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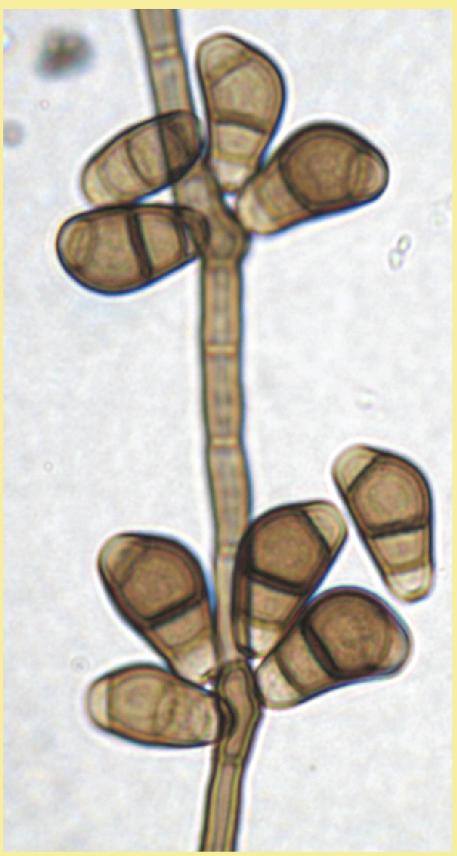

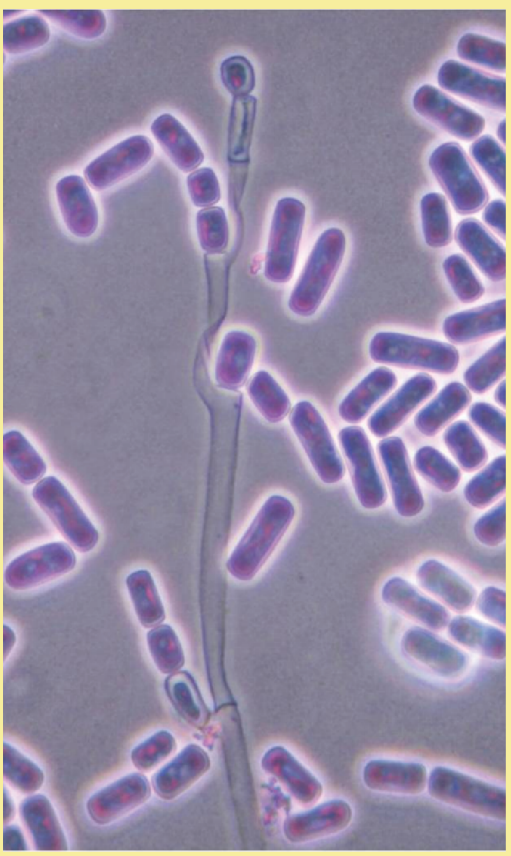

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Synergistic effect of mycosynthesized titanium oxide and silver nanoparticles in combination with tioconazole against some pathogenic microorganisms

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ABSTRACT

The chemical and physical techniques applied for nanoparticles synthesis are mostly high cost and may have harmful effects. So, there is an indigence to synthesize a low cost, high yield, most effective and most stable nanoparticles. The antimicrobial activity of mycosynthesized titanium oxide (TiO_2NPs) and silver (AgNPs) nanoparticles from mycelial extract of *Penicillium chrysogenum* AUMC 6092 was the main target of this study. The antimicrobial activity was assayed against 5 pathogenic Gram- positive (*Staphylococcus aureus* and *Streptococcus* sp.) and Gram- negative (*Escherichia coli, Klebsiella pneumoniae*, *Pseudomonas aeruginosa*) bacteria. In addition, 12 human pathogenic fungal strains including some multidrug resistant yeast strains and dermatophytic fungal strains (*Epidermophyton floccosum*, *Microsporum canis*, *Trichophyton mentagrophytes* and *T. rubrum*) were tested. TiO_2NPs and AgNPs synthesized from culture filtrate of *Penicillium chrysogenum* AUMC 6092 are uniform, spherical and small. The results showed that, generally TiO_2NPs and AgNPs had high inhibitory activity against most tested microorganisms, especially when mixed with Tioconazole. TiO2NP2 had the highest inhibitory effect against *C. glabrata* AUMC 13382, followed by *Escherichia coli*, *Staphylococcus aureus* and *C. tropicalis* AUMC 13378. On the other hand, *Staphylococcus aureus* and *C. glabrata* AUMC 13382 were the most sensitive microbial strains towards AgNPs.

