



## EFFICIENCY OF *PLEUROTUS OSTREATUS* IN BIO-SYNTHESIS OF SILVER NANOPARTICLES AND PRODUCT EFFICACY AGAINST *CANDIDA ALBICANS* ISOLATES

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### Abstract

Quality of Silver Nanoparticles AgNPs biosynthesized from a silver nitrate solution using the oyster *Pleurotus ostreatus* was assessed and the efficacy of silver produced at different concentrations (100, 200, 300, 400, 500 and 600)  $\mu\text{g/mL}$  was also evaluated for its effectiveness against *Candida albicans* isolates. The results indicated the efficacy of the fungal filtrate of *P. ostreatus* in production of silver nanoparticles. Findings also confirmed product quality based on Characterization of bio synthesized AgNPs depending on the physical characteristics including UV- Spectrophotometer analysis Scanning Electron Microscopy Test (SEM), Energy Dispersive Spectroscopy (EDS), X-Ray Diffraction Technology (XRD). The results showed that all the different concentrations of silver nanoparticles (100-600)  $\mu\text{g/ml}$  did not inhibit the growth of any *Candida albicans* isolates. Generally, higher concentrations of Nano-silver might be required to reduce *C. albicans* resistance to silver Nanoparticles.

**Keywords:** Nano-Silver, Multi-resistance, *Candida albicans*, Oyster Mushroom

### Introduction

As a result of infection with multiple resistance pathogens, traditional antibiotics used to treat them have become ineffective, which has increased health problems. Multiple resistance organisms to antibiotics have become a major threat to the safety of human life, and the increased rate of disease or death may depend on the most common microbial infections. The most important step in eliminating resistance is to reduce the use of antibiotics and restrict their use to cases of extreme necessity when other treatment options fail. Therefore, the use of non-traditional treatments in combating resistant microbes has become an alternative to the treatments that have caused resistance. However, these alternatives are required to be non-toxic, safe and effective and may be used against pathogens of multiple resistance (Franci *et al.*, 2015).

Recently, many properties have been discovered in the metals nanoparticles that have proven in many types to be effective in treating diseases caused by multiple resistance pathogens (Tomar *et al.*, 2014). Nowadays, nanoparticles are occupied high interest in scientific fields and considered a viable antibiotics alternative because of their ability to reduce problems related to multiple antibiotic resistance (Franci *et al.*, 2015). The use of non-microscopic fungi (Macro-Fungi), especially mushroom fungi, in silver Nano-particles synthesis is a new method with broad prospects for producing larger quantities of Nano-materials and in relatively short time periods.

*Pleurotus* is one of the most edible oyster mushrooms. All species of this species are edible and most of them have been commercially produced and developed as the third largest fungus cultivated in the world. It is one of the healthy foods rich in proteins and polyunsaturated fats is low in calories, and it contains keratin and a group of vitamins B which are Thiamin (B1), Riboflavin (B2), Niacin (B5), Pyridoxine (B6) and Biotin (B7) as it is an important source of minerals And breaking enzymes of lignins and phenols. In fact, mushroom fungi produce significant amounts of proteins and external enzymes that are involved in reduction process of nitrate silver mainly during the process of forming

nanoparticles. In addition, mushrooms are among the most easy to propagate and cultured fungi under laboratory conditions or in industrial farms with high level of production (Al-Bahrani *et al.*, 2017). The study aimed to biosynthetically produce silver nanoparticles from AgNO<sub>3</sub> salts using the oyster *Pleurotus ostreatus* and to evaluate the antimicrobial activity of the resulting silver nanoparticles against different isolates of *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

### Materials and Methods

Oyster fungal filtrate was prepared using 0.5 cm disk from the fungal colony after being cultured on PDA medium and incubated for 7 days. The disk was placed in 250 ml Potato Dextrose Broth medium in a 1000 ml glass beaker, incubated in dark conditions at  $27 \pm 1$  C°. for 21 days (Mutlaq *et al.*, 2017). After incubation, fungal biomass was removed while the liquid was centrifuged at 4000 rpm for 10 minutes and filtered through Whatman No.1 filter paper. The filtrate was sterilized using the 0.45 $\mu\text{m}$  Millipore Filter System, and the resulting filtrate was used to synthesize silver nanoparticles (Al-Askar *et al.*, 2013).

