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Production of Silver Nanoparticles from *Curvularia tuberculata* Fungus and Evaluate its Antimicrobial Activity in Laboratory

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KEY WORDS:
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ABSTRACT

The creation of proteins in huge amounts and low toxicity of the residues make the fungi as the reducing agents in manufacture of silver nanoparticles.Silver nanoparticles are produced from fungi allow for the control of infections. They can use as antimicrobials in health and agriculture. Current study aimed to produce silver nanoparticles from *Curvularia tuberculata* fungus that was isolated from the soil. *C. tuberculata* mycelium filtrate had developed in glass flasks contain liquid fermentation medium for 10 days and resolved with silver nitrate salt AgNo3.The biosynthesis procedure was found by watching the color turns yellow to brown or dark brown by using Ultraviolet spectrometry (UV)that showed absorption peaks at wavelengths 420- 430 nm. A scanning electron microscope (SEM) was revealed the nanoparticles in spherical shapes with dimensions 8–100 nm. The spectrum revealed the existence of amide groups and protein residues, as well as carboxyl groups. The chemical groups in charge of the biosynthesis and encapsulation of nanoparticles were also found by the infrared spectrum (IR) for both fungal filtrates and the resulting particles after converting them into powder. The C. *tuberculata* showed its ability of silver nanoparticles biosynthesis after 42 hours of incubation. The silver particles produced by fungi detected inhibitory action against the pathogenic bacteria including *P. aeruginosa* and *S. typhi*, they also showed scavenging activity against *C. albicans* yeast. In conclusion: The fungus *C. tuberculata* showed the capability to produce silver nanoparticles and the possibility of using particles as antibiotics against pathogenic bacterial isolates and yeasts.

انتاج دقائق الفضة النانوية من الفطر *Curvularia tuberculata* وتقييم نشاطها المضاد للميكروبات في المختبر

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الخلاصة

إن إنتاج كميات هائلة من البروتينات، وارتفاع الانتاج، وانخفاض سمية المخلفات يجعل الفطريات عوامل اختزال في تصنيع جسيمات النانو الفضية. يتم إنتاج جسيمات النانو الفضية من الفطريات مما يسمح بالسيطرة على العدوى. ويمكن استخدامها كمضادات للميكروبات في مجالات الصحة والزراعة. تهدف الدراسة الحالية إلى إنتاج جسيمات نانوية فضية من فطر *Curvularia tuberculata* المعزول من التربة. تم تطوير ترشيح فطر *C. tuberculata* في وسط تخمير سائل في قوارير زجاجية لمدة 10 أيام وتم حله باستخدام ملح نترات الفضة AgNo3. تم اكتشاف إجراء التخليق الحيوي من خلال مشاهدة اللون يتحول من الأصفر إلى البني أو البني الداكن باستخدام مطيافية الأشعة فوق البنفسجية (UV) التي أظهرت قمم الامتصاص عند أطوال موجية 420-430 نانومتر. كشف المجهر الإلكتروني الماسح (SEM) عن الجسيمات النانوية في أشكال كروية بأبعاد 8-100 نانومتر. كشف الطيف عن وجود مجموعات أميد وبقايا بروتينية، بالإضافة إلى مجموعات كربوكسيل. كما تم تحديد المجموعات الكيميائية المسؤولة عن التخليق الحيوي وتغليف الجسيمات النانوية باستخدام طيف الأشعة تحت الحمراء (IR) لكل من المرشحات الفطرية والجسيمات الناتجة بعد تحويلها إلى مسحوق. أظهر فطر *C. tuberculata* قدرته على التخليق الحيوي لجسيمات الفضة النانوية بعد 42 ساعة من الحضانة. أظهرت جسيمات الفضة التي أنتجها الفطر نشاطًا مثبطًا ضد البكتيريا المسببة للأمراض، بما في ذلك الزائفة الزنجارية (*P. aeruginosa*) والسالمونيلا التيفية (*S. typh*i)، كما أظهرت نشاطًا كاسحًا ضد خميرة *C. albicans*. ختامًا: أظهر فطر *C. tuberculata* قدرته على إنتاج جسيمات الفضة النانوية، وإمكانية استخدامها كمضادات حيوية للبكتيريا المسببة للأمراض والخميرة.

