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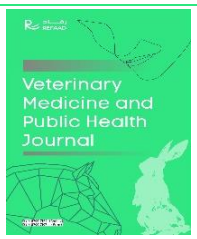

Biosynthesis of Silver Nanoparticles using *Aspergillus Niger* Isolated from Soil and Studying its Antimicrobial Effect Against Some Multidrug-Resistant Bacterial Species and *Candida Albican*

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Abstract:

Objectives: Biosynthesis of silver nanoparticles using the fungus Aspergillus niger, isolated from the soil for the purpose of studying its ability to produce nanoparticles.

Methods: Grown in a liquid fermentation medium in glass flasks for ten days. The biosynthesis process was detected by observing the color changes of the filtrate. The properties of silver nanoparticles were determined by UV-measurement. Use SEM to discover the size, shape and distribution of nanoparticles. The chemical groups responsible for the biosynthesis and encapsulation of nanoparticles were detected using FTIR for both the fungal biomass filtrate and the resulting particles, after turning them into powder. Silver nanoparticles produced from mushrooms using the Well-Diffusion-Method showed antibacterial activity against MDR-bacteria for both Gram-positive and Gram-negative Bacteria, which are: (E. coli, K. Pneumonia, S. aureus, and P. aeruginosa), at two concentrations (50, 100). µg/ml) for AgNPs.

Results: The biosynthesis was detected by observing the color changes of the filtrate from transparent to light yellow to dark brown. The properties of silver nanoparticles were determined by measurement (UV). Which showed absorbance values at wavelengths (450 nm). The results showed SEM The shape was spherical with dimensions ranging from (17.84-64.03) nanometers.

Conclusions: The fungus Aspergillus niger showed the ability to biosynthesise silver nanoparticles after 24 hours of incubation of the fungal filtrate treated with AgNO3.

Keywords: *Aspergillus niger; AgNPs; Biosynthesis; Multidrug resistant MDR.*

1 Introduction

Nanotechnology is one of the most promising modern research fields in modern medical sciences and has emerged as a multidisciplinary science to solve several problems in the fields of biomedical sciences, pharmacy and food processing (Rother, 2016; Rajiv, 2010). It deals with a material measuring one billionth of a meter, so nanoparticles are the basic element in manufacturing nanostructures. Nanometer-sized particles exhibit unique new properties (Raffaele and others, Chakraborty; *et al*., 2016). There are multiple methods for obtaining nanoparticles, including chemical, physical, and biological (Gupta *et al*., 2016). It is not usually preferable to produce nanoparticles using chemical or physical methods because they have negative effects on human health and the environment, in addition to their high economic cost and the complexity of their production mechanisms, as they require special conditions of toxic chemical raw materials, an energy source, and high pressure. Therefore, attention has turned to preparing nanoparticles using biological methods that are easy to prepare, safe, inexpensive, and environmentally friendly and do not have negative effects on health or the environment (Zhang *et al*., 2016). The process of creating silver nanoparticles by fungi is one of the biological methods that can be used successfully, as it is simple, low-cost, and energy-efficient. Fungi have the ability to produce enzymes that act as reducing and coating agents by forming stable, shape-controlled silver nanoparticles (Zaho *et al*., 2018). The use of antibiotics has risen dramatically in recent years. The excessive use and misuse of antibiotics not only increases the resistance of microorganisms but also has a negative impact on the immune system, and the antibiotics used now have severe harmful effects in addition to their high production costs, which prompted researchers to search for sources. New antibiotics that are safe and effective (Schillaci *et al*., 2017). Among the applications of silver nanoparticles (AgNPs) is their effectiveness against a wide range of pathogenic bacteria, fungi, and some strains resistant to antibiotics, which have become a real problem. Therefore, nanoscience technology has opened the horizons in the field of nanomedicine and the preparation and manufacture of nanoparticles, which is an alternative that can solve the problem of multiple resistance drugs (MRD) shown by most microorganisms (Emily, 2010).

2 Materials and Methods

2.1 Laboratory Isolation of Fungi

I adopted the fear method (1959, Johnson). To isolate fungi from soil from different areas of Basra Governorate, weigh 10 grams of soil sample, add 90 ml of sterilized distilled water in a glass beaker, and shake the suspension in an electric shaker for 10 minutes. Transfer 1 ml of the suspension using a sterile pipette and add it to 9 ml of sterile distilled water to obtain a 10-1 dilution. The process was repeated to obtain the best concentration of 10-4. Then 1 ml of this dilution was transferred using a sterile pipette, placed in sterile Petri dishes, and added to each dish. 20 ml of dissolved culturemedium (PDA). Prepared and added to the antibiotic Chloramphenicol. The plates were incubated after hardening in the incubator at a temperature of 25 ± 2 °C for 5-7 days. Use three replicates for each sample. The fungi were then purified and diagnosed.

2.2 Identification of isolated Fungi

2.2.1 Morphological Identification

After isolating the fungus from the soil, it was diagnosed based on the colony's external appearance (morphological features), such as shape and color.