For the production of silver nanoparticles, silver nitrate solution was mixed with oyster mushroom filtration and the mixture was incubated for 24 hours (Al-Askar *et al.*, 2013). A solution of silver nanoparticles was prepared at a concentration of 300 $\mu\text{g/ml}$  by dissolving 0.03 g of purified and dried nanoscale silver in 20 ml of distilled water and subjected to an ultrasound water bath for 30 minutes. Then 30 ml of distilled water was added and the mixture was also exposed to ultrasound for 30 minutes, after which the volume was completed to 100 ml. The final solution was applied to the ultrasound machine for half an hour to ensure the spread of the Nano-powder and avoid any sediment formation (Pradhan *et al.*, 2016).

### Quality of the produced silver Nano-particles

Quality characteristics of the bio-synthesized AgNPs were evaluated depending physical characterizing tests, including UV-Spectrophotometer analysis (Trepathi,2014), Scanning Electron Microscopy Test (SEM) for determining

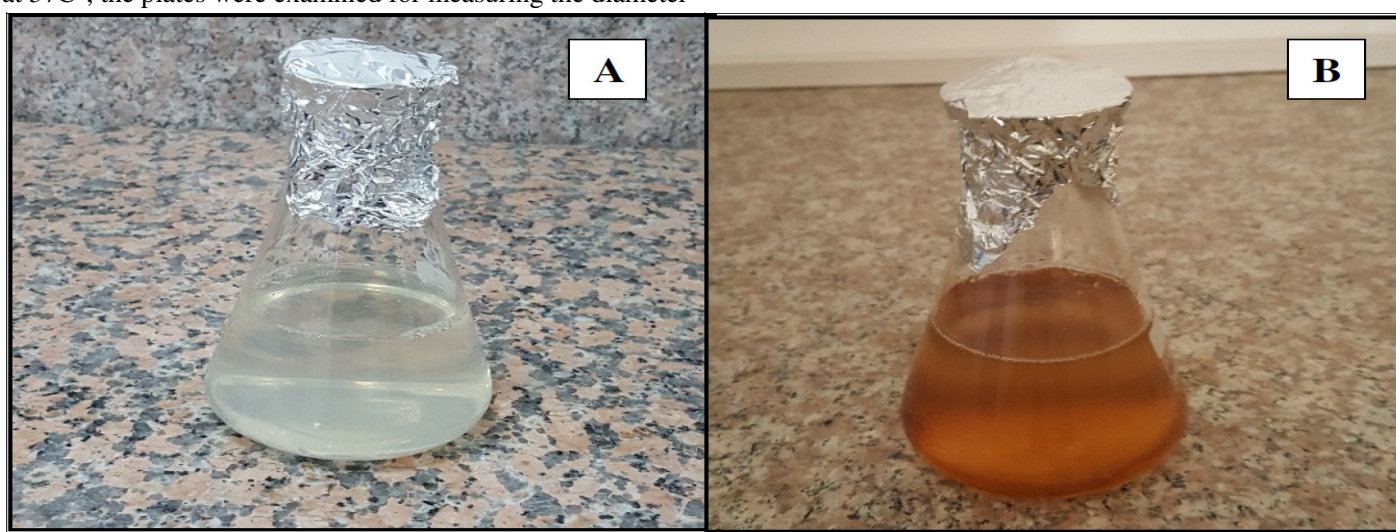
and evaluating silver particle size, particle surface texture, permeability, distribution and uniformity (Palmqvist, 2017; Caroling *et al.*, 2013). Energy Dispersive Spectroscopy (EDS) was also nano-particles component analysis (Smuleac *et al.*, 2013). Some other characteristics of the produced silver Nano-particles were also evaluated including particle structure, crystallization, particle size and some other physical characters using X-Ray Diffraction Technology (XRD) (Castillo-Michel *et al.*, 2017).

Cultures for the 5 fungal isolates were purified and propagated by spreading 0.02 ml of the fungal suspension ( $10^6$  cell/ml) on sprout dextrose agar medium in 9 cm Petri dishes. Three holes were made in each plate on the medium using a 9 mm diameter cork-borer. The holes were then filled with a solution of nanoparticles at concentration of 100, 200, 300, 400, 500 or 600  $\mu$ g/ml. After 24-hour incubation period at 37C°, the plates were examined for measuring the diameter

of the inhibitory zone due to the diffusion silver nanoparticle solution. The inhibition zone was calculated by subtracting the total inhibition zone from the diameter of the hole. As for the control treatments, the holes were filled with non-ionizing distilled water, the fungal filtrate or a mixture of silver nitrate solution and fungal filtrate without incubation period.

## Results and Discussion

The results of the visual examination showed the ability of *P.ostreatus* to bio-synthesize the silver nanoparticles, as the color of the reaction mixture (solution of the fungus mixed with silver nitrate) changed from pale yellow to yellowish brown and then to dark brown color continuously during the incubation period (Figure 1). This is an indication of the biological reduction of silver ions due to the filtrate components and the formation of nanoparticles.