الكلمات الافتتاحية: *Curvularia tuberculata*، جزيئات الفضة النانوية، احياء مجهرية مسببة للأمراض ، النشاط المضاد للميكروبات.

INTRODUCTION

The range of nanotechnology is today among the highest important areas of investment among the current science subjects due to the fast and steady daily progress of its concepts and the comprehensiveness and breadth of its applications that cover all of human life aspects, which formed a competition and excitement feeling. Republics, researchers and research institutions should invest effort and money in further nanotechnology development (Singh *et al.*, 2010), especially in the field of biotechnology and health technologies and at the level of devices and treatments (Prabhu *et al.*, 2010). Due to their high metal tolerance and ease of handling, fungi are suitable agents for the biogenic production of silver nanoparticles. Additionally, they release a lot of extracellular proteins that help keep the nanoparticles stable (Du *et al.*, 2015; Netala *et al.*, 2016). Aditionally, fungi can be used to produce nanoparticles through an internal or extracellular biogenic synthesis process. (Gudikandula *et al.*, 2017). Because of their ability to absorb and bioaccumulate metals, as well as their tolerance to heavy metals, these organisms are frequently employed as stabilizing and reducing agents. Additionally, it is simple to grow fungus on a big scale (in "nanofactories") and they can create nanoparticles with precise size and shape(Ahluwalia *et al.*, 2014; Khan *et al.*, 2017). The ability to synthesize enormous amounts of enzymes and proteins, some of them can be utilized for the maintainable and quick synthesis of nanoparticles, gives fungi an advantage over other microbes. (Alghuthaymi *et al.*, 2015). Fungi are considered one of the most important sources in the production of antibiotics. Many antibiotics have been identified from different types of fungi when grown in appropriate cultural media during the last three decades (Pohanka, 2006). The current research aimed to produce silver nanoparticles

utilizing local fungal species, represented by the fungus *Curvularia tuberculata*, detection and diagnosis of secondary particles using secondary technologies including: Scanning Electron Microscope (SEM), Infrared Spectroscopy (FTIR), and Ultraviolet Spectroscopy (UV-VIS) evaluating the biological efficacy of nanoparticles against isolates of pathogens of bacteria and yeasts.

MATERIALS AND METHODS

Growing of Fungi in Fermentative Media

The isolated fungi were grown on previously prepared MGYP, PGB media with the PH value adjusted to 6.4 as they tainted the 500 ml capacity glass flasks. A container containing 200 ml of each medium was transferred to two flasks with a diameter of 6 mm using a sterilized cork auger from pure fungal cultures, and all of the flasks were incubated for ten days at 25°C. (Sadowski *et al.*, 2008).

Biosynthesis of Silver Nanoparticles

An approach presented through (Karbasian *et al.,* 2008) was followed in order to the silver nanoparticles biosynthesis external the cell. Following the end of the incubation period time, the fungal cultures were then filtered by using Whitman (No.1-filter papers). Then, the raw filtrate was excluded, and then kept in refrigerator for further uses. To eliminate any suspended materials from the media, sterile deionized water was used to wash the fungal mycelium of each diameter three times.. 10 grams of each mycelium were wet weighed under sterile conditions, resuspended in one hundared ml of sterile deionized water, and incubated. All in an electric vibrator at 120 r/m at a temperature of 25°C for 72 hours. After the incubation period finished, the fungal cultures were filtered using Whatman No. 1 type filter papers. After that, using centrifugation at a rate of 6000 revolutions for ten minutes, one hundared ml of the filtrate of the fungi culture supernatant (c.s.) was taken for each fungus and silver nitrate salt Alfa Aesar AgNo3 99.9% was added to it. Germany with a (0.017) gram weight was added to arrive a (0.001 M) final concentration , and left (100) ml of the culture filtrate not including it. Additionally, for the control purpose, all of the flasks were incubated at (25°C) for 96 hrs. in dark conditions.