***Keywords**:* Titanium dioxide nanoparticles, Silver nanoparticles, *Penicillium chrysogenium*, Multi drug resistant yeast, Dermatophytic fungi, Antimicrobial activity.

1. Introduction

The chemical and physical techniques applied for nanoparticles synthesis are mostly high cost and may have harmful effects. So, there is an indigence to synthesize a low cost, high yield, most effective, eco-friendly and most stable nanoparticles. This could be obtained using fungi as simple, low cost, due to their toleration, high wall-binding and metal bioaccumulation capacity good intracellular metal uptake, easy to scale up, large biomass capacity, ease biomass handling, secretion of reducing enzymes, economic livability and large scale biosynthesis of NPs [1, 2]. The small size of biosynthesized nanoparticles has an advantage for potential applications over other chemical methods [3-9]. Some studies focused on the utilization of yeast and filamentous fungi in the biosynthesis of nanoparticles (NPs) and their

applications [10, 11]. The advantage of fungi in nanoparticles biosynthesis over other biogenic sources is that, their mycelia are able to resist the flow pressure, agitation, high temperature and other harsh conditions and finally the mycosynthesized nanoparticles can be directly used in various applications due to the high purity [12, 13]. Biological methods for synthesis of titanium nanoparticles would thus help mitigate the shortcomings of chemical methods [14]. TiO_2NPs were synthesized previously from the strain of *F. oxysporum* [15]. In the myco-nanotechnological process of NPs synthesis, the fungus mycelium is treated with a metal salt solution, which leads fungi to produce their enzymes to catalyst the reduction of metal ions into nanoparticle form [13]. Recently, fungus-mediated synthesis of metal nanoparticles is getting much attention due to their extensive application in various sectors, antimicrobial [16], textile fabrics [17], catalytic activity [18], vegetable and fruit preservation [19], water hygiene management [20], live cell imaging and diagnostics [11, 21], as catalysts [22], semiconductors [23], biosensors [24], encapsulation of drugs and others. TiO_2 is a non-toxic white pigment for use in manufacture of paints, plastics, paper, ink, rubber, textile, cosmetics, leather, and ceramics [25] and water remediation [26]. NPs are also used to cut flower storage areas to prevent spoilage [2, 27]. Raliya *et al.* [2] could synthesize TiO_2NPs using *Aspergillus flavus* TFR 7 as an ecofriendly biological approach.

With the increase of the multidrug resistance of pathogenic microorganisms to numerous antibiotics in the recent time, there is an urgent need to create alternative antiseptics. Several reports indicated differences in the distribution of *Candida* spp. associated with Vulvovaginal candidiasis (VVC) and their antifungal drug susceptibility patterns from different geographic locations [28-30]. Recently, several studies done on AgNPs as antimicrobial agents against pathogenic bacteria and *candida* species [31-33]. Furthermore, it was suggested that the antimicrobial activity of mycosynthesized TiO_2, AgNPs and other NPs can be a new antibacterial materials [10,34]. The antibacterial activities of Ampicillin, Kanamycin, Erythromycin, and Chloramphenicol were improved when mixed with AgNPs [10]. Also, the antifungal properties of Fluconazole against *Phoma glomerata*, *P. herbarum*,

Fusarium semitectum, *Trichoderma* sp. and *Candida albicans* could be improved in the presence of AgNPs biosynthesized by *Alternaria alternata* [35]. The substituted imidazole antimicrobial agent, Tioconazole has a broad spectrum against some dermatophytes, pathogenic yeasts, and Gram-positive bacteria [36].

The great attention with metallic nanoparticles as antimicrobial agents may refer to their large surface area to volume ratio and the growing microbial resistance against metal ions, antibiotics, and the development of resistant strains [37]. The present work aimed to synthesize low cost titanium and silver nanoparticles from TiO_2 and $AgNO_3$, respectively using mycelial extract of a benign *Penicillium chrysogenum* AUMC 6092 strain for their application as antimicrobial agents against pathogenic and highly drug resistance microorganisms. Synergistic effect of the mycosynthesized NPs in combination with Tioconazole was also evaluated.