**Fig. 1** : Color reaction of the mixture (*P. ostreatus* filtrate and silver nitrate solution) before (A) and after (B) 3 days incubation

### Characterization of Biosynthesized Nanoparticles

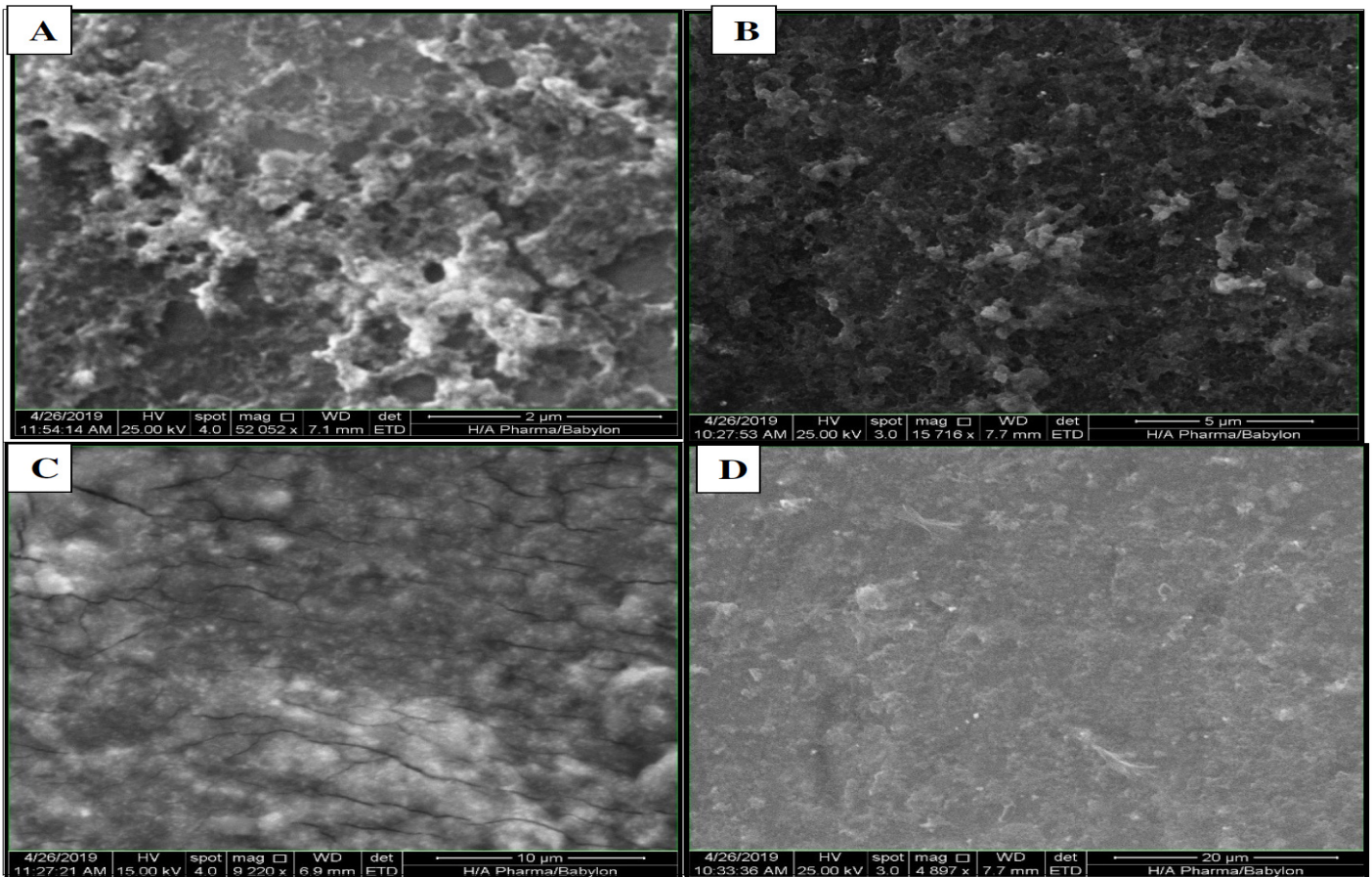
Results of the produced silver analysis using UV-Spectrophotometer analysis at the wavelength range 200-800 nm after 24-hour incubation showed that the absorption intensity gradually increased to the highest peak point at the wavelength of 400 nm. This indicates the continuous decrease in the concentration of  $AgNO_3$  salts and the formation of nanoparticles in the reaction mixture and their increased concentration (Figure1).

