2014). The correlation between the concentration of H₂O₂ and the decrease in absorbance of SNPs as a function of time could be used as a measure to rapidly detect the H₂O₂ present in various samples (Raja *et al.*, 2015).

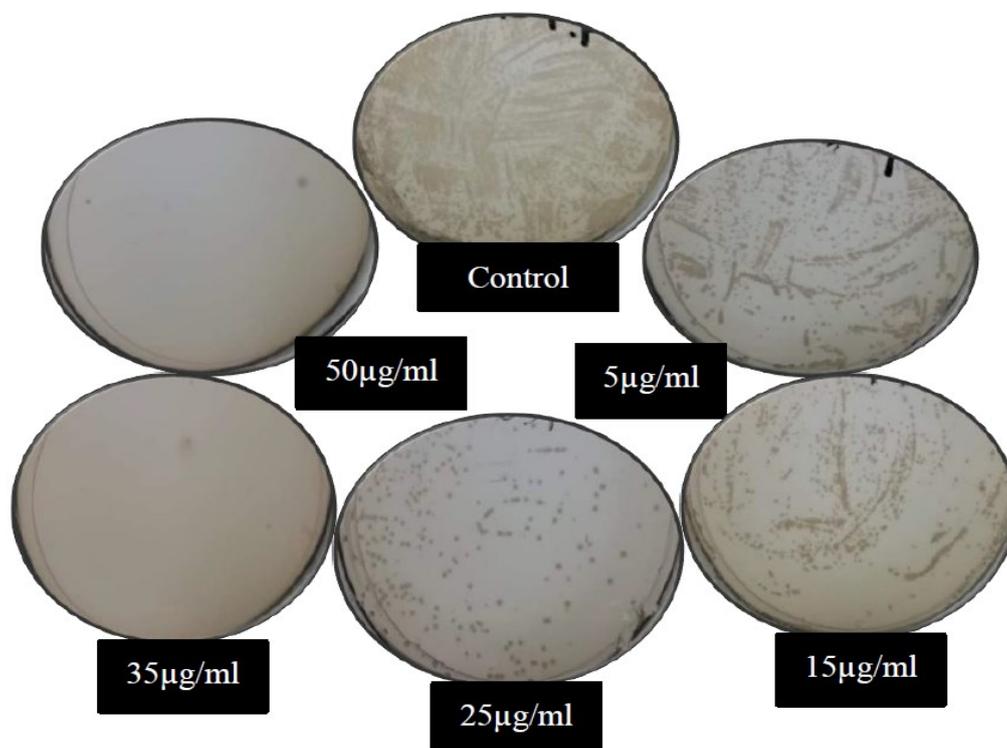


Fig. 7. Antimicrobial activity of silver nanoparticles at different concentrations against pathogenic microorganisms

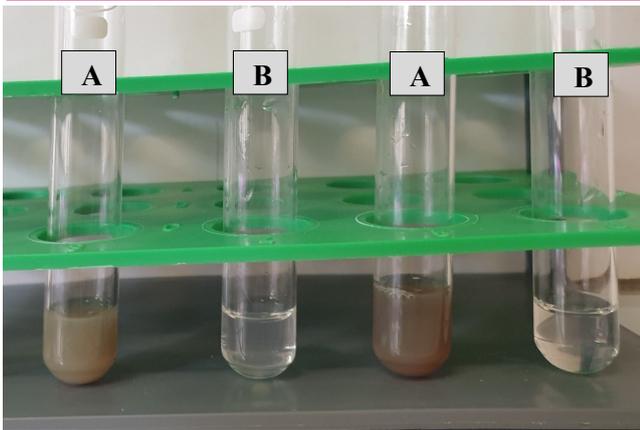


Fig. 8. Detection of H_2O_2 by silver nanoparticles. **A:** AgNPs. **B:** AgNPs+ H_2O_2