2. Materials and Methods

2.1. Mycosynthesis of nanoparticles

Strain used: *Penicillium chrysogenum* AUMC 6092 strain, isolated from strawberry fruits, Assiut, Egypt was used in this study for biosynthesis of Titanium (TiO_2NPs) and Silver (AgNPs) nanoparticles from Titanium oxide (TiO_2) and Silver nitrate ($AgNO_3$), respectively. The strain was recultured on potato dextrose agar medium and incubated at 28ºC for 5 days.

2.1.1. Synthesis of NPs

Penicillium chrysoginum AUMC 6092 was inoculated in Erlenmeyer flask containing 100 ml potato dextrose broth medium. The flasks were incubated in a rotary incubator at 150 rpm and 25ºC for 72 h. Then, the biomass was separated by filtration through sterilized filter paper (Whatman no. 1), washed several times with sterilized bi-distilled water. The biomass was transferred to 100 ml sterilized bi-distilled water under stirring conditions at 150 rpm for 72 h at 25°C. The fungal mycelia were excluded by filtration using sterile filter paper (Whatman no. 1). For TiO_2NPs production, the cell free filtrate was treated with 100 mM TiO_2 salt and the mixture was incubated at 28ºC under shaking condition at 150 rpm till the appearance of white to orange precipitate [2]. For synthesis of AgNPs, 1.0 mM of aqueous solution of AgNO3 was added to the cell free fungal filtrate in an Erlenmeyer flask [38], the mixture was kept in the dark at room temperature till the

colour changing into pale to reddish brown. Filtrate free from TiO_2 or $AgNO_3$ solution, as well as solutions of TiO_2 and $AgNO_3$ were used as a control. All mixtures were then centrifuged under cooling at 14,000 rpm for 20 min. The precipitate was resuspended in distilled water and subjected to washing three times using sterile bi-distilled water. The biotransformation particles were then monitored for characterization [7].

2.1.2. Characterization of the mycosynthesized nanoparticles

UV–visible spectrophotometry

The fungal reduction of TiO_2 or $AgNO_3$ solutions and the formation of TiO_2NPs or AgNPs, respectively by fungal mycelia were detected visually and by scanning the resulting solutions using Ultraviolet-Visible Spectroscopy (UV-Vis) (Evolution 300- UV-Visible spectrophotometer-England) [7].

Atomic absorption spectroscopy (AAS)

The concentration of AgNPs obtained from fungal mycelia was assessed using the Buck Scientific atomic absorption spectrophotometer (Model 210 VGP; Norwalk, CT, USA) equipped with a silver hollow cathode lamp and air-acetylene flame. Standard silver solutions were also measured [7].

Transmission electron microscopy (TEM)

To determine the shape and size of mycosynthesized TiO_2NPs and AgNPs, the JEOL TEM 100 CXII (Electron Microscope Unit, Assiut University, Egypt) was used. The samples were centrifuged, resuspended in distilled water (100 µg/ml), placed on a carbon-coated copper grid and dried in air at room temperature.

2.2. Antimicrobial activity

2.2.1. Microorganisms tested and growth conditions

Bacterial strains: Two Gram-positive (*Streptococcus* sp. and *Staphylococcus aureus*) and three Gram-negative (*E. coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*) bacterial strains were tested. All bacterial strains were clinically isolated from the infection control unit (Assiut University Hospital). They were grown in nutrient broth or nutrient agar medium at 37°C. Final concentration of 10^8 colony-forming units (CFU)/ml was prepared.

Fungal strains: Eight yeast strains were selected: *Candida albicans* AUMC 11588, *C. albicans* AUMC 13380, *C. glabrata*

AUMC 11592, *C. glabrata* AUMC 13382, *C. tropicalis* AUMC 11593, *C. tropicalis* AUMC 13378, *Clavispora lusitaniae* AUMC 11591, *Pichia kudriavzevii* AUMC 11590. These strains were isolated previously from patients suffering from Vulvovaginal candidiasis or respiratory diseases and some of which were recorded as multidrug resistant strains [39]. Moreover, four filamentous fungal dermatophytes (*Epidermophyton floccosum* AUMC 6185, *Microsporum canis* AUMC 13575, *Trichophyton mentagrophytes* AUMC 10945 and *T. rubrum* AUMC 1804) were also tested. All fungal strains (12) were kindly provided by Assiut University Moubasher Mycological Center (AUMC).