The wavelength of 400 nm falls within the range associated with the surface of the silver metallic particles (Wani *et al.*, 2010; Vidhu *et al.*, 2011) and the appearance of brown and yellowish solutions gave the absorbance intensity above the highest peak of the solutions of a dark brown color at the same wavelength of the radiation Ultraviolet (Owaid *et al.*, 2017). This indicates an increase in the concentration of nanoparticles in solutions (Verma and Mehata, 2016). Rangunath *et al.* (2017) also found the highest UV absorption peak for nanoparticle silver particles manufactured by *P. florida* was at 410 nm indicating that a spectroscopic characterization study based on the UV absorption scale of nanoparticle silver manufactured by some types of mushrooms (*Trametes*), *Gonoderma*, *Pleurotus*) lies within the wave range (430-420) nm.

As for Scanning Electron Microscopy using different magnification forces, the analysis showed the presence of different sizes and shapes of nanoscale silver particles

manufactured using the ostrich mushroom *P.ostreatus*. Generally, the majority of the particle shape is spherical, homogeneous in size with dimensions 59-36 nm, distributed evenly with little agglomeration (Figure2). The particles produced at pH (6.8) were larger than the ones manufactured at pH (9). The spherical shape is also considered one of the desirable properties and specifications in the form of nanoparticles for its important role in the way it relates to the cell membranes of microorganisms (Al-Khzai, 2019). Several studies using scanning electron microscopy have indicated that nanoparticles are spherical in sizes between (50-40) nm (Devika *et al.*, 2012). Balashanmugam *et al.* (2013) showed that the average size of spherical nanoparticles produced using mushrooms was 40 nm. The results of the study showed that the particle size of the Nano-silver was within the average in previous studies and was 20 nanometers (Kaur *et al.*, 2018), or (50-20) nanometers (Rangunath *et al.*, 2017), or (70-10) nanometers produced Using the fungus *A. biosporus* (Banerjee and Rai, 2018).

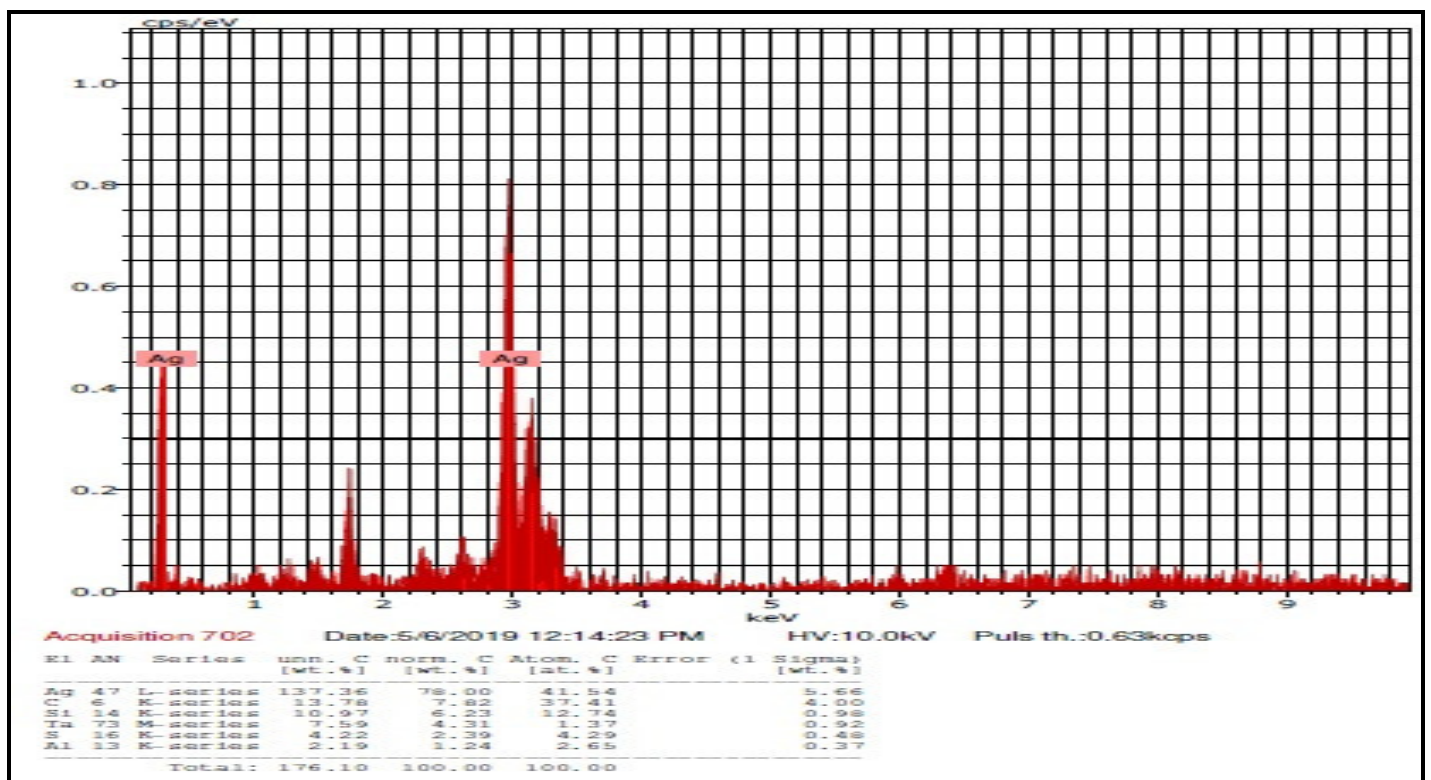
Energy Dispersive X-Ray Spectroscopy (EDX) Analysis showed the percentage weight of the constituent weight of the nanoscale silver particles produced by the oyster mushroom *P. ostreatus* was 78% for the silver and (7.8, 6.2, 4.3, 2.3, 1.2)% for each of the elements (C, Si, Ta, S, Al) respectively (Figure 3). This indicates dominant silver structure of nanoparticles and silver atoms that make up the nanoparticle.