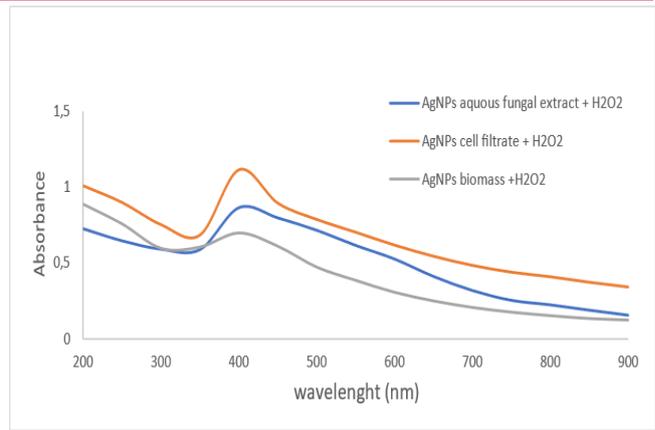


Fig. 9. Effect of AgNPs synthesized using *F. oxysporum* on H_2O_2 solution after 10 min of reaction

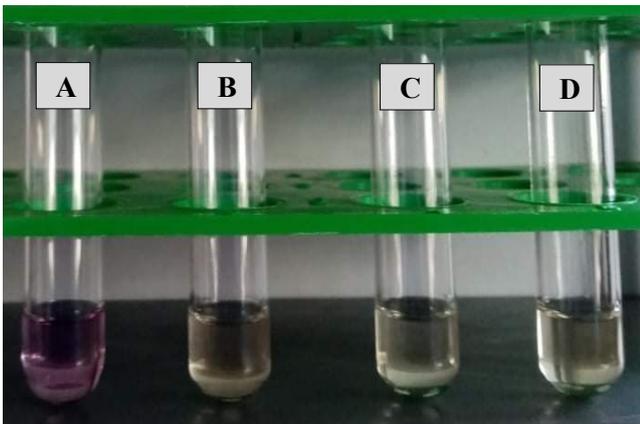


Fig. 10: Antioxidant activity of the biosynthesized AgNPs.

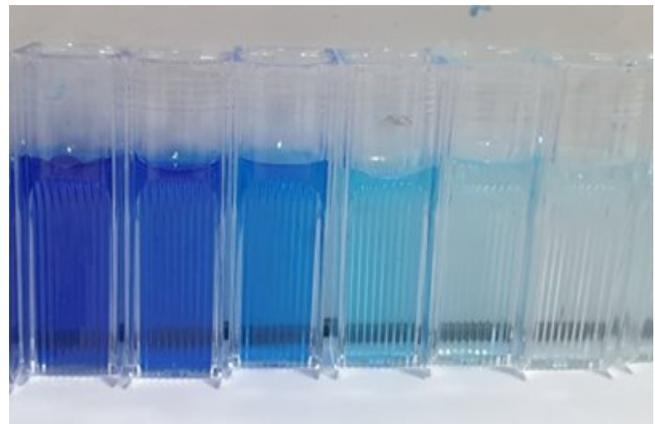


Fig. 11. Colour change of the reaction mixtures depending

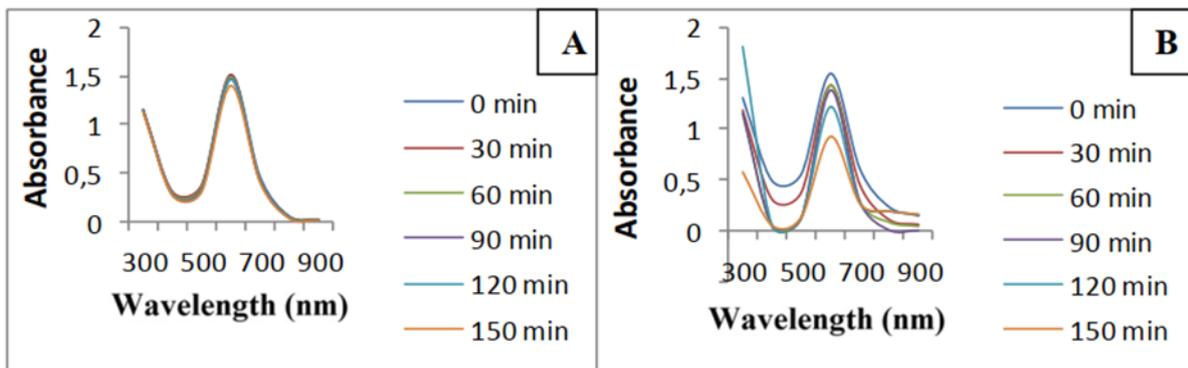


Fig. 12. Absorption spectra of MB after treatment for different times of irradiation. **A:** without AgNPs. **B:** in the presence of the synthesized AgNPs.

