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BIOSYNTHESIS OF SILVER NANOPARTICLES USING LIGHT AND DARK OF *CLADOSPORIUM CLADOSPORIOIDES* AND ANTIFUNGAL ACTIVITY AGAINST PATHOGENIC FUNGI CAUSING ONYCHOMYCOSIS

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Abstract

Recently, Fungal isolates showed increasing resistance to antifungal agents and this had contributed to high morbidity among the patients. For that this study aimed biosynthesis of silver nanoparticals (AgNPs) using light and drak *Cladosporium cladosporioides* and evolution of antifungal against pathogenic fungi causing onychomycosis. The isolates from patients with Onychomycosis were distributed as Dermatophyte fungi, non-dermatophyte mold and yeast. Bio synthesis of AgNPs using light and drak of *C. cladosporioides*. The XRD for AgNPs Biosynthesis by *C.cladosporioides* in dark condition (C. dark) showed three peaks refer to $AgNO_3$, NaOH and Ag nanoparticals (111). In UV-Visible Spectroscopy showed A strong absorption peak at 440nm indicated the formation of AgNPs. In (SEM) showed aggregation surface morphology. The particle sizes were ranging from (2-150nm). While FTIR of AgNPs showed absorption bands at 2938.63 cm^{-1}, 2887.63cm^{-1}, 1541.41 cm^{-1} and 1114.21 cm^{-1}. While XRD for AgNPs Biosynthesis by *C. cladosporioides* in light condition showed two peaks refer to $AgNO_3$ and Ag nanoparticals. In UV-Visible Spectroscopy showed a strong absorption peak at 440nm indicated the formation of AgNPs. In (SEM) showed spherical surface morphology. The particle sizes were ranging from 1-750nm. While FTIR measurement showed absorption bands at 2938.63 cm^{-1}, 2887.63cm^{-1}, 1541.41 cm^{-1} and 1114.21 cm^{-1}. The effect of AgNPs at concentrations (25, 50 and 100) µg/ml against (2) isolates of *Trichophyton* spp and (5) isolates of mold study by Food poising method. Also study the effect of AgNPs against (4) isolates of *Candida* spp and (1) isolates of *Rhodotorula* spp by agar plate well diffusion assay. The results showed that all *Trichophyton* spp, mold isolates and some species of yeast were susceptible to AgNPs and the inhibition rate increases with the increase of concentration.

Key words: *Cladosporium cladosporioides*, Onychomycosis, silver nanoparticals.

Introduction

Onychomycosis (Tineaungium) is a denomination used to describe nail infection usually caused by dermatophytes, yeast and non-dermatophytic molds. These fungi cause onychomycosis particularly as secondary invaders after damage by trauma or disease (Soltani *et al*.,2015).The term onychomycosis is derived from the Greek word "onyx", a nail and "mykes" a fungul when George Meissner in1853 naked hyphae in a potassium Hydroxide preparation of "a thick finger nail" taken from 80 year old man. But is now used general term to signify all fungal infections of the nail.The a superficial fungal is keratinophylic meaning use the keratin found in the nail and hair for growth and development because it's contain enzyme called keratinize able utilize the keratin and caused infection. The factors that increase the prevalence of onychomycosis include increasing age, female gender, underlying conditions such as diabetes, immune deficiency, peripheral arterial disease and psoriasis, environmental and behavioral factors such as sporting and religious practices and certain professions. Genetics has also been identified as a factor governing the epidemiology of Onychomycosis (Neupane, 2009). Nanotechnology is one of the application most thoroughly investigated nanomaterials and owes its popularity to its biocidal properties (Kalaiselvam, 2013). Nanosilver is one of the most thoroughly investigated nanomaterials and owes its popularity to its biocidal properties (Kalaiselvam, 2013; Devi and Joshi, 2015). Silver nanoparticles (AgNPs) play an important role in the field of biology and medicine due to their attractive physiochemical properties and antibacterial activity antifungal, anti inflammatory, antiviral, anti-angiogenesis and antiplatelet activity also not shown to cause microbial resistance currently complicating

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antibiotic therapy of pathoginc fungi the biological synthesis of silver nanoparticals best than chemical synthesis of nanoparticals because Biosynthesis of nanoparticals give only small particals of silver nanoparticals (AgNPs) also lower environmental impact and non toxic effect on the patient but cost effective Although well-known toxic effects of silver in microorganisms (Logeswari *et al*., 2015).

Material and Method

Fungal isolates

In the period of November 2017 to March 2018, a total of 70 patients attending in Baghdad /Iraq. All isolates Cultivation on different media Sabouraud's dextrose agar (SDA) with chloramphenicol and cycloheximide for isolation dermatophyte fungi (*Trichophyton rubrum* and *Trichophyton mentagrophytes*; SDA with chloramphenicol for mould (*Aspergillus niger, Mucor* spp, *Penicllium* spp, *Syncephalastrum* spp and *Cladosporium* spp) and yeast isolates *Candida*spp and *Rhodotorula*. The culture were incubated at 28°C for 5-7 days to isolate pure colonies to examine their shape, size, color.