Detection and Characterization of Silver Nanoparticles

Ultra Violet Spectrum (UV- Vis) Test

After noting the color alterations in the fungal farms treated filtrate with nitrate silver, one ml of solution taken and used at regular intervals of 24-48–72 hours and diluted in a ratio of 1-3 using deionized water, then centrifuged at a rate of 800 rpm for 5 minutes, and then 1 ml of the supernatant was taken and examined with a spectrophotometer. Ultraviolet radiation type APEI PD303 Japan at wavelengths (300–900) nm (Bharathidasan and Panneerselvam, 2012).

Infrared Red Spectrum (FT IR)

Record Spectrum Infrared at wavelengths (500–4000 cm) using a SHIMA DZU-Japan device after converting the silver nanoparticles dissolved in the solution into a powder based on the method described by (Raheman *et al.,* 2011) and as follows: The solution containing the silver

particles was taken. Most of it was distributed in test tubes and mixed with a 5% acetone solution, then centrifuged at a rate of 4500 rpm/m. For 15 minutes after completion, the tubes were shaken well, Then the centrifugation process was repeated three times, after which the supernatant was emptied and 21 ml of acetone was was incorporated to the sedimented pellets and shaken properly. After that, the contents of the tubes were emptied into sterile glass Petri dishes and left at laboratory temperature for the purpose of evaporation. Then the precipitate was collected from the nano-silver powder, and via compressing a solid disk of (potassium bromide- KBr). The supernatant of the fungal culture was also used after drying it into powder as a control agent in the FT-IR test.

Scanning Electron Microscope Examination (SEM)

A scanning electron microscope (SEM) type inspector Netherlands was used for the objective of determining the shape and size of the molecules. samples were prepared according to (Vanmathi Selvi and Sivakumar, 2012) Filter the solution containing Ag NPs using sterile (0.2 µm millipore) filters and hold approximately 5 microliters of the solution was putted on an electron microscope holder made up of a gold and carbon clip. Room temperature was maintained for the samples for the purpose of drying and were subjected to examination using different magnification powers.

Estimation of the Biological Activity of Silver Particles Against Bacteria and Fungi

A specialist followed the etching diffusion method (Magaldi *et al.,* 2004) used the well diffusion method for the purpose of measuring the effectiveness of inhibition of the nanoparticles against two types of isolates of bacteria including; *Pseudomonas aeruginosa* and *Salmonella typhi*, by preparing a sterile petri -dishes with a (ninity mm) diameter comprising (MHA) media and inoculating them by the diffusion method with 0.1 mm of suspension. Bacterial (cell count 10^6 cells and optical density 0.1 at a wavelength of 540 nm) after leaving the dishes for 5 minutes, then making (3) holes in each petri- dish with a six mm in diameter by the using a sterile core dig and placing 50, 100 microliters of Ag NPs solution and one hundred microliters of the supernatant of the fungal culture was used as the control- agent. Also, the dishes containing (SDA medium) was prepared and incubated them with 0.1 ml (10^6 cells) of the fungal suspension of the standard yeast *Candida albicans* ATCC10231. Then, the dishes left for about five minutes, then two holes was made on each dish and placed one hundard microliters of AG NPs solution as well. The fungal culture supernatants were kept at 37°C degrees, and the results were recorded after 24 and 48 hours by calculating the zones of inhibition around the holes.

Statistical Analysis

The statistical analysis was done using the (Statistical Package for Social Science - SPSS version 11) was employed to examine obtaind information statistically by the using of complete randomized design (C.R.D.). The means of the coefficients were contrasted using the least adjusted significant differences (RLSD) at the (<0.05 p) probability level , according to what was stated in (Al-Rawi and Khalaf Allah, 1980).

RESULTS AND DISCUSSION

The isolated fungal species demonstrated the capacity to synthesize silver nanoparticles after their growth on fermentative media and the treatment of the filtrate (FCS) of fungal culture with the silver nitrate salt and (AgNo3) within first optical observation of the process of bio -reduction, that is accountable for the alters of color in the treated fungal cultures filtrate after (24) hrs. After an hour at 25°C in dark conditions, the C. *tuberculata* mushroom culture filtrate displayed a color change from colorless to brown color as shown in (Figure 1).