Antioxidant activity

The antioxidant activity of synthesized AgNPs was evaluated by DPPH assay. Ascorbic acid was used as a standard antioxidant. This method has been the most accepted model for evaluating any new drug's free radical scavenging activity (Patel Rajesh and Patel Natvar, 2011).

The DPPH is a free radical, stable, characterized by its purple color, with a maximum adsorption at ethanol or methanol at 517 nm (Bentabet et al., (2014) and

Bhakya et al., (2016). When a solution of DPPH is mixed with a substance that can donate a hydrogen atom, then this gives rise to the reduced form from the DPPH to the DPPH-H form (2,2-diphenyl-1-picryl-hydroxyl-hydrate), with decolorization and loss of violet colour to yellow colour (Fig.10). More the decolorization more is the reducing ability. The percentage inhibition of free radicals of synthesized AgNPs using aqueous fungal extract, cell filtrate and biomass were $82,12 \pm 0,42\%$, $70,46 \pm 1,53\%$ and $72,65 \pm 1,33\%$ respectively

at a concentration of 160 µg/ml of biosynthesized AgNPs solution. The results are appreciable compared to the previous report (Kharat and Mendhulkar, 2016; Sumitha and Senthil, 2020).

Photocatalytic activity

Methylene blue (MB) is a toxic cationic colorant, widely applied in major fields including medical, chemical, pharmaceutical, and aquaculture industries (Miri *et al.*, 2018). The photocatalytic activity was carried out using our biosynthesized silver nanoparticles as photocatalyst, exposing the MB solution containing the AgNPs to radiation. UV.

Fig.12 demonstrates the UV spectra of MB solution with different irradiation times in the presence and absence of the synthesized Ag-NPs as photocatalyst, where the UV light exposure time is considered as a parameter. The absorbance peak of MB was observed at around 600 nm, but after exposure to UV light and in the presence of AgNPs, a decrease in the intensity of this peak was noticed rapidly in the first 30 min and it can be seen by the reduction of colour intensity (Fig.11), which indicates the degradation of MB (Fig.12 B), comparing with the control (Fig.12 A) which did not show any change of colour or peak intensity over the experimental period. According to Ozcelik Kazancioglu *et al.* (2021), the band observed in the visible region at 612 nm corresponds to the chromophore group in the MB molecules due to the sulfur-nitrogen conjugated system. In a similar experiment, Gupta *et al.* (2021) showed nanoparticles' ability to catalyse MB, whose absorption peak of this dye was at 664 nm. Upon exposure to UV light, the characteristic peak at 664 nm decreases significantly over time.

Conclusion

In the present study, *F. oxysporum* showed potential for extracellular and intracellular biosynthesis of silver nanoparticles, as conformed by UV-Vis spectroscopy. The AgNPs thus synthesized proved an important technological aptitude and potential for the different biological activities investigated, such as antimicrobial activity, antioxidant activity, photocatalytic activity and detection of H₂O₂. The present study allows the opening of new perspectives. To put in value of these nanomaterials industrially and for better application, it is necessary to complete their physico-chemical characterisation using the TEM, XRD, FTIR and SEM. These methods will determine the structural properties of nanoparticles that impact biological systems. However, it is necessary to continue the cytotoxicity study of these silver nanoparticles and their influence on normal cells and cancer cells to prove the non-toxicity of this synthesis method and use it as an alternative chemical method in different fields, such as treating resistant bacteria to

antibiotics, toxic heavy metal biosensors and others.

Conflict of interests

The authors declare that they have no conflict of interest.

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