Biosynthesis of Silver Nanoparticles

The mycelia of *C. cladosporioides* were inoculated in 250 mL Erlenmeyer flasks, each containing 100 mL of potato dextrose broth (PDB) medium and incubated at 25 ± 2°C for 7 days. Later, mycelia were harvested by filtration through whatman filter paper no. 1 and washed thrice with sterilized distilled water to remove the traces of medium on fungal biomass. The washed mycelia were suspended into 100mL sterilized distilled water and incubated at 25°C for 24hrs. Again, mycelia were harvested by filtration through whatman filter paper no.1 suspensions were filtrated through a 0.22µm filter (Millipore). Then cell filtrate was treated with 1mM silver nitrate solution and incubated at room temperature. Positive controls containing cell free filtrate without silver nitrate and only 1mM silver nitrate as negative control were also maintained (Pandey *et al*., 2011). Effect of different temperatures, light and pH on the rate of synthesis of (AgNPs) was studied. The pH of the fungal filtrate was adjusted to 5 and 9 then treated with 1mM silver nitrate and kept at 28°C and 60°C in darkness and light till the synthesis of SNP was observed.

Characterization of silver Nanoparticals

1. XRD Analysis: The structure and composition crystallite size of synthesis of silver nanostructure determine by X-Ray Diffraction spectroscopy (Philips PNA analytical). The synthesis AgNPs were studies with cu-ka radiation at voltage of 30 KV and current of 20 mA with scan rate of 0.030/s.

2. UV-Visible Spectrophotometer: Biotransformation of silver ions was monitored by UV-visible spectroscopy measurement of the reaction medium. Three milliliters of supernatant were taken and absorbance was scanned by Labomed, UV-Vis double beam (Labomed, Inc, USA) within the wavelength ranging from 300 to 900 nm. The absorption of the visible depends directly on color of the chemicals in solution (Husseiny *et al*., 2015).

3. Scanning electron microscopy: Morphological characterization and elemental analysis of Bio-AgNPs were performed with an ultrahigh-resolution field emission scanning electron microscope (Nano SEM-FEI Nova 200-FEG/SEM: OR, USA), with integrated microanalysis X-ray system (energy dispersive spectrometer (EDS)) and electron backscatter diffraction (EBSD, EDAX-Pegasus X4M (EDS/EBSD)). For SEM analysis, a portion of freeze-dried of each C Bio-Ag-NPs was loaded in the specimen holder of the equipment and afterwards analysed (Pereira *et al*., 2014).

4. Fourier Transform Infrared Spectroscopy (FTIR): FTIR analysis of the dried powder of AgNPs was carried out by scanning the spectrum in the range 400-4,000 cm^{-1} at a resolution of 4 cm^{-1} (8400S/Shimadzu / Japan). FTIR measurements were made to locate the possible biomolecules, which are responsible for the reduction of silver ions to AgNPs and stabilization of AgNPs. To prepare dried powder of AgNPs, the fungal treated broth was centrifuged at 12000 g for 15 minutes. Supernatants were discarded and pellets of AgNPs were washed three times with autoclaved distilled water. The dried powder of AgNPs was subjected to FTIR analysis.

Antagonistic activity of Silver Nanoparticles against fungi

1. Inhibitory effect of silver Nanoparticales against Dermatophyte and Non-dermatophyte by food poising Method.

The antifungal activity of silver nanoparticles on most resistance isolates of dermatophytes fungi (*Trichophyton rubrum* and *T. mentagrophtes*) and non dermatophyte mold (*Aspergillus niger*, *Penicillium* spp, *Cladosporium cladosporioides*, *Mucor* spp and *Syncephalastrum* spp) were assay by food poising method. SDA disk (5mm diameter) of a pure culture of fungal isolates taken from the margins of (4-7 days -old culture, dermatophyte and non dermatophyte respectively were placed on to the critter of AgNPs at concentration (25, 50, 100) µg/ml (3ml of each concentration mixed with 27ml of SDA). Control petri plates contained only SDA. All plates were

incubated at 28°C for 4-7 days. Then growth measurements were taken using ruler. Three petri dish for each treatment were considered this evolution.

2. Inhibitory effect of Silver Nanoparticles against yeasts by agar plate well diffusion assay.

The antifungal activity of the silver nanoparticles on four most resistance isolates of *Candida* spp (*C. albicans, C. famata, C. guilliermondii* and *C.lusitaniae*) and one isolate of *Rhodotorula* spp were assayed by agar plate well diffusion assay. Method described by Abood, (2014) was followed to detect of AgNPs inhibition activity by spreading 0.1 ml of the yeast suspension on the surface of SDA and left to dry at room temperature. 100 µL of an AgNPs solution, 25, 50 and 100 µg/mL concentrations were added into 5 mm diameter wells, Later that incubated for 24 hrs at 37°C then the zone of inhibition has been measured using ruler.