2.2.2 Microscopic Identification

Microscopic Diagnosis was made using a compound-light-microscope for examination, by preparing glass slides by taking a portion of the fungal hyphae from the pure-growing-colony using a sterile loop-inoculation needle, then placing them on a clean glass slide containing 2–3 drops of Lactophenol Cotton Blue dye and mixing with The dye was then placed on the slidecover and gently pressed on it for the purpose of brushing the sample. The sample was examined under an optical microscope using (10X) power, then at (40X) power, in order to observe the fungal hyphae, their shapes, branches, and dimensions, and the conidia in their different shapes and sizes, and the fungal isolates were diagnosed using taxonomic keys (Summerell et al., Bennett & Kwon, 1992).

2.3 Molecular Identification

Diagnosing the fungal isolate using the technique of determining the sequence of the nitrogenous bases of the genetic segment (ITS-DNA sequencing) is as follows:

2.3.1 DNA Extraction

DNA was extracted from the fungus using the method described in (Palanisamy and Alshehri 2020). The protocol instructions included in the diagnostic kit (PrestoTM Mini gDNA Yeast Kit) were followed and then photographed using the 0.8% agarose-gel-electrophoresis technique.

2.3.2 Polymerase Chain Reaction (PCR) using Universal Primers

The fungus' internal transcribed spacer region was amplified using PCR. The ITS region of the 5.8S rDNA gene was amplified by PCR reaction using universal forward and reverse primers ITS1-F:5'-TCCGTAGGTGAACCTGCGG-3' and ITS4-R:5'-TCCTCCGCTTATTGATATGC-3'. (Raja *et al*,2017).

2.3.3 NCBI BLAST

Basic Local Alignment Search Tool (BLAST) and National Center for Biotechnology Information (NCBI) both provided insurance for Phoma tropica.

2.4 Preparation of fungal biomass and synthesis of silver nanoparticles

2.4.1 Biomass preparation

The fungal mass was prepared by taking four discs of the pure, growing fungal colony at 7 days old using a sterile cork perforator with a diameter of 6 mm, piercing the edges of the growing colony on the fungal growth plate (potato dextrose agar (PDA). Place it in a 250 ml conical flask containing 100 ml of the previously prepared potato dextrose broth (PDB) with the addition of 125 mg of the antibiotic Chloramphenicol to ensure that bacteria do not grow. Then it is placed in a rotary shaker (120 revolutions/min) at 26°C for a period of time. 10 days for fungal mat with different weights (Karbasian et al., 2008).

2.4.2 Biosynthesis of Silver Nanoparticles

The method described by (Al-Shammari, 2015). was followed for the purpose of biosynthesis of silver nanoparticles outside the cell, with some modifications, as follows: Mushroom biomass was harvested after 10 days of incubation using a glass funnel and filter paper (Whatman filter paper No 1). The raw filtrate was removed and stored in the refrigerator for other uses. Dieses was followed by washing the biomass with sterilized distilled water three times, followed by washing it with deionized water twice, to get rid of all the remaining nutrient media. Weighed 10 g of mushroom biomass based on wet weight and in sterile conditions using a sensitive balance and transferred it to a 250-ml-conical flask containing 100 ml. Deionized water and also incubated under the same conditions as above with daily shaking with a shaker (120 rpm) for 72 hours. After the expiration of the period, the biomass was filtered using Whatman No. 1 filter papers, then using Millipore filters with a size of 0,45 and a weight of 0,017 g to reach a final concentration of 1 mM, and 100 ml of the culture filtrate was left without addition for the purpose of control. All flasks were incubated at a temperature of 28 °C in dark conditions for 72 hours.

2.5 Detection and Characterization of Silver Nanoparticles

2.5.1 Colour Changing

After 72 hours of incubation in dark conditions, the fungal culture filtrate solution (AgNO3 solution) turns brown. Diese means, that the process of synthesis of silver nanoparticles was confirmed by the color change. The AgNPs were then concentrated by centrifuging the reaction mixture at 6000 rpm for 20 minutes three times, then washed with distilled water and placed in a hot air oven at 40 °C for drying. After drying, the nanoparticles were collected, and the sample was stored in a glass vial.

2.5.2 Absorption UV-Visible Light Spectroscop

The sample was prepared to examine the absorbance spectrum after 72 hours of placing the AgNPs solution at 26–25 °C in dark conditions. Then 2 ml of the prepared AgNPs solution was taken, after filtering it from the mushrooms using sterile filter paper (Whatman No. 1), shaken well to homogenize the solution, and examined with a spectrophotometer at wavelengths of 300-900 nm. Untreated fungal cell filtrate was used as a control agent. The examination was carried out at the Polymer Research Center/University of Basrah after rinsing the device with sterile distilled water.

2.5.3 FT – Infra Red Spectrum (FT IR)

The infrared spectrum was recorded to determine the functional groups of the biomolecules that cover and stabilize the AgNPs (Umoren, 2014). by compressing a hard disk in the presence of potassium bromide (KBr) for the purpose of examination. The FTIR examination was conducted at the Polymer Research Center at the University of Basrah.