**Fig. 2 :** Scanning Electron Microscopy analysis for silver Nanoparticles Bio-produced by oyster mushroom *P.ostreatus*. Silver particles structure and distribution shown at different magnification forces at measurement scale of (A) 2, (B) 5, (C) 10 and (D) 20 μm.

It was observed that the peak optical absorption was at about 3KeV, which indicates the presence of pure silver nanoparticles and that silver where the energy level of 3KeV represents the highest absorption level for the primary silver (Hytham, 2015; Elgorbana *et al.*, 2016). Although the

mechanism for creating nanoparticles using fungi has not been clearly defined yet, a number of researchers have reported that some proteins produced by fungi in the outer cellular filtrate can play a role in the synthesis and stability of nanoparticles (Mukherjee *et al.*, 2001).

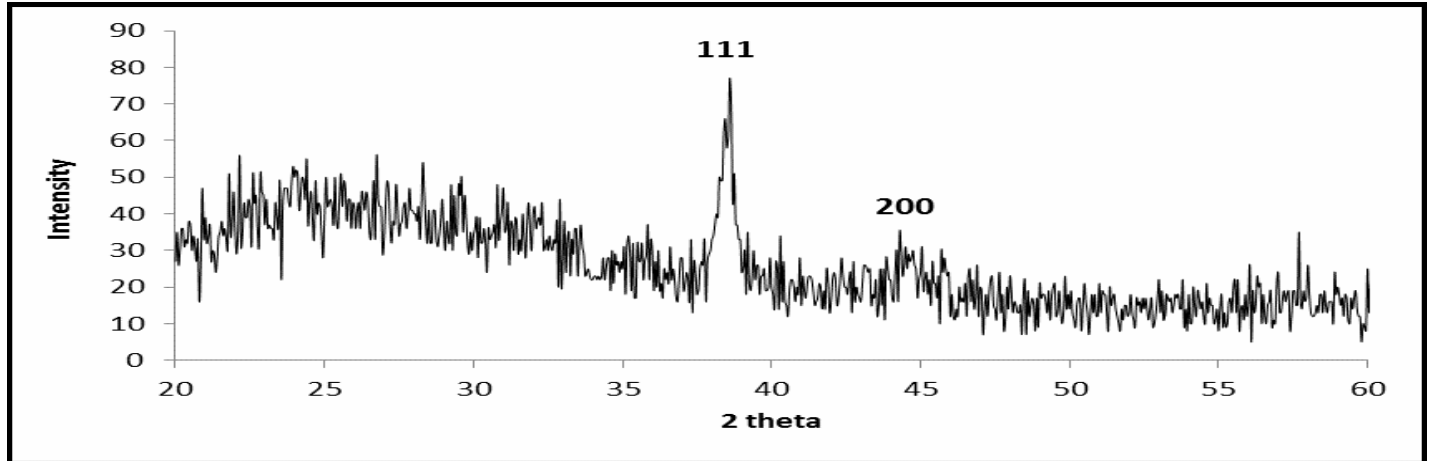


**Fig. 3 :** Energy Dispersive X-Ray Spectroscopy (EDX) Analysis for silver

### Nanoparticles Bio-produced by Oyster fungus *P.ostreatus*

On the other hand, the results of X-Ray Diffraction (XRD) showed that the highest peaks of diffraction are (111) and (200) at angles of 38.59 and 44.51, respectively (Figure 4). These values are close to the angles mentioned in the JCPDS card File No. (04-0783) attributed to the Face Centered Cubic (FCC) silver multi-phase crystallized cubic structure (Velusamy and Ggopinath, 2013). From Figure (4) it appears that the top (111) is more and higher than the top