Statically analysis

Minitab software version 6 was used for analyzing data, the ANOVA-test has been done to calculate the P value between the control and test groups in the previous studies. Least significant difference -LSD test was done also to compare means between groups in this study the results were presented as mean ± SD.AP value equal or less than 0.05 was considered as the level of statistical significance.

Results and Discussion

Properties of silver Nanoparticles

1. X-Ray Diffraction: Further confirmation of synthesized AgNPs was examined by the XRD Diffraction spectroscopy the XRD pattern of 1Mm silver nitrate solution prepared in dark condition which deposited on glass substrate by drop casting method which it can be observed a narrow and sharp peak at diffraction angle 29.46° refer to Hydroxide sodium, the second peak located at 31.94° refer to $AgNO_3$, also, the third peak located at 38.14° refer to the AgNPs with plane of (111) according to (JCPDS No 04-0783) like in fig. 1.

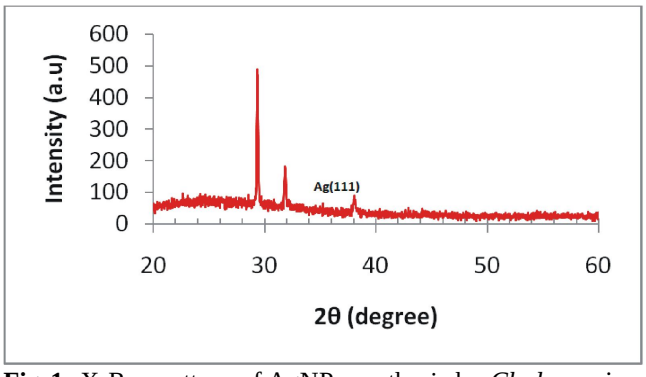

Fig. 1: X-Ray pattern of AgNPs synthesis by *Cladosporium cladosporioides* in Dark in pH9 (C. dark).

In fig. 2 showed XRD pattern of the 1mM of silver nitrate prepared in light condition which revealed polycrystalline nature with a cubic structure the observed tow narrow and sharp beak, the first peak at 32.32° indicated to silver nitrate and the second peak is refer to Ag nanostructure at 38.14° with plane (111).

Pereira *et al*., (2014) show the XRD pattern of diffraction peaks showed at 2 values of 32.32°, 45.99°, 66.72° and 75.76° assigned to the planes of (111), (200), (220) and (311) faced centre cubic (FCC) of silver were obtained ranging from 20 to 80. The values agree well with those reported for silver (face centric cubic) by JPCDS No. 04-0783.

2. UV-Visible Spectrophotometer: The presence of nanoparticles was confirmed by obtaining a spectrum in the visible range of 30nm-600nm using UV visible spectrophotometer. From this analysis, absorbance peak 440nm which is specific for the AgNPs. In fig. (2a,b) and fig. (3a,b) showed single peak indicating synthesis of spherical nanoparticals in dark condition at 60°C and light condition. These results agree with Husseiny *et al*., (2015) AgNPs synthesized shown maximum absorbance peak at 420 nm by UV-Visible spectroscopy.

3. SEM Analysis: Morphological characterization and elemental analysis of Bio-AgNPs were performed with an ultrahigh- resolution field emission scanning electron microscope (Nano SEM-FEI Nova 200-FEG/SEM: OR, USA), fig. 4 showed Aggregation surface morphology. The particle sizes were ranging from nm in dark condition. While in fig. 5 showed spherical surface morphology. The particle sizes were from 92nm in light condition. (Rathna *et al*., 2013) show the SEM images and their size distributions revealed that, the mean diameters and standard deviation of silver nanoparticles were about 20-80 nm with the different morphologies. Electron microscopy data indicate that the extracellular particles produced.

4. Fourier Transform Infrared Spectroscopy (FTIR): FTIR measurements of the samples of dried powder were

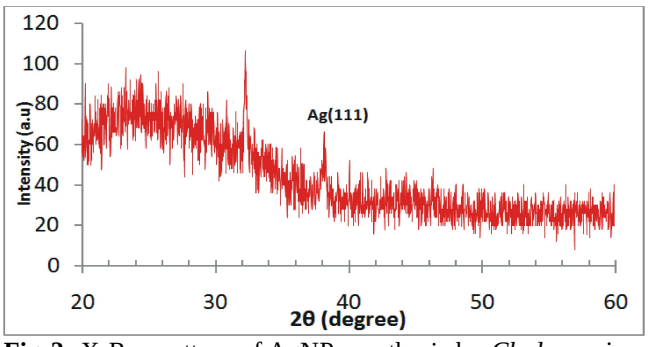

Fig. 2: X-Ray pattern of AgNPs synthesis by *Cladosporium cladosporioides* in light in pH9.