2.5.4 Scanning electron Microscoopy (SEM)

A scanning electron microscope (SEM) was used at the Electron Microscopy Unit in Iran at the University of Tehran to characterize the

morphology and size of silver nanoparticles (Caroling, *et al*., 2013).

2.6 Clinical specimen collection

Four pathogenic bacterial isolates were obtained from the central laboratory of Qurna Hospital, isolated from urinary tract patients (uropathogenic), belonging to Gram-negative and Gram-positive bacteria, which included *E. coli*, *K. pneumonia*, *P. aeruginosa*, and *S. aureus*. The diagnosis of the four isolates obtained was confirmed, and their sensitivity to antibiotics was tested using the Vitek2 Compact System (bioMerieux, Marcy I'Etoile, France) in the Al-Bayan Laboratory. Likewise, a pathogenic isolate of *Candida albicans* yeast was obtained from the Physiology Laboratory. Fungi in the Department of Life Sciences, College of Science, and University of Basrah, isolated from the skin.

2.7 Identification of Pathogenic Bacteria Isolates by Using Vitek 2 System

Bacterial isolates were identified using the Vitek 2 system at Al-Bayan Laboratory using the Vitek 2 NG REF 21341 kit (Harvey *et al*., 2013).

2.8 Antimicrobial activity of AgNPs

The Well Diffusion Method was used to measure the inhibitory effectiveness of silver nanoparticles against four pathogenic bacterial isolates, namely *E.coli, k.pneumonia, p. aeruginosa, and S. aureus*. The isolates were grown in sterile Petri media with a diameter of 90 mm, containing MHA-Medium, at 37 degrees Celsius for 24 hours. The bacterial suspension (inoculum) was diluted with sterile normal saline to reach 18 × 10 cells/cm^3. In comparison with the McFarland-standard solution for Turbidity. The bacterial suspension was then spread with a sterile cotton swab on the medium, and the dishes were left for 5 minutes. Sonication of AgNPs was performed for 3 minutes, after suspending them using sterile distilled water. Then, make three holes in each dish with a diameter of 6 ml using a sterile metal-cork-drill, then transfer 100 microliters of each concentration of silver nanoparticles (50, 100) micrograms/ml and 100 microliters of the fungal-culture-supernatant as a control agent. I also prepared dishes containing PDA-Medium and inoculated (108 x 1 cell)/cm3 of Candida albicans-Yeast-Fungal-Suspension. I left the dishes for 5 minutes, then made three holes for each dish and transferred 100 microliters of each concentration of silver nanoparticles at concentrations (50, 100) (Balouiri *et al*., 2016).

2.9 Statistical Analysis

The ready-made statistical program (Statistical Package for Social Sciences) (SPSS) version 26 was used to analyze the data statistically using Guide. The significant differences between the means were compared with the least significant difference (RLSD) test. At a probability-level of $p<0.01$ (SPSS 2020).

2.10 Statistical Analysis

We utilized the Statistical Package for the Social Sciences (SPSS), Version 2020 (Copyright IBM Inc., USA). The least substantial difference (LSD) test was used in the statistical data analysis that was performed using the Guide. Dieses Test was used to compare the substantial differences that existed between the averages with a probability threshold of p less than 0.01.

3 Results and Discussion

3.1 Isolation of Fungi

The current study showed the isolation of Aspergillus niger fungi from the soil of different locations in the city of Basra (Al-Qurnah - Al-Deir - Al-Faw - Shatt Al-Arab - Abu Al-Khasib - Al-Zubair). The isolated fungi showed the ability to biosynthesise nanosilver. The isolated species showed good growth on PDA-medium at a temperature of 28°C. The dominance of the genus Aspergillus was observed, as it appeared in all study sites. The reason for this is due to the wide spread of the species of this genus and the large quantities. of Conidia) (Al-Suhaili *et al* ,1982)

3.2 Phenotypic and microscopic diagnosis of the fungus

Characteristics and Microscopical Culture:

The results of laboratory culture showed that colonies of the A. niger fungus appeared after 3-5 days of incubation at a temperature of 25°C on soft or slightly woolly PDA-medium, with a black color resembling the color of the conidia. The conidial head appears in its greenish-black color (Figure A), and the results showed that microscopic examination has a head that appears in the form of a spherical, radial, or branched form. The Conidia also appear with thick, smooth, transparent walls. Either the Conidia appear spherical or elliptical in shape, brown in color, or numerous in number (Moubasher, 1993.) (Figure B).