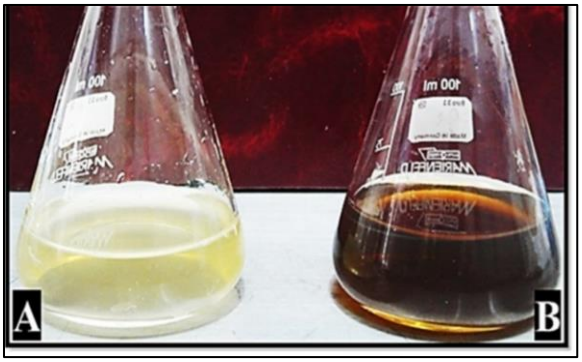

Figure 1. The color change of the C. *tuberculata* filtrate culture after the incubiotion for 24 hrs. A-The untreated culture filtrate -control. B- Silver nanoparticles culture filtrate

The results of the (UV-VIS) spectrum at wavelengths (300–900) nm, recorded after 24 hours, showed the appearance of different absorption peaks at specific wavelengths, where the fungus C. *tuberculata* showed a wide absorption section at the wavelength of 420 nm, which increased in it peaked over time, Reaching the highest absorbance value of 2.284 max at 72 hours as in Figure 2, while the filtrate of farms not treated with silver nitrate and utilized as a control agent did not show any absorption peaks at the aforementioned wavelengths.

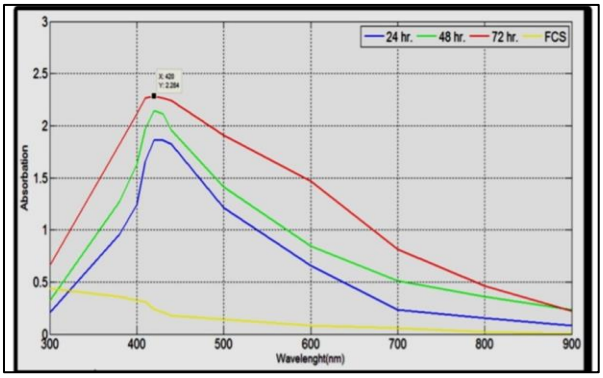

Figure 2. UV Spectrum of the fungus C. *tuberculata* at regular intervals (24-48-78) hours using fungal culture filtrate (FCS) as a control agent.

The results of the IR spectrum indicate the presence of a number of biomolecules in the filtrate of fungal farms before treatment (control) and after the construction of silver nanoparticles (Table 1). The IR spectrum of the fungus C. *tuberculata* showed a shift in the absorption bands at wavenumbers (3404.11 cm to 3398.34 cm), Which is due to the amplitude oscillation of a group NH. There is also a shift in the absorption bands due to the amplitude oscillation of the amide carbonyl group from wave number (1649cm^{1}- 1654 cm^{1}).

Table 1: Effective absorption bands and compositional aggregates for FCS fungal culture filtrate and silver nanoparticles.

Functional group	***C. tuberculata***	
	Fcs cm^{-1}	**Ag NPs cm^{-1}**
N-H, O-H	3404.11	3398.34
CH_2CH_2	2854.45	2854.45
	2925.81	2925.81
C=O-OH	1741.60	1733.89
C=O-NH	1649.02	1654
C-O	1257.50	1261.36
C-N	1809.02	1380.94

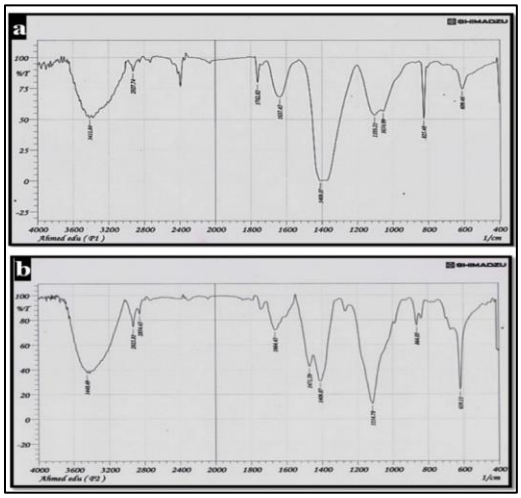

Figure 3. IR spectrum of the fungus C. *tuberculata*.
A-Fungal culture filtrate- control. B- Silver nanoparticles.