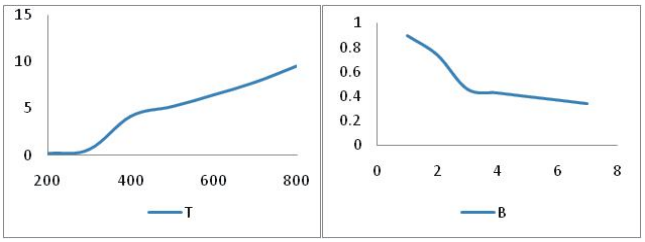

Fig. 2: (a, b) Absorptions and translation spectrum of biosynthetic AgNPs using *Cladosporium cladosporioides* dark condition and pH9.

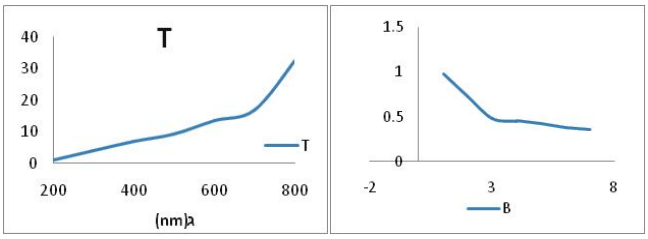

Fig. 3: (a, b) Absorptions and translation spectrum of biosynthetic AgNPs using *Cladosporium cladosporioides* in light condition and pH9.

carried out to identify the probable interactions between silver and bioactive molecules, which may be responsible for synthesis and stabilization (capping material) of AgNPs. In fig. (6 and 7), the FTIR spectrum of AgNPs synthesis by *Cladosporium cladosporioides* in dark (C. dark) and light (C. light) with six main peaks at different locations including at 822.85 cm^{-1}, 1397.47cm^{-1}, 1541.41cm^{-1}, 2854.90 cm^{-1}, 2887.63 cm^{-1} and 2938.63 cm^{-1} which are associated with the several oxygen-comprising functional groups. The overall observation confirms the presence of protein in samples of silver nanoparticles from *Cladosporium cladosporioides* it has also been reported earlier that protein can bind to silver nanoparticles through their free amine groups or cysteine residues (Gole *et al*., 2001 and Jeevan *et al*., 2012).

Inhibitory effect of AgNPs Synthesis *C. cladosporioides* Against Onychomycosis fungi.

1. Inhibitory effect of AgNPs Against dermatophytes by poising food assay.

The inhibitory effects of various concentrations of AgNPs (25, 50 and 100µg/mL)were assayed on two dermatophyte isolates (*T. rubrum, T. mentagrophtes*) by poising food assay.the effect of AgNPs synthesis *Cladosporium cladosporioides* in dark (C. dark) in pH 9 and *Cl. Cladosporioides* in light (C. light) in pH 9. In 100µg/ml concentration the high inhibition effect of AgNPs synthesis by (C. dark) which presented in *T. mentagrophytes* with growth rate (10.0mm), While found effect of AgNPs was on *T. rubrum* with growth rate (13.0mm). While 50µg/ml. The high inhibition effect of AgNPs which presented in *T. mentagrophtes* at growth rate (15.0mm), while low effect of AgNPs was on *T. rubrum* with growth rate (17.0mm).

In 25µg/ml the high inhibition effect of AgNPs which presented in *T.rubrum* and *T.mentagrophtes* with growth rate (20.0mm). The results of statistical analysis showed significant ($p \leq 0.05$) between *Trichophyton*sppisolates. While 100 µg/ml the high inhibition effect of AgNPs synthesis by (C. light) AgNPs which presented in *T.mentagrophtes* with growth rate (15.0mm). But found the low effect in *T.rubrum* with growth rate (18.0mm). In the 50µg/ml concentration the inhibition effect AgNPs which presented *T.rubrum* and *T.mentagrophtes* with growth rate (20.0mm). While in 25µg/ml concentration found the inhibition effect AgNPs which presented in *T.rubrum* and *T.mentagrophtes* with growth rate (25.0)mm. The results of statistical analysis showed significant ($P \leq 0.05$) between *Trichophyton* spp isolates like in table 1.

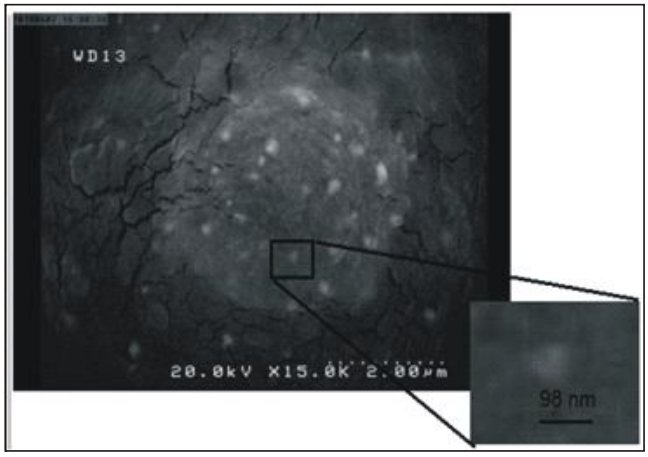

Fig. 4: Scan Electron microscope (SEM) image of silver nanoparticals synthesis by *Cladosporium cladosporioides* in dark condition with size 98 nm.