(200), and this shows that the AgNPs produced by reducing the Ag + silver ions by the filtrate of *P. ostreatus* are of a crystalline nature. Also, the absence of other peaks shows that the silver produced material is free from any bundles of silver oxides, which indicates the role of the filtrate leachate components in the synthesis and the reduction of most silver ions in the reaction mixture and its role in stability and chemical stability of the nanoparticles (Gajendran *et al.*, 2014).



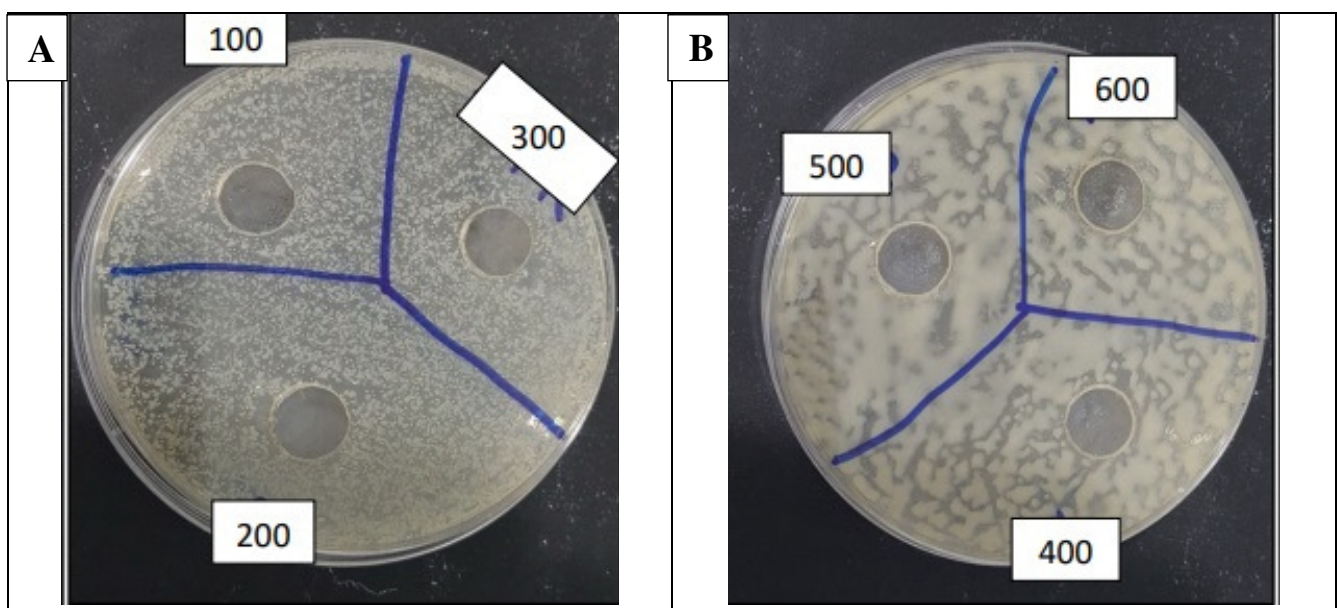
**Fig. 4 :** X-Ray Diffraction (XRD) Analysis for silver Nanoparticles Bio-produced by Oyster fungus *P. ostreatus*

The expansion of the bundles of peaks (111) and (200) indicates that the produced particles have nanoscale dimensions, and some unknown peaks that are less intense than the crystal tops of the nanoscale silver may appear due to the presence of some organic and biological filtrate components that surround a massive surface Nanofunding (Anandalakshmi *et al.*, 2016).

The results in Gajendran *et al.* (2014) showed Nano-scale silver particles produced using oyster mushrooms *P. djamor* resulted in peak 111 that stronger and higher than those of 200 and 220, while the particles made using *P.*

*ostreatus* were more crystallized in which 4 peaks appeared at level 111 (Devika *et al.*, 2012). This is mostly due to the amount of filtrate used and pH conditions that generally affect the dimensions and properties of the produced nanoparticles (Remya *et al.*, 2015).