2.2.2. Disc diffusion method

The antimicrobial activities of the mycosynthesized TiO_2NPs and AgNPs were evaluated on nutrient agar plates (for bacteria) or Sabouraud dextrose agar (for yeast and dermatophytes) inoculated each with 200 µl of $1x10^5$ CFU/ml of bacterial or yeast strain [40]. Sterile filter paper discs (6 ml diam), impregnated each with 10 µl of nanoparticles dispersion were transferred to the surface of agar plate. The plates were then incubated at 30ºC for 24 hours, the inhibitory effect was estimated by measuring the diameters of inhibition zones.

2.2.3. Agar-well diffusion method

To evaluate the antifungal activity of TiO_2NPs and AgNPs against 4 dermatophytic species (*Epidermophyton floccosum, Microsporum canis, Trichophyton mentagrophytes* and *T. rubrum*), 100 µl of the fungal suspension were incorporated with 20 ml Sabouraud dextrose agar medium in sterile Petri dishes. After solidification, 0.5 cm diam. agar wells were filled with the tested NPs dispersion (20 µl of 100 µg /ml) after sonication to ensure complete dispersion of the particles and the plates were incubated at 28ºC for 7 days. The antifungal activity of NPs was assessed by determining the diameters of inhibition zone. All experiments were carried out in triplicates, and the average values were calculated.

2.2.4. Minimum inhibitory concentration (MIC):

E. Coli, K. pneumonia, Streptococcus sp. *S. aureus, C. albicans* AUMC 13380, *C. glabrata* AUMC 13382 and *C. tropicalis* AUMC 13378 were selected to evaluate the minimum inhibitory concentrations of biosynthesized NPs

solution. The double fold micro-dilution method was used [32]. Eight different concentrations of two-fold serial dilution of nanoparticles in the 96-well microtitre plate were prepared starting with 200 µg/ml of TiNPs or AgNPs. Hundred µl of 5×10^5 cfu/ ml of the microbial suspension were added to each NPs concentration giving the final volume of 200 µl. Pure microbial suspension in broth and NPs solution were used as positive and negative controls, respectively. The plates were incubated at 30ºC for 24 hours, after that, the lowest inhibiting NPs concentration (MIC) showing no detectable growth was recorded.

2.2.5. Minimum bactericidal concentration (MBC):

Aliquots of 10 µl from all concentrations with no detectable microbial growth (MIC) were inoculated in NA (for bacterial isolates) and SDA (for fungal isolates). The plates were incubated overnight at 30°C and the MBC was determined by detecting the lowest concentration of NPs with no microbial growth.

Statistical analysis

The obtained data were analyzed using the SPSS 11 Software.

3. Results

3.1. Mycosynthesis and characterization of nanoparticles

The biosynthesis of nanoparticles from fungal extract is a promising approach in nanotechnology. In the current study, Titanium dioxide (TiO_2NPs) and Silver (AgNPs) nanoparticles were synthesized by exposure of salt solution of TiO_2 or $AgNO_3$ to mycelial extract of *Penicillium chrysogenum* AUMC 6092.

3.2. UV-Vis spectral analysis

UV spectral analysis revealed that TiO_2NPs have broad absorption band in the range of 290 - 500 nm according to the Plasmon absorbance of Titanium dioxide nanoparticles (Fig. 1). A typical silver nanoparticles absorption band was located in the visible region between 350 and 550 nm, Plasmon resonance peak obtained at 420 nm is well-indicated for various metal nanoparticles with sizes between 2-100 nm (Fig. 2).

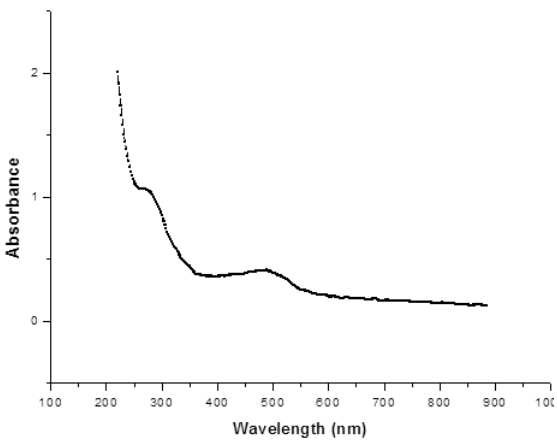

Fig. 1. UV-Vis absorption spectrum of TiNPs shows absorption band between 490 nm after 7 days of incubation in dark.