The images of Scanning Electron Microscope (SEM) indicated the sizes, shapes of the nanoparticles of silver created from the fungal cultures filtrate by the using of different magnification-powers, the images revealed that silver nanoparticles produced from C. *tuberculata* fungus are spherical shape and have a diameter of (50.7 nm), with dimensions ranging from (10 to 50 nm) as in Figure 4.

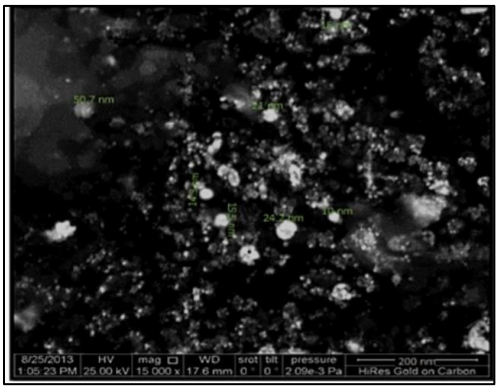

Figure 4.Electron microscope images of AgNps nanoparticles produced from the fungus C. *tuberculata* 15000x magnification.

The results of bioactivity showed that silver nanoparticles (AgNps) produced from the secondary metabolism of fungi species possess inhibitory activity against pathogenic bacteria were tested with significant differences at the probability level of 0.05 p (Table 2). Agnes showed the C. *tuberculata* diameter. The inhibition ability against *P. aeruginosa* bacteria was highest, and the inhibition zones average diameter was reached (25-28) mm as in Figure 5.

Table 2. The growth inhibition zones (mm) average diameter of sliver nanoparticles produced by fungi against the pathogenic bacteria using in this study.

Rate of growth inhibition diameters (mm)				**Fungi producing silver nanoparticles**
P. aeruginosa		***S. typhi***		
100 mµ	50 mµ	100 mµ	50 mµ	
28	25.5	20	17	*C. tuberculata*

RLSD At a significant level ($P \leq 0.05$) = 1.1070.

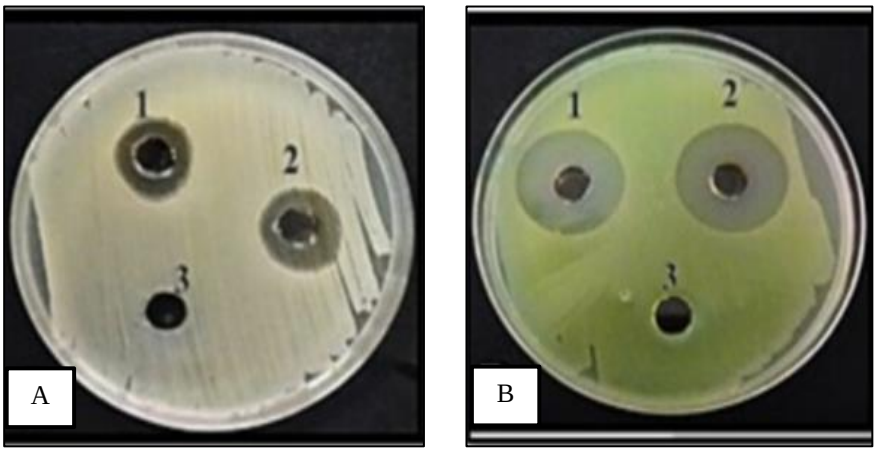

Figure 5. The inhibition zones in mm average diameter for the silver nanoparticles solution of the C. *tuberculata* against the pathogenic bacterial strains A- *S. typhi* B- *P. aeruginosa*.

1. The silver nanoparticle solution concentration is (50 µl/ml).
2. The silver nanoparticle solution concentration is (100 µl/ml).
3. Filtrate of fungal culture (100 µl/ml).

Silver nanoparticles produced from the tested fungi also showed bactericidal capability against the *Candida albicans*, as the nanoparticles produced from the C. *tuberculata*. The damping rate reached 36.5 mm. Figures 6.