In case Antifungal Activity of Silver Nanoparticles, the results (Figure5) showed that all the different concentrations (100-600)  $\mu\text{g/ml}$  of silver nanoparticles used did not inhibit the growth of the pathogenic fungal isolates used. Which, means that all of them were resistant to the particles produced in this stud



**Fig. 5 :** Silver Nano-particles bio-synthesized using *P. ostreatus* at different concentrations (100-600  $\mu\text{g/ml}$ ) did not affect growth *Candida albicans*

Some studies have indicated that AgNP activity depends not only on its concentration and size but also on its shape. *E. coli* bacteria were found to respond better to triple nanoparticles and were inhibited at low concentrations (Lin *et al.*, 2014). Pal *et al.* (2007) showed that triple nanoparticles are more qualitatively effective, because the triangular shape gives a greater positive charge to the nanoparticles, ensuring greater activity than rod and spherical shapes.

### References

- Abdel-Hafez, S.I.; Nafady, N.A.; Abdel-Rahim, I.R.; Shaltout, A.M. and Mohamed, M.A. (2016). Biogenesis and optimisation of silver nanoparticles by the endophytic fungus *Cladosporium sphaerospermum*, Int J Nanostruct Chem., 2: 11–19.
- Al-Askar, A.A.; Hafez, E.E.; Kabeil, S.A. and Meghad, A. (2013). Bioproduction of silver-nanoparticles by *fusarium oxysporum* and their Antimicrobial activity against some plant pathogenic bacteria and fungi. Life Sci. J., 10(3): 2470-2475.
- Al-Bahrani, R.; Raman, J.; Lakshmanan, H.; Hassan, A.A. and Sabaratnam, V. (2017). Green synthesis of silver nanoparticles using tree oyster mushroom *Pleurotus ostreatus* and its inhibitory activity against pathogenic bacteria. Materials Letters, 186: 21-25.
- Anandalakshmi, K.; Venugobal, J. and Ramasamy, V. (2016). Characterization of silver nanoparticles by green synthesis method using *Petalium murex* leaf extract and their antibacterial activity. Applied Nanosci., 6(3): 399-408.
- Balashanmugam, P.; Santhosh, S.; Giyaullah, H.; Balakumaran, M.D. and Kalaichelvan, P.T. (2013). Mycosynthesis, characterization and antibacterial activity of silver nanoparticles from *Microporus xanthopus*: a macro Mushroom. Int. J. Innov. Res. Sci. Eng. Technol, 2(11): 6262-6270.
- Banerjee, K. and Rai, V.R. (2018). A review on mycosynthesis, mechanism, and characterization of silver and gold nanoparticles. BioNanoSci., 8(1):17-31.
- Caroling, G.; Tiwari, S.K.; Ranjitham, A.M. and Suja, R. (2013). Biosynthesis of silver nanoparticles using aqueous broccoli extract-characterization and study of antimicrobial, cytotoxic effects. Asian J Pharm Clin Res., 6(4):165-172.
- Castillo-Michel, H.A.; Larue, C.; Pradas Del Real, A.E.; Cotte, M. and Sarret, G. (2017). Practical review on the use of synchrotron based micro- and nano- X-ray fluorescence mapping and X-ray absorption spectroscopy to investigate the interactions between plants and engineered nanomaterials. Plant Phys. and Biochem., 110: 13-32.
- Elgorbana, A.M.; Al-Rahmaha, A.N.; Sayedc, S.R.; Hirada, A.; Mostafaa, A.A. and Bahkalia, A.H. (2016). Antimicrobial activity and green synthesis of silver nanoparticles using *Trichoderma viride*.. Biotechnolog and biotechnological equipment, 30(2): 299-304.
- Devika, R.; Elumalai, S.; Arumugam, P. and Kasinathan, K. (2012). Biosynthesis of silver nanoparticles using the fungus *Pleurotus ostreatus* and their antibacterial activity. Journal of NanoScience.NanoEngineering & Applications, 2(2).
- Franci, G.; Falanga, A.; Galdiero, S.; Palomba, L.; Rai, M.; Morelli, G. and Galdiero, M. (2015). Silver nanoparticles as potential antibacterial agents. Molecules, 20(5): 8856-8874.
- Gajendran, B.; Chinnasamy, A.; Durai, P. and Raman, J. (2014). Biosynthesis and characterization of silver nanoparticles from *Datura innoxia* and its apoptotic effect on human breast cancer cell line MCF7, 122: 98–102.
- Gopinath, V. and Velusamy, P. (2013). Extracellular biosynthesis of silver nanoparticles using *Bacillus* sp. GP-23 and evaluation of their antifungal activity towards *Fusarium oxysporum*. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 106: 170-174.
- Kaur, T.; Kapoor, S. and Kalia, A. (2018). Synthesis of Silver Nanoparticles from *Pleurotus florida*, Characterization and Analysis of their Antimicrobial Activity. Int. J. Curr.Microbiol. App. Sci., 7(7): 4085-4095.
- Mukherjee, P.; Ahmad, A.; Mandal, D.; Senapati, S.; Sainkar, S.R.; Khan, M.I. and Sastry, M. (2001). Fungus-mediated synthesis of silver nanoparticles and their immobilization in the mycelial matrix: a novel biological approach to nanoparticle synthesis. Nano letters, 1(10):515-519.
- Mutlag, N.H.; AlShukri, A.Y. and Hussein, A.M. (2017). Assessment of the addition of oyster mushroom (*Pleurotus ostreatus*) on the weights sectors ratios and giblets weight of broiler chicken and microbial contents. Biochem. Cell. Arch, 17(2): 739-743.
- Owaid, N.M.; Al-Saeedi, S.S. and Abed, I.A. (2017). Study on UV-visible for detection of biosynthesis of silver nanoparticles by oyster mushroom's extracts. Journal of Water and Environmental Nanotechnology, 2(1): 66-70.
- Palmqvist, M. (2017). Nanoparticles. Diss. (sammanfattning/summary) Uppsala : Sveriges lantbruksuniv., Acta Universitatis agriculturae Sueciae, 105: 1652-6880.
- Rades, S.; Hodoroba, V.; Salge, T.; Wirth, T.; Lobera, M.P.; Labrador, R.H.; Natte, K.; Behnke, K.; Gross, T. and Unger, W.E.S. (2014). High-resolution imaging with SEM/T-SEM, EDX and SAM as a combined methodical approach for morphological and elemental analyses of single engineered nanoparticles. RSC Adv., 4: 49577-49587.
- Ragunath, A.; Parveen, N.A. and Kumuthakalavallir, A. (2017). Mycosynthesis of Silver Nanoparticles: characterization, Antioxidant and Anti-Inflammatory activity from *Pleurotus florida* (mont) singer: a macro fungi. Asian J. pharm. Clin.Res., 10(9):186-191.
- Raman, J.; Reddy, G.R.; Lakshmanan, H.; Selvaraj, V.; Gajendran, B.; Nanjian, R. and Sabaratnam, V. (2015). Mycosynthesis and characterization of silver nanoparticles from *Pleurotus djamor* var. roseus and their in vitro cytotoxicity effect on PC3 cells. Process Biochemistry, 50(1): 140-147.
- Remya, R.R.; Rajasree, S.R.; Aranganathan, L. and Suman, T.Y. (2015). An investigation on cytotoxic effect of bioactive AgNPs synthesized using *Cassia fistula* flower extract on breast cancer cell MCF-7. Biotechnology Reports, 8: 110-115.