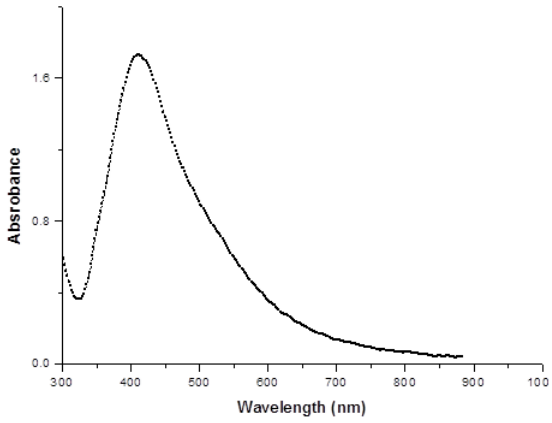

Fig. 2. UV-Vis absorption spectrum o AgNPs shows absorption band at 415 nm 7 days of incubation in dark.

3.3. TEM analysis of NPs

Micrographs taken by TEM showed that the Titanium dioxide nanoparticles are uniform in distribution, well-dispersed; mostly have rounded shapes, smaller than 7.89 nm and up to 17.9 nm in size (Fig. 3). Representative TEM micrographs of AgNPs showed that, most of the synthesized nanoparticles are homogenous with spherical shape, and the particle size ranged between 6-30 nm. Most nanoparticle sizes were smaller than 15 nm (Fig. 4).

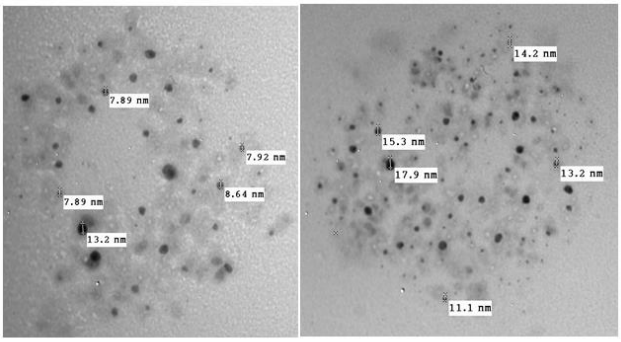

Fig 3. Transmission electron microscopy (TEM) photographs of TiNPs.

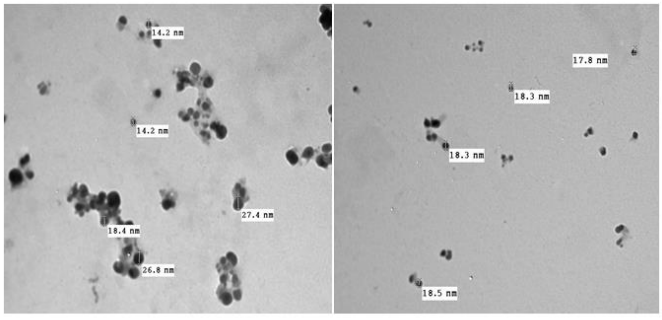

Fig 4. Transmission electron microscopy (TEM) photographs of AgNPs.

3.4. Determination of antimicrobial activity

The main target of nanoparticles biosynthesis is that their applications in nanomedicine as a potential antimicrobial agent against pathogenic microorganisms. The antimicrobial activity of the mycosynthesized nanoparticles (TiO_2NPs

and AgNPs) was studied against 3 strains of Gram-negative (*Pseudomonas aeruginosa, Klebsiella pneumoniae* and *E.coli*), 2 of Gram-positive (*Streptococcus* sp. and *Staphylococcus aureus)* bacteria, 8 yeast strains; *C. albicans, C. glabrata, C. tropicalis, Clavispora lusitaniae*, *Pichia kudriavzevii*, in addition to, 4 dermatophytic fungal strains (*Epidermophyton floccosum*, *Microsporum canis*, *Trichophyton mentagrophytes* and *T. rubrum*). Also, tioconazole, singly or mixed with nanoparticles, was tested against the same microorganisms.