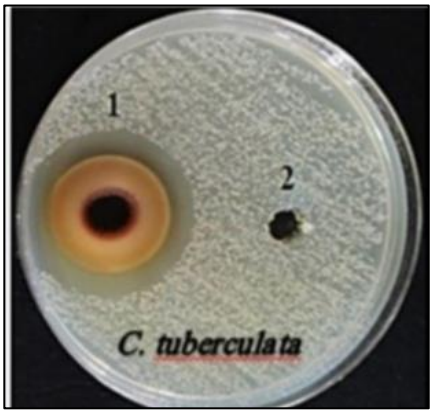

Figure 6. The inhibition zones in mm diameter for the silver nanoparticles solution of the C. *tuberculata* against the *C. albicans*.
1-The silver nanoparticle solution concentration is (100 µl/ml).
2-The fungal culture filtrate (144 µl/ml).

The Fungi are one of the vital tools used in biotechnology, and with the development of technologies during the past decade, Fungi have been used in bio nanotechnology as an alternative to chemical and physical methods. A number of fungi have been used in the biosynthesis of secondary metal particles, especially silver nanoparticles. They have been widely used in many applications, particularly medical and biological ones. (Kearns *et al.,* 2006; Sharma *et al.,* 2009; Silambarasan and Abraham, 2013). In the extracellular synthesis, the metal- precursor was added to the aqueous filtrate that contains just the fungal biomolecules, lead to in the formation of free nanoparticles in the dispersion, this method is the most popular because no steps are needed to release the nanoparticles from the cells. The mechanism of biogenic synthesis of nanoparticles using fungi can be either intracellular or extracellular. (Sabri *et al.,* 2016; Costa Silva *et al.,* 2017; Gudikandula *et al.,* 2017). However, to get rid of fungal residues and contaminants, the nanoparticle dispersion needs to be filtered. This can be done with techniques such simple filtering, membrane filtration, gel filtration, dialysis, and ultracentrifugation. (Qidwai *et al.,* 2018; Yahyaei and Pourali, 2019). The fungus is first cultured on agar and then transferred to a liquid media in order to create silver nanoparticles. The molecules involved in the synthesis are then released by transferring the generated biomass to water. Silver nitrate is added to the filtrate after the biomass has been filtered and disposed of. (Kearns *et al.,* 2017; Ottoni *et al.,* 2017).Even though employing fungus to synthesize silver nanoparticles is easy and efficient, the process's parameters need to be adjusted to produce particles with acceptable mono dispersity, stability, and biocompatibility. (Balakumaran *et al.,* 2015). Depending on the fungus being utilized, parameters like temperature, agitation, light, culture and synthesis periods can be changed to produce the required nanoparticle properties. The parameters employed for the fungus's culture and the synthesis process must be changed in order to control the size and shape of the nanoparticles. (Birla *et al.,* 2013). According to studies, nanoparticles with various physicochemical properties can be produced by varying the temperature, pH, culture medium, biomass amount, and concentration of the metal precursor. (Rajput *et al.,* 2016; Saxena *et al.,* 2016; Liang *et al.,* 2017).In the present investigation, a type of fungus isolated from local soil samples was selected for its ability to biosynthesize silver nanoparticles and detected and distinguished them using ultraviolet spectroscopy, scanning

electron microscopy, and infrared spectroscopy, and the resulting particles were used. Color changes were observed after 24 hours of incubation in the filtrate of the treated fungal cultures to a dark yellow and dark brown color, and an increase in color intensity was observed with increasing incubation time indicates the occurrence of the process of bio reduction of silver ions and the formation of secondary silver particles in the droplet filtrate, and this is the result of surface plasmon resonance of silver nanoparticles, which is consistent with the results of previous studies. (Ingle *et al.,* 2008 ; Fayaz *et al.,* 2010; Singh *et al.,* 2013).