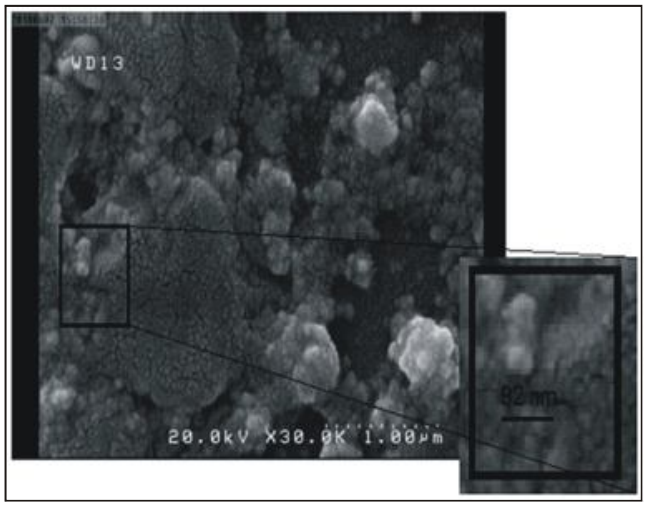

Fig. 5: Scan Electron microscope (SEM) image of silver nanoparticals synthesis by *Cladosporium cladosporioides* in light condition with size 92 nm.

For non-dermatophyte fungi in 100µg/ml concentration the high inhibition effect of AgNPs synthesis by (C. dark in) which presented in *Aspergillusniger* and *Penicillum* spp with growth rate (13.2±0.4 mm) and (13.4±1.1mm) respectively. Followed by *Cladosporium* spp with growth rate (15.0mm), while found effect of AgNPs was on *Mucor* spp and *Syncephalastrum* spp with growth rate (20.0mm). The results of statistical analysis showed significant (P≤0.05) between *Mold* spp isolates. While 50µg/ml The high inhibition effect of AgNPs which

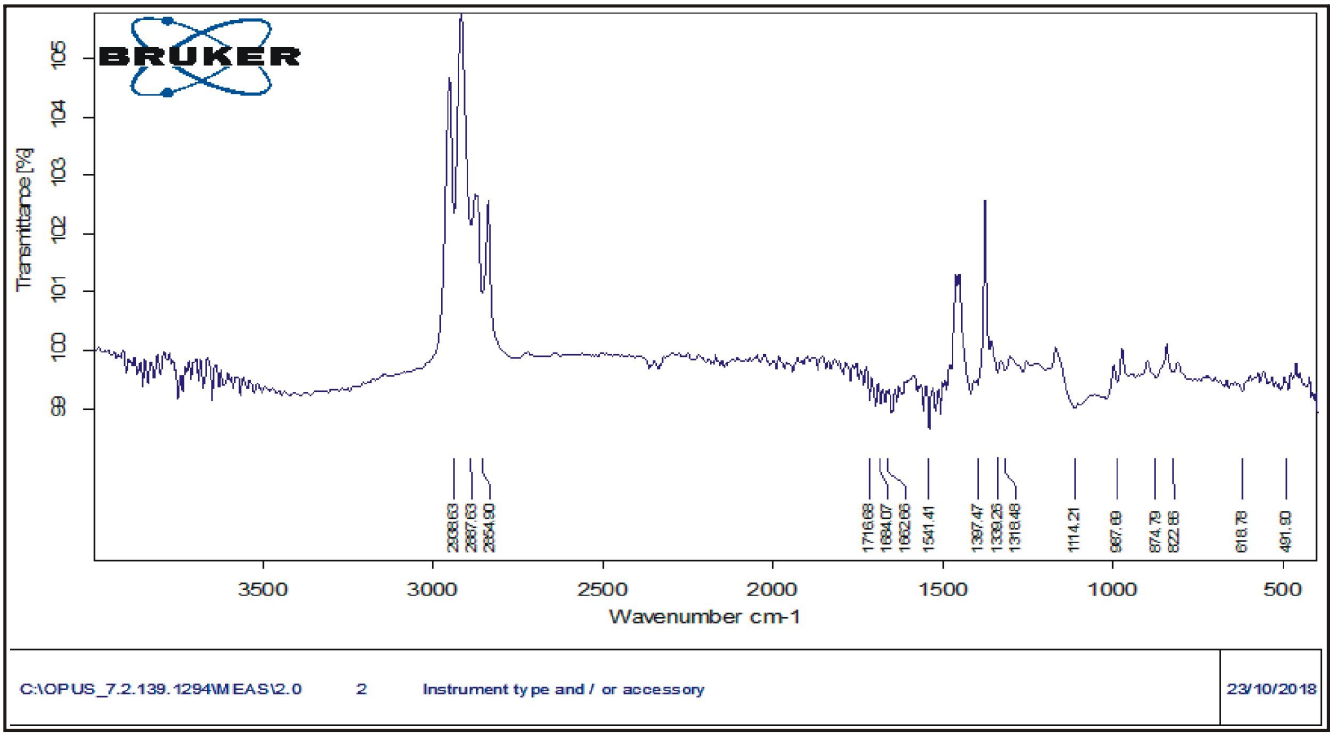

Fig. 6: Fourier transform infrared (FT-IR) spectrum of Bio-AgNPs synthesized by *cladosporium* in dark in pH9.