- Smuleac, V.; Xiao, L. and Bhattacharyya, D. (2013). Greener and Other Approaches To Synthesize Fe and Pd Nanoparticles in Functionalized Membranes and Hydrogel. ACS Symposium Series. Vol. 1124. Chapter 3: 41–58.
- Szweda, P.; Gucwa, K.; Kurzyk, E.; Romanowska, E.; Dzierzanowska-Fangrat, K.; Jurek, A.Z. and Milewski, S. (2015). Essential oils, silver nanoparticles and propolis as alternative agents against fluconazole resistant *Candida albicans*, *Candida glabrata* and *Candida krusei* clinical isolates. Indian journal of microbiology, 55(2):175-183.
- Tada, R.; Latge, J.P. and Aimanianda, V. (2013). Undressing the fungal cell wall/cell membrane-the antifungal drug targets. Current pharmaceutical design, 19(20): 3738-3747.
- Tomar, R.S.; Agarwal, M. and Jyoti (2014). A. Metallic Nanoparticles nextgen antimicrobial agents. The Scitech. J. Sci. and Tech., 3(2): 2-9.
- Valverde, M.E.; Hernández-pérez, T. and Paredeslópez, O. (2015). Review Article Edible Mushrooms: Improving Human Health and Promoting Edible Mushrooms: Improving Human Health and Promoting, 99:(97.22), 100.
- Verma, A. and Mehata, M.S. (2016). Controllable synthesis of silver nanoparticles using Neem leaves and their antimicrobial activity. Journal of radiation Research and applied sciences, 9(1): 109-115.
- Vidhu, V.K.; Aromal, S.A. and Philip, D. (2011). Green synthesis of silver nanoparticles using *Macrotyloma uniflorum*. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 83(1): 392-397.
- Wani, I.A.; Khatoon, S.; Ganguly, A.; Ahmed, J.; Ganguli, A.K. and Ahmad, T. (2010). Silver nanoparticles: Large scale solvothermal synthesis and optical properties. Materials Research Bulletin, 45(8): 1033-1038.