One of the most crucial species for the manufacture of metal nanoparticles is fungus, as myco-synthesis is an important aspect of myco-nanotechnology (Kashyap *et al.,* 2013). On the other hand (Natarajan *et al.,* 2010), fungi have been used to construct nanoparticles for many metals such as gold, silver, platinum, cadmium, zirconia, iron, and silica, whether inside the cell or outside it (Krumov *et al.,* 2009 ;Gade *et al.,* 2010). One of the reasons for the success of fungi in nanotechnology is that they possess many advantages for biosynthesis compared to other living organisms. In addition to their great diversity in nature, they are relatively easy to isolate and develop in large quantities in the laboratory and using media of a green chemical nature (Vahabi *et al.,* 2011). Given the increase in pathogenic microorganisms such as bacteria and fungi and the development of antibiotic-resistant strains, there is an urgent need to search for inorganic agents and natural resources as antibacterials, as silver was considered one of the most powerful antiseptic agents (Prakash and Thiagarajan 2012, Gavanji *et al.,* 2013). Applications for silver nanoparticles include antibacterial and anti-inflammatory activity. Therefore, they have been used in medical tools during surgeries and organ transplant operations, in addition to textiles, bio-learning and sensing devices, as well as their anti-cancer activity (Seil and Webster, 2012; Silambarasan and Abraham, 2013; Patle *et al.,* 2013). Based on (Henglein, 1993), UV-V is spectroscopy is an important analytical tool for detecting silver nanoparticles in solution as a result of the optical properties exhibited by the nanoparticles.

A study by (Mukherjee *et al.,* 2008) using IR spectroscopy stated that the carboxylic carbonyl groups and free amine groups are perpendicular to the silver nanoparticles and are directly linked to them, leading to their encapsulation process. The research by (Zhang *et al.,* 2011) also mentioned that the metabolic products of fungi, such as enzymes and antioxidants, are responsible for the process of bio-reduction of metal ions and the formation of secondary particles. The Scanning Electron Microscope (SEM) that used in the current study, which is considered one of the important tools in determining the size of silver nanoparticles produced from the studied fungi.Images using different magnification powers showed that the majority of silver particles were spherical in shape, with some clusters appearing for those particles. As indicated in a study conducted by (Sadowski *et al.,* 2008). In addition, the current study results was consistent with a number of previous particle studies conducting by (Nithya and Ragunathan, 2009; Ghodake *et al.,* 2011; Vanmathi Selviand Sivakumar, 2012; Mahendran Vanaja *et al.,* 2011). Recent studies also indicated that the size of secondary silver ranges between (7-89) nanometers and is in spherical shapes at wavelengths (420–450) nanometers (Martínez-Castañón *et al.,* 2008; Pal *et al.,* 2007). It was noted through the study that there was a significant difference in the inhibitory effectiveness of the diameter-producing secondary particles in the inhibitory effectiveness of positive and negative bacteria. For Gram stain, this is consistent with a number of previous studies (Nithya and Ragunathan, 2009; Sunkar and Nachiyar, 2013; Jain *et al.,* 2010). The current study results

revealed a significant difference in the antibacterial effectiveness of the biosynthesized nanoparticles against Gram-positive and Gram-negative bacteria, as indicated by the variation in inhibition zone diameters. This enhanced inhibitory activity is particularly noteworthy when compared to the findings of a previous study, which demonstrated high levels of resistance among the same bacterial isolates to several conventional antibiotics, including Amoxicillin-Clavulanic acid, Cefotaxime, and Erythromycin (Mahdi *et al.,* 2024),. These results suggest that the nanoparticles developed in this study offer a more promising alternative for combating antibiotic-resistant bacterial strains. The results of these studies are mentioned in such a way that the nanoparticles produced from the fungus under study indicated high bactericidal activity against *C. albicans* yeast, and this supports the research carried out by (Sadhasivam *et al.,* 2010).

CONCLUSIONS

The great diversity of fungi, their ability to grow in low-cost media, and their production of many primary and secondary metabolites make them amongst the most significant microorganisms that currently used in nanotechnology and its medical applications. The C. *tuberculata* fungus showed the ability to produce silver nanoparticles and the possibility of using secondary particles as antibiotics for pathogenic bacteria and yeasts.

CONFLICT OF INTEREST

This study authors declare that they have no conflict of interest.

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