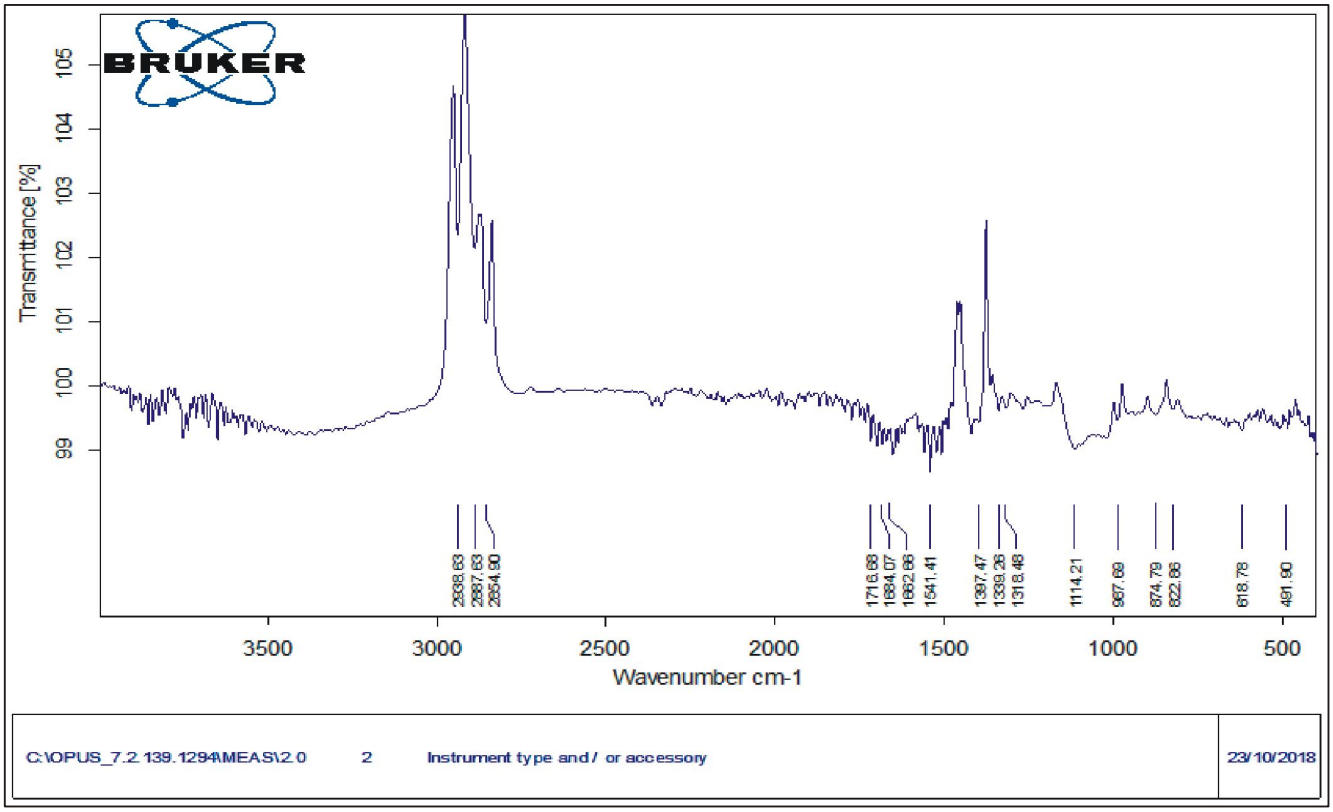

Fig. 7: Fourier transform infrared (FT-IR) spectrum of Bio-AgNPs synthesized by *cladosporium* in light in pH9.

Table 1: Effect of silver nanoparticles synthesis by *Cladosporium cladosporioides* against Dermatophytes fungi by poising food assay.