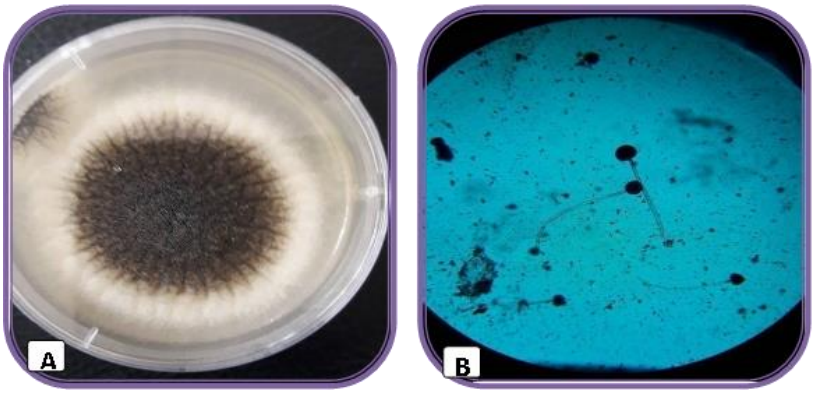


Figure 1: A colony of the fungus Aspergillus niger growing on PDA medium
B: Conidia and conidia under 40X magnification

3.3 Genetic identification of *A. niger*

3.3.1 GenomicDNAextraction

The DNA of the fungus Aspergillus niger was extracted in order to diagnose it molecularly using polymerase chain reaction (PCR) technology. The results showed the efficiency of the diagnostic kit used in extracting DNA, known as the PrestoTM Mini gDNA Yeast Kit (Genaid, USA), which is used in extracting DNA. The DNA was extracted according to the method). Alshehri and Palanisamy ,2020) The DNA bands appeared clearly after the electrophoresis process was performed on the extracted DNA when examined under ultraviolet rays, as shown in Figure 2.

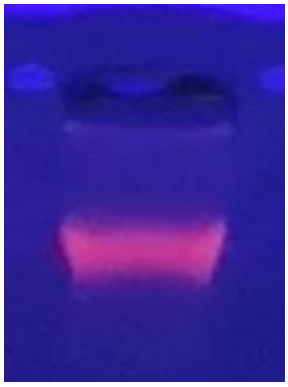


Figure 2: 0.8% of agarose gel Electrophoresis showed total DNA band of *Aspergillus niger*

3.3.2 PCR amplification

The results of amplification of the DNA obtained from the isolated fungi through PCR-technology and electrophoresis on a 1%-agarose-gel showed that the primers (ITS1-ITS4) amplified the genetic strand of the fungal isolates, whose DNA was extracted, in light of the positive results obtained when amplification was performed using these primers. The molecular size of the amplified band of the *A. niger* fungus was pb540, as shown in Figure 3. Diese Studie (Al-Sudani, 2015) showed that the molecular weight of the DNA replication band of the fungus *A. niger* was estimated.

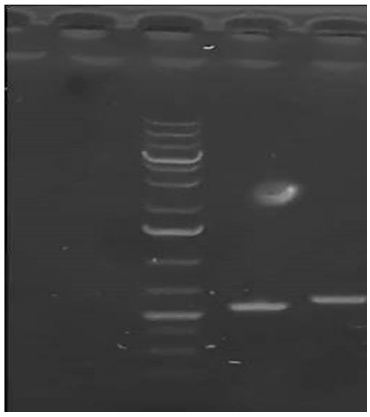


Figure 3: The electrophoresis of PCR

3.3.3 Sequencing of ITS gene

The Sequences of the nitrogenous bases of the interstitial region (ITS1-ITS4) of the fungal isolate were sent to the American company Psomagen, to determine the sequences and match them with the isolates in the NCBI GenBank and were analyzed using the website (http://www.ncbi.nlm.nih.gov). The results showed that the isolate belongs to the Aspergillus genus, *A. niger*. The results, using the BLAST tool program, also showed a 100% match with the global isolates registered in the database of the National Center for Biotechnology Information (NCBI) and GenBank, and the serial number of the isolate is MT273953.1.

3.4 Biosynthesis of Silver Nanoparticles (AgNPs)

3.4.1 Colour Changing

The isolated fungi showed the ability to biosynthesise silver nanoparticles, after growing them on PDB-fermentation media and treating the fungal culture filtrate (FCS) with the silver nitrate salt AgNO3, through preliminary detection by visual observation of the bioreduction process, which is responsible for the color changes in the culture filtrate. Fungal treatment after 24 hours of incubation in the dark at a temperature of 28°C The filtrate of the A. niger mushroom culture showed a color change from light yellow to dark brown (Figure 4). These results are similar to what the researcher reached (Abeer, 2015). (Figure 5) shows the amount of AgNPs obtained from mushrooms.

Figure 4: Color change observed in fungal cell filtrate (FCF) of ***A. niger*** *after* exposure to $AgNO_3$, a- without 1mM AgNO3 b- after 24 h treated with $AgNO_3$

Figure 5: Nanoparticles synthesized by *A.niger*

3.4.2 UV-Visible Spectrophotometry Analysis

By employing a UV-visible spectrophotometer to conduct qualitative testing on the supernatant, the reduction of silver ions was verified. After 24 hours, 1 ml of the sample supernatant was removed, and the absorbance has been determined between 300 and 900 nm, utilizing a UV-visible spectrophotometer (fig.6). At 450 nm, the absorbance peak was noted.