3.4.1. Titanium dioxide nanoparticles (TiO_2NPs)

The mycosynthesized TiO_2NPs at 100 µg/ml had antibacterial effect against all human pathogenic bacterial strains tested with variable degrees. Presence of TiO_2NPs in combination with tioconazole lead to highly inhibitory effect against *Staphylococcus aureus*, resulting 20 mm inhibition zone (Table 1).

Table 1. Antibacterial activity of TiO_2NPs and tioconazole against pathogenic bacteria

Bacterial strains	Diameters of inhibition zone in mm (Mean ± SE)		
	TiO_2NPs (100 µg)	**Tioconazole (50 µg)**	**Tioconazole + TiO_2NPs (1 : 1)**
Escherichia coli	8 ± 1	0	11± 1
Klebsiella pneumoniae	7± 1	0	10 ± 2
Pseudomonas aeruginosa	8 ±2	0	10 ± 1
Streptococcus sp.	10 ± 2	7 ± 1	12 ± 1
Staphylococcus aureus	12 ± 2	13 ± 2	20 ± 3

All fungal strains tested, except *E. floccosum* and *T. mentagrophytes*, were affected by TiO_2NPs (Table 2). The most valuable results were obtained when nanoparticles were mixed with tioconazole, where, the mixture considerably improved the inhibitory action against *C. albicans* AUMC 13380 from 7 mm (for TiO_2NPs singly) and 13 mm (for tioconazole singly) into 28 mm (Table 2). The highly affected bacterial strains, *S. aureus* showed its MIC at 25 µg/ml, while MBC at 12.5 µg/ml TiO_2NPs concentration, while, *C. glabrata* AUMC 13382 was the most sensitive strain against TiO_2NPs, with 12.5 µg/ml for both MIC and MBC (Table 3).

Table 2. Antifungal activity of TiO_2NPs and tioconazole against pathogenic fungi

Fungal strains	AUMC number	Clinical diagnosis	Diameters of inhibition zone in mm (Mean ± SE)		
			TiO_2NPs (100 µg)	Tiocon. (50 µg)	Tiocona. + TiO_2NPs (1 : 1)
Candida albicans	11588	Vulvovaginal candidiasis **[39]**	12 ±2	10± 1	20 ± 2
C. albicans	13380	Respiratory disease	7 ± 1	13 ± 2	28± 3
C. glabrata	11592	Vulvovaginal candidiasis **[39]**	7± 1	0	18 ± 2
C. glabrata	13382	Respiratory disease	7± 1	10 ±1	16± 3
C. tropicalis	11593	Vulvovaginal candidiasis **[39]**	12 ± 1	8 ± 1	18± 2
C. tropicalis	13378	Respiratory disease	7± 1	9 ± 2	17± 3
Pichia kudriavzevii	11590	Vulvovaginal candidiasis **[39]**	7 ± 1	8 ± 2	18 ± 2
P. kudriavzevii	11591	Vulvovaginal candidiasis **[39]**	8 ± 1	12 ± 2	24 ± 2
Epidermophyton floccosum	6185	Onychomycosis	0	25±3	32±4
Microsporum. canis	13575	Tinea capitis	7 ± 1	20±3	25±2
Trichophyton mentagrophytes	10945	Onychomycosis	0	20±2	25±5
Trichophyton rubrum	1804	Human skin disease	16±2	15±3	17±3

Table 3. Minimum inhibition concentration (MIC) and minimum bactericidal concentration (MBC) values of TiO_2NPs against some pathogenic microorganisms

Microorganisms	MIC	MBC
	(Conc., µg/ml)	
Escherichia coli	25	12.5
Klebsiella pneumoniae	50	25
Streptococcus sp.	50	25
Staphylococcus aureus	25	12.5
C. albicans AUMC 13380	50	25
C. glabrata AUMC 13382	12.5	12.5
C. tropicalis AUMC 13378	25	12.5

3.4.2. Silver nanoparticles (AgNPs)

AgNPs at 100 µg/ml concentration could inhibit the five human pathogenic bacterial strains tested with the maximum inhibitory effect against *Sterptococcus* sp. (12 mm), followed by *Escherichia coli* and *Staphylococcus aureus* (11 mm) (Table 4). The resultes also indicated that, tioconazol had weak, against *Streptococcus* sp., or no, against *E. coli*, *Klebsiella pneumonia* and *Pseudomonas aeruginosa*, antibacterial activity. It is worthy to mention that, mixing AgNPs with tioconazol improved the antibacterial activity against *S. aureus*, *Sterptococcus* sp., *E.coli* and *K. pneumonia* (Table 4).