Fungi type	Mean colony diameter (mm) ± SD						
	Concentration of nanoparticles (µg/ml)						
	Control	Clado.dark			Clado.light		
		100	50	25	100	50	25
T.rubrum	90.0± 0.0	13.0±0.0	17.0± 0.0	20.0±0.0	18.0±0.0	20.0±0.0	25.0±0.0
T. mentagrophytes	90.0±0.0	10.0±0.0	15.0±0.0	20.0±0.0	15.0±0.0	20.0±0.0	25.0±0.0
P value	0.05	0.05	0.05	0.05	0.05	0.05	0.05

presented in *A.niger* at growth rate (10.0mm). followed by *Penicllium* spp and *Cladosporium* spp with growth rate (20.0 mm), while low effect of AgNPs was on *Mucor spp and Syncephalastrum* spp with growth rate (25.0mm). The results of statistical analysis showed significant ($p \leq 0.05$) between *Mold* spp isolates. But in 25µg/ml the high inhibition effect of AgNPs which presented in *A.niger* with growth rate (20.0mm) and *Penicllium* spp with growth rate (25.0mm). While the growth rate for *Mucor* spp, *cladosporium* spp and *Syncephalastrum* spp are (30.0mm). The results of statistical analysis showed significant ($p \leq 0.05$). The high inhibition effect of AgNPs synthesis by (C. light) AgNPs which presented in *A.niger* with growth rate (12.3±0.4mm) and *Cladosporium* spp with growth rate (17.0mm). But found the growth rate of *Penicllium* spp are (18.0 mm). While the low effect in *Mucor* spp and *Syncephalastrum* spp with growth rate (25.0mm). The results of statistical analysis showed significant ($P \leq 0.05$) between Mold sppisolates. While 50µg/ml the high inhibition effect of AgNPs which presented in *A.niger* at growth rate (15.0mm). Followed by *Penicllium* spp with growth rate (20.0 mm) and *Cladosporium* spp with growth rate (25.0mm). While low effect of AgNPs was on *Mucor spp and Syncephalastrum* spp with growth rate (28.0mm), the results of statistical analysis showed significant ($p \leq 0.05$) between Mold spp isolates. But in 25µg/ml the high inhibition effect of AgNPs which presented in *A.niger* and *Penicllium* spp with growth rate (20.0mm) and (25.0mm). While the growth rate for *Mucor* spp, *cladosporium* spp and *Syncephalastrum* spp are (30.0mm). The results of statistical analysis showed significant ($p \leq 0.05$) like in table 2.

This results agree with Fird house and Lalitha, (2013) who find efficacy of synthesized nanoparticles was tested against *T. rubrum, E. floccosum* and *T. mentagrophytes* by well diffusion method at different concentration levels are (10±0.03)mm for *T.rubrum*, (10±0.02) mm *E. floccosum* and *T. mentagrophytes* (9±0.1)mm. While Pereira *et al*., (2014) find Bio-AgNPs produced by the fungal cell-free filtrate showed an antifungal activity higher than fluconazole but less than terbinafine, itraconazole and Chem-AgNPs. Noorbakhsh *et al*., (2011) who find Ag-NPs can inhibited the mentioned fungus at 10 microgeram per mili liter (µg/ml). Regards to this concentration, Ag-Nps shows less inhibitory efficiency than griseofulvin (0.8 µg/ml), but more efficiency than fluconazole (40µg/ml). For non-dermatophyte fungi Xu *et al*., (2013) showed that the activity of nano-silver against *Aspergillus*spp. Is tow times greater than of Amphotericin B.Also Agree with Pulit *et al*., (2013) showed that even a low concentration of Nano silver particals makes it possible to achieve a high percentage of growth inhibition.

2. Inhibitory effect of AgNPs Against.

• Yeast by Agar plate well Diffusion assay

In table 3 In (100, 50, 25 µg/ml) concentration of

Table 2: Effect of silver nanoparticles synthesis by *Cladosporium cladosporioides* against Non-Dermatophytes fungi by poising food assay.

Type of mold	Mean colony diameter (mm) ± SD						
	Concentration of nanoparticles (µg/ml)						
	Control	Clado.dark			Clado.light		
		100	50	25	100	50	25
Cladosporium spp	90.0±0.0	15.0±0.0	20.0±0.0	30.0±0.0	17.0±0.0	25.0±0.0	30.0±0.0
Aspergillusniger	90.0±0.0	13.2±0.4	10.0±0.0	20.0±0.0	12.3±0.4	15.0±0.0	20.0±0.0
Penicllium. spp	90.0±0.0	13.4±1.1	20.0±0.0	25.0±0.0	18.0±0.0	20.0±0.0	25.0±0.0
*Mucor.*spp	90.0±0.0	20.0±0.0	25.0±0.0	30.0±0.0	25.0±0.0	28.0±0.0	30.0±0.0
Syncephalastrum. spp	90.0±0.0	20.0±0.0	25.0±0.0	30.0±0.0	25.0±0.0	28.0±0.0	30.0±0.0
LSD	0.3	1.4	2.1	2.2	3.4	5.1	3.3
P-value	0.05	0.05	0.05	0.05	0.05	0.05	0.05

Table 3: Effect of silver nanoparticles synthesis by *Cladosporium cladosporioides* against yeast by cell diffusion method.