It was identical to what was obtained by (Soni and Prakash,2013). when preparing silver nanoparticles using the Aspergillus iiniger fungus, as the test result showed an absorbance value at the wavelength (450) nm when examining the UV.

While the result was close to what I found (Abeer, 2015). using a spectrophotometer, a band of 440 nm was recorded for silver nanoparticles manufactured from the fungus ATCC 16404 *A. niger*.

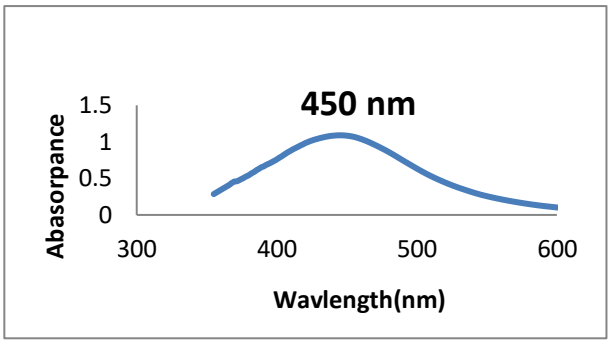


Figure 6: UV-Visible spectra of produced AgNPs by *A. Niger*

3.4.3 FTIR Analysis

An FTIR examination was conducted for the nanosilver solution biosynthesized by the fungus A. niger after mixing with silver nitrate to determine the possible interaction between silver and the biomolecules secreted by the fungi (proteins, enzymes) that are responsible for the reduction of silver ions and the formation of AgNps silver nanoparticles. (Shivaraj *et al*., 2013) The IR spectrum was shown, in relation to (Figure 7), as it gave absorption bands at (3405.67) cm-1, which belong to the ninth frequency of the (O-H) group, and also at the location (1638.23) cm-1, which returns to the ninth frequency of the bond (C=C), and the band. At the location (1384.64) cm-1, it returns to the (C-H) chain. As for the absorption beam at the location (107.01) cm1, it returns to the (C-O) chain frequency. At the location (619.038) cm-1, it returns to the (C-H) chain. The reductive activity of silver ions is due to the multiple active groups that appeared in the solution, as several studies have proven the ability of these groups to reduce in the process of preparing silver nanoparticles in previous studies (Joshi, 2012., Nabila *et al*., 2014). (Figure 7)

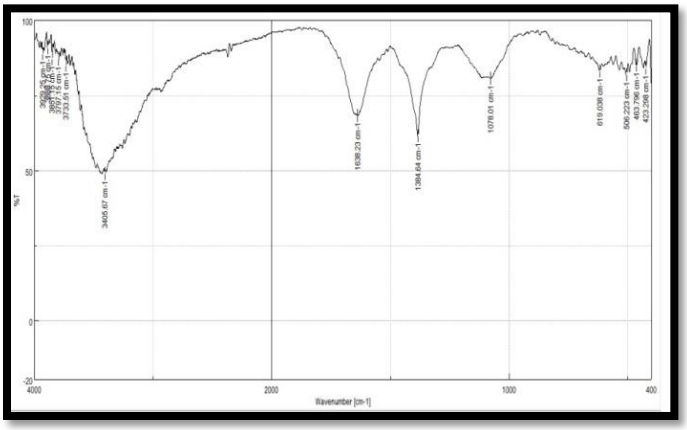


Figure 7: FTIR spectrum of AgNPs biosynthesized by *A. niger* with distinct peaks

3.4.4 Scan Electrons Microscope (SEM)

Scanning electron microscope images showed the shapes and sizes of silver nanoparticles produced from the filtrate of fungal cultures, using a magnification power of 200 kx. The microscopic images showed that the AgNps produced from the fungus A. niger were in the form of spherical aggregates with dimensions ranging between 17.84 and 64.03 nanometers (Figure 8) and were similar to the study conducted by who (Khalilor *et al*., 2013). found that the size of the nanoparticles ranges between 20 and 51 nm.

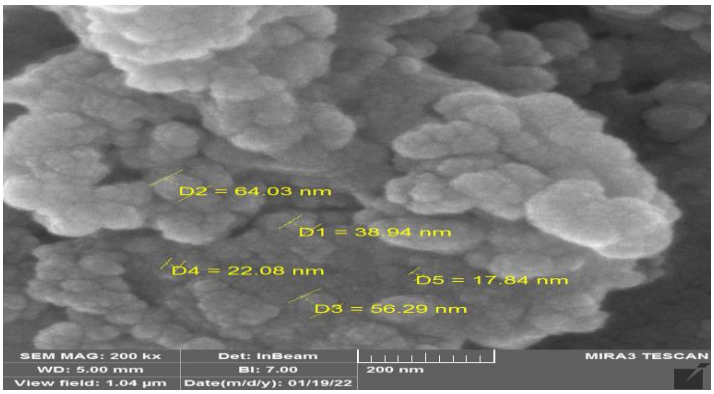


Figure 8: The biosynthesized AgNPs in the fungal free-cell filtrate were depicted in a SEM micrograph as spherical shapes aggregated with size ranges from 55 to 99 nm (magnification 200 K X)