Type yeast	Inhibition zone (mm) mean± SD						
	Concentration of nanoparticles (µg/ml)						
	Control	Clado.dark			Clado.light		
		100	50	25	100	50	25
Candida albicans	6.0±0.0	17.7±2.2	17.7±2.2	6.0±0.0	20.0±0.0	14.5±2.2	10.0±0.0
Candida famata	6.0±0.0	6.0±0.0	6.0±0.0	6.0±0.0	6.0±0.0	6.0±0.0	6.0±0.0
Candida guilliermondii	6.0±0.0	19.2 ±1.1	19.1±1.1	0.0±0.0	6.0±6.0	6.0±0.0	6.0±0.0
Candida lusitaniae	6.0±0.0	6.0±0.0	6.0±0.0	6.0±0.0	6.0±0.0	6.0±0.0	6.0±0.0
Rhodotorula. spp	6.0±0.0	6.0±0.0	6.0±0.0	6.0±0.0	6.0±0.0	6.0±0.0	6.0±0.0
LSD	0	0.8	0.8	0	5.5	4.4	3.4
P value	NS	0.05	0.05	NS	0.01	0.01	0.01

AgNPs synthesis by (Clado in dark and light conditions), shows the effect of AgNPs synthesis by *Cladosporium cladosporioides* on different species of *Candida* the results showed that some *Candida*spp isolate were susceptible to AgNP and the inhibition rate increases with the increase of concentration. In 100µg/ml concentration of AgNPs synthesis by (Clado in dark) the high inhibition effect of AgNPs which presented in *C.guilliermondii* followed by *C.albicans* with inhibition zone (19.2± 1.1 mm) and (17.7± 2.2 mm) respectively. While not found any effect of AgNPs was on *C. famata* and *C. Lusitania* and *Rhodotorula* spp with inhibition zone (6.0 mm). The results of statistical analysis showed significant ($P \leq 0.05$) between *Candida spp* and *Rhodotorulaspp* isolates. While in 50µg/ml concentration. The high inhibition effect of AgNPs which presented in *C.guilliermondii* followed by *C.albicans* with inhibition zone (19.2± 1.1 mm) and(17.7± 2.2 mm) respectively, while not found resistance effect of AgNPs was on *C.famata*, *C.lusitaniae* and *Rhodotorula* spp with inhibition zone (6.0mm). The results of statistical analysis showed significant ($p \leq 0.05$) between *Candida* spp and *Rhodotorula* spp. In 25µg/ml concentration was found all *Candida*spp and *Rhodotorula*spp are resistance effect of AgNPs. The results of statistical analysis showed Non significant between *Candida* spp and *Rhodotorula* spp. While in 100µg/ml concentration of AgNPs synthesis by (Clado in light) the high inhibition effect of AgNPs which presented in *C.albicans* with inhibition zone (20.0mm). But found the other *Candida* spp and *Rhodotorula* spp are resistance effect to AgNPs. The results of statistical analysis showed significant ($P \leq 0.01$) between *Candida* spp and *Rhodotorula* spp isolates. In50µg/ml concentration found the high inhibition effect AgNPs which presented in *C.albicans* with inhibition zone (14.5± 2.2mm). But found the other *Candida* spp and *Rhodotorula* spp are resistance effect to AgNPs. The results of statistical analysis showed significant ($P \leq 0.01$) between *Candida* spp and *Rhodotorula* spp isolates. In 25µg/ml concentration found the high inhibition effect AgNPs which presented in *C.albicans* with inhibition zone (10.0mm). But found the other *Candida* spp and *Rhodotorula* spp are resistance effect to AgNPs. The results of statistical analysis showed significant ($P \leq 0.01$) between *Candida* spp and *Rhodotorula* spp isolates. This results partially agree with the results Nasrollahi *et al*., (2011) show the antifungal effects of silver nanoparticles on *C. albicans* by attach AgNPs with cell membrane and penetrate in the fungi then produce a site witch little molecular weight in center of fungi and then AgNps attach to respiratory sequence and finally cell division stop lead to cell death. (Morones *et al*., 2005) showed the antimicrobial effects of AgNPs depended on their size and the rate of silver ion release. The antifungal activity of Ag-NPs against *Candida* spp as models for fungi was investigated and Ag-NPs has been used as a comparable antifungal drug by antifungal drugs like Amphotericin B, Fluconazole. Ag-NPs exhibited a potent antifungal activity against fungal strains tested (Lara *et al*., 2015). Mechanisms of antifungal effect of silver nanoparticles: Effect On cell membrane By inhibition cell wall formation. Cell wall is composed of ergosterol the silver nanoparticles disrupt the cell membrane by inhibiting ergosterol synthesis or binding with sterol forming pits and causing the membrane permeability to become leaky leads to cell death (Panáek *et al*., 2009). On the mitotic spindle cell division by targeting the microtubule and also inhibit DNA transcription Rai and Gade, (2009).

Conclusion

In conclusion, silver nanoparticales had been synthesized by the fungus *Cladosporium cladosporioides* in deffrent condition in dark and light condition, Moreover Ag NPs proved excellent antimicrobial activity against pathogenic fungi causing onychomycosis.

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