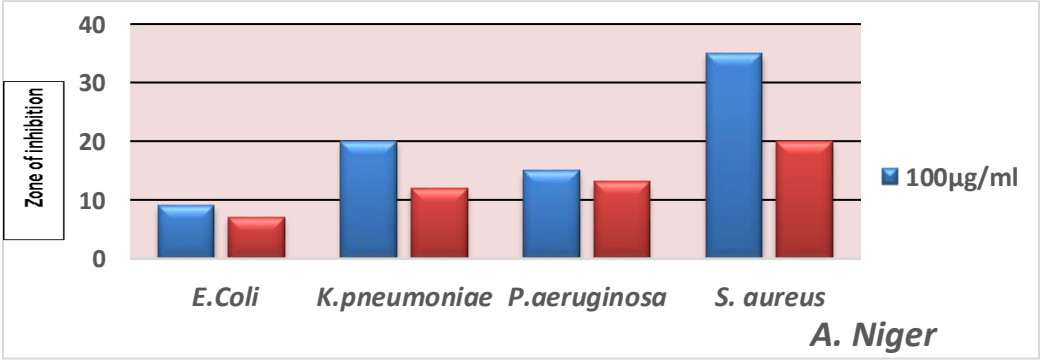
3.5 Antmicrobial Activity of AgNPs

3.5.1 Antibacterial Activity of Ag NPs on pathogenic Bacteria

Inhibitory activity of AgNPs synthesized from A. niger against the tested bacteria:

The results on the effect of concentrations of (50, 100) micrograms/ml of silver nanoparticles produced from the fungus *A. niger* on the growth of the resistant bacteria under study showed that there were differences in the rate of inhibitory effect at a significant level (1%), as shown in Figure 9. Dies was confirmed by the results of the statistical analysis, where the highest inhibitory capacity of AgNPs was against S. aureus bacteria, and the average diameter of the inhibition zone reached 20 mm at a concentration of 50 µg/ml, and the lowest inhibitory value against *E. coli* bacteria was 7 mm at the same concentration (Figure 10), and this is The result is consistent with the study carried out by the researcher .Which recorded the diameter of inhibition of silver nanoparticles against bacteria against *S. aureus*-bacteria as 24 mm at a concentration of 50 µg/ml. (Hussein, 2016). also noted that the therapeutic effectiveness of silver nanoparticles against *E. coli* bacteria was about 12 mm, which is similar to the results. The current study. The average diameter of inhibition reached 35 mm against S. aureus-bacteria at a concentration of 100 micrograms/ml.

AgNPs, and an average diameter of inhibition of 9 mm against *E. coli* bacteria at the same concentration, which is a result in agreement with the researcher (Vasudeva *et al*., 2015). Who recorded 12 mm? The result was consistent with the study he conducted (Hussein, 2016). While the average diameters of inhibition for both *K. pneumonia* and *P. aeruginosa* bacteria reached 13 and 12 mm, respectively, at a concentration of 50 micrograms/ml, while they recorded an inhibitory diameter of 20.15 mm, respectively, at a concentration of 100 micrograms/ml, These results agree with the findings (Hemashekhar *et al*., 2017) . Which recorded 20 mm inhibitory diameter for *K. pneumonia* bacteria, while it was recorded (C. Sundaramoorthi *et al*., 2009). The average inhibitory diameter of P. aeruginosa-bacte ria at 14 mm is a result that is similar to the current study.



RLSD at a significance level of P≤ 0.01.
L.S.D. for concentrations = 1.402; L.S.D. for bacteria = 1.983; L.S.D. for interference = 2.804

Figure 9: Zones of growth inhibition shown by four strains of human pathogenic bacteria in response to two concentrations (100.50) µg/ml of silver AgNPs produced by *A. niger*.

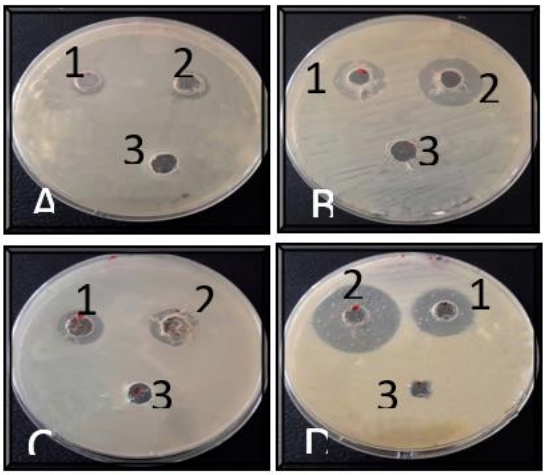


Figure 10: Average diameter of inhibition zones (mm) for a solution of silver nanoparticles of the fungus *A. niger*. Against tested pathogenic bacteria

***St.aureus* :D** ***P.aeruginosa* :C** ***K.pneumoniae* :B** ***E.coli* :A**

1. The concentration of the silver nanoparticle solution is 50 µg/ml.
2. The concentration of the silver nanoparticle solution is 100 µg/ml
3. Fungal culture filtrate, 100 µl/ml

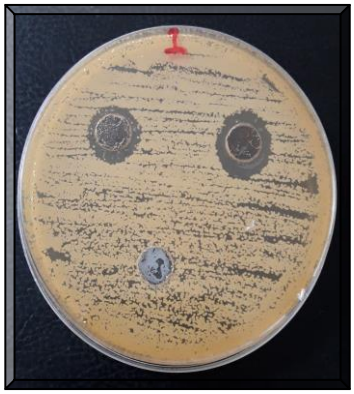


Figure 11: Average diameter of the inhibition zones (mm) of a solution of silver nanoparticles of the fungus A. niger Against C. albicans

3.5.2 Bioactivity of silver nanoparticles against C.albicans

Silver nanoparticles produced from mushrooms showed inhibitory ability against the yeast C. albicans, with significant differences at the probability level (1%), as at a concentration of 50 micrograms/ml for AgNPs synthesized from A. niger, the highest rate of inhibitory diameter reached 14 mm. While silver nanoparticles synthesized from nano-producing fungi at a concentration of 100 µg/ml for AgNPs showed an inhibition rate of 17.5 (Figure 11), These results are similar to many studies conducted by a number of researchers. (Gaikwad and Bhosale, 2012).

4 Conclusions

The fungal isolates showed Aspergillus niger, Aspergillus flavus, and Aspergillus. Sp, Phoma tropica isolated from local soil. Ability to produce silver nanoparticles. Resistance of pathogenic bacterial isolates (*E. coli*, *K. pneumonia*, *P. aeruginosa*, *S. aureus*) to many groups of antibiotics. Efficiency of silver nanoparticles in affecting bacteria. Diseases with multiple resistance to antibiotics and pathogenic yeasts (*C.albicans*). Increased joint effectiveness in inhibiting the growth of pathogenic bacteria in combination with the antibiotics Ampicillin and chloramphenicol at two concentrations (50 and 100) micrograms/ml for AgNPs.

Recommendations:

Testing other fungal species to produce silver nanoparticles. 2- Studying the effect of nanoparticles on other types of microorganisms, such as parasites, viruses, and fungi. Reducing the indiscriminate use of antibiotics without consulting doctors to reduce the resistance of bacteria to

antibiotics. Testing the effectiveness of silver nanoparticles against laboratory animals to determine their side effects. 5- Testing the ability of fungi to biosynthesise other metals such as gold and copper.

References:

Abeer. A.B. M. (2015). Biosynthesis and Size of Silver anoparticles Using *Aspergillus niger* ATCC 16404 as antibacterial activity, 4 (2), 522-528.

Al-Shammari, H. A. (2015). *Predator effect Dicrodiplosis manihoti HarrisDiptera:Cecidomyiidae and silvernanoparticles Planococcus citri (Risso) Hemiptera: Pseudococcidae.* PhD thesis, College of Agriculture, University of Baghdad.

Alshehri, B. & Palanisamy, M. (2020). Evaluation of molecular identification of Aspergillus species causing fungal keratitis. *Saudi J Biol Sci,* 27(2), 751-756.

Al-Sudani, A.A.M. (2015). *Phenotypic and molecular characteristics of Aspergillus niger and evaluation of its ability to produce citric acid under different conditions.* PhD thesis. College of Education. Al-Qadisiyah University. 63 p.

Al-Suhaili, I. A., Salih, Q.N. & Salem, I. A. (1982). *Fungi*. University of Marsal, Iraq.

Balouiri, M., Sadiki, M., & Ibnsouda, S. K. (2016). Methods for in vitro evaluating antimicrobial activity: A review. *Journal of pharmaceutical analysis, 6*(2), 71-79. https://doi.org/10.1016/j.jpha.2015.11.005

Caroling, G., Tiwari, S.K., Ranjitham, A.M. & Suja, R. (2013). Biosynthesis of silver anoparticles using aqueous broccoli extract-characterization and study of antimicrobial, cytotoxic effects. *Asian J Pharm Clin Res.* 6 (4), 165- 172.

Chakraborty, P., Prithiviraj, B., Selvaraj, S., & Kumar, B. (2016). Polychlorinated biphenyls in settled dust from informal electronic waste recycling workshops and nearby highways in urban centers and suburban industrial roadsides of Chennai city, India: levels, congener profiles and exposure assessment. *Science of the Total Environment*, *573*, 1413-1421. https://doi.org/10.1016/j.scitotenv.2016.07.129

Emily, A.; Morell, B. A.& Balkin, D. M. (2010). Methicillin-resistant *Staphylococcus aureus*: a pervasive pathogen highlights the need for new antimicrobial development. *Yale J. Biol.Med,* 84(4), 223-233.

Gaikwad, S. & Bhosale, A. (2012). *Green Synthesis of Silver Nanoparticles Using Aspergillus niger and Its Efficacy Against Human Pathogens*, 1675, 1654-1668.

Gupta, A., Eral, H. B., Hatton, T. A., & Doyle. P. S. (2016). Nanoemulsions: formation, properties and applications. *Soft matter*, 12(11), 2826-2841. https://doi.org/10.1039/c5sm02958a

Haddadine, N. & Chalal, S., Bouslah, N., Souilah, S., Benaboura, A. & Barille, R. (2014). Preparation and characterization of carbopol/silver nanoparticles composites obtained by heating process for antimicrobial application. *Journal of Polymer Research,* 21: 477.

Harvey, R.A., Cornelissen, C.N. & Fisher, B.D. (2013). *Lippincott's Illustrated Review Microbiology. 3d ed. Lippincott Williams and Wilkins Wolters Kluwer business*. USA.

Hemashekhar, B., Chandrappa, C P, Govindappa, M, Chandrasekhar, N., Nagaraju, Ganganagappa R. (2017). *Green synthesis of silver nanoparticles from Endophytic fungus Aspergillus niger isolated from Simarouba glauca leaf and its Antibacterial and Antioxidant activity*, 21,17-24.

Hussein, A. R. (2016). *Efficiency of some local isolates of Fungi and Bacteria in the production of nano particles and usability inhibitory to the growth of Bacteria pathogenic.* M.Sc, college of science, Department of Biology, University of Tikrit.

Johnson, L. E., Curl, E. A., Bond, J. H. & Fribourgh, H.A. (1959). *Methods for studying soil microflora – plant disease relationships.* Burgess. publ.Co. Minneapolis. USA.

Joshi, P. (2012). The anticancer activity of chloroquine-gold nanoparticles against MCF-7 breast cancer cells. *Colloids and Surfaces B: Biointerfaces,* 95, 195–200. https://doi.org/10.1016/j.colsurfb.2012.02.039

Karbasian, M., Atyabi, S.M., Siyadat, S.D., Momen, S.B., & Norouzian, D. (2008). Optimizing Nano-silver Formation by *Fusariumoxisporum* PTCC 5115 Employing Response Surface Methodology. *AM.J.Agi and boil,* 3(1); 433-437. https://doi.org/10.3844/ajabssp.2008.433.437

Khalil, N. M. (2013). Biogenic silver nanoparticles by Aspergillus terreus as a powerful nanoweapon against Aspergillus fumigatus. *Afr. J. Microbiol. Res, 7* (50), 5645-5651. https://doi.org/10.5897/ajmr2013.6429

Kown-Chung, K.J. & Bennett, J.E. (1992). *Medical Mycology.* Lea and Febiger, Philadelphia.

Moubasher. A.H. (1993). *Soil fungi in Qatar and other Arab countries*. Doha, Qatar.

Raffaele, M., Li Volti, G., Barbagallo, I. A., & Vanella, L. (2016). Therapeutic efficacy of stem cells transplantation in diabetes: role of heme oxygenase. *Frontiers in cell and developmental biology*, *4*, 80. https://doi.org/10.3389/fcell.2016.00080

Raja, H.A., et al., (2007). Fungal Identification Using Molecular Tools: A Primer for the Natural Products Research Community. *J Nat Prod,* **80**(3), 756-770. https://doi.org/10.1021/acs.jnatprod.6b01085

Rajiv K, S., Sunita, A., Krunal, C., Vinod, C., & Brijal Kiranbhai, S. (2010). Vermiculture technology: reviving the dreams of Sir Charles Darwin for

scientific use of earthworms in sustainable development programs. *Technology and Investment, 2010*. https://doi.org/10.4236/ti.2010.13019

Rother, M., Nussbaumer, M. G., Renggli, K., & Bruns, N. (2016). Protein cages and synthetic polymers: a fruitful symbiosis for drug delivery applications, bionanotechnology and materials science. *Chemical Society Reviews*, *45*(22), 6213-6249. https://doi.org/10.1039/c6cs00177g

Schillaci, D., Cusimano, M. G., Cascioferro, S. M., Di Stefano, V., Arizza, V.,Chiaramonte, M., . . . Gargano, M. L. (2017). Antibacterial activity of desert truffles from Saudi Arabia against Staphylococcus aureus and Pseudomonas aeruginosa. *International Journal of Medicinal Mushrooms, 19*(2). https://doi.org/10.1615/intjmedmushrooms.v19.i2.30

Shivaraj, N., Vandana Rathod, Jyoti. Dattu, I, Parema, K. & Manzoor, -UL-HAQ1. (2013). *Extracellular biosynthesis of silver Nanoparticles. using Aspergillus flavus and theis antimicrobial actifity against negative MDR strains,* p 227, 222-229.

Sundaramoorthi, C., Kalaivani. M, Dhivya Mariam Mathews, S. Palanisamy, V. Kalaiselvan, A. Rajasekaran, (2009). *Biosynthesis of silver nanoparticles from Aspergillus niger and evaluation of its wound healing activity in experimental rat model.* p: 1526, 1523-1529.