Table 4. Antibacterial activity of AgNPs and tioconazole against pathogenic bacteria

Bacterial strains	Diameters of inhibition zone in mm (Mean ± SE)		
	AgNPs (100 µg/ml)	Tioconazole (50 µg/ml)	Tioconazole + AgNPs (1 : 1)
Escherichia coli	11 ± 2	0	12± 1
Klebsiella pneumoniae	9± 2	0	12 ± 2
Pseudomonas aeruginosa	8 ± 1	0	10 ± 2
Streptococcus sp.	12 ± 1	7 ± 1	14 ± 2
Staphylococcus aureus	11 ± 1	13 ± 2	20 ± 2

With respect to pathogenic fungi, 10 strains (out of 12 tested) were inhibited weakly by AgNPs singly, but when mixed with tioconazol, an increase in the inhibitory effect against *Candida albicans, C. glabrata, C. tropicalis, Clavispora lusitaniae* and *Pichia kudriavzevii* could be achieved (Table 5). AgNPs showed high antibacterial activity against *S. aureus* in very low MIC (6.25 µg/ml) and MBC (3.125) (Table 6).

Table 5. Antifungal activity of AgNPs and tioconazole against pathogenic fungi

Fungal strains	AUMC number	Clinical diagnosis	Diameters of inhibition zone in mm (Mean ± SE)		
			AgNPs (100 µg/ml)	Tiocon. (50 µg/ml)	Tiocon. + AgNPs (1 : 1)
Candida albicans	11588	Vulvovaginal candidiasis **[39]**	10 ±2	10± 1	22 ± 3
C. albicans	13380	Respiratory disease	9 ± 2	12 ± 3	20± 2
C. glabrata	11592	Vulvovaginal candidiasis **[39]**	9± 1	0	12 ± 2
C. glabrata	13382	Respiratory disease	9± 1	9 ±1	16± 3
C. tropicalis	11593	Vulvovaginal candidiasis **[39]**	10 ± 1	8 ± 1	15± 2
C. tropicalis	13378	Respiratory disease	13± 2	8± 2	17± 1
Clavispora lusitaniae	11591	Vulvovaginal candidiasis **[39]**	10 ± 1	12 ± 2	20 ± 2
Pichia kudriavzevii	11590	Vulvovaginal candidiasis **[39]**	12 ±2	8 ± 2	15 ± 2
Epidermophyton floccosum	6185	Onychomycosis	0	25 ± 3	23± 2
Microsporum canis	13575	Tinea capitis	7± 1	20 ± 3	21 ± 1
Trichophyton mentagrophytes	10945	Onychomycosis	0	20± 2	22 ± 2
Trichophyton rubrum	1804	Human skin disease	8 ±2	15 ± 3	15 ± 2

Table 6. Minimum inhibition concentration (MIC) and minimum bactericidal concentration (MBC) values of AgNPs against some pathogenic microorganisms.

Microorganisms	MIC (Conc., µg/ml)	MBC (Conc., µg/ml)
Escherichia. coli	12.5	6.25
Klebsiella pneumoniae	25	12.5
Streptococcus sp.	12.5	12.5
Staphylococcus aureus	6.25	3.125
C. albicans AUMC 13380	25	12.5
C. glabrata AUMC 13382	6.25	3.125
C. tropicalis AUMC 13378	25	12.5

4. Discussion

The current study provided valuable nanoparticles (TiO_2NPs & AgNPs) synthesized from mycelial extract of *Penicillium chrysogenum* AUMC 6092. Nanoparticles were mycosynthesized using a simple, fast, clean, benign, and ecofriendly alternative method to the complex chemical synthetic methods, where the crude fungal extract acts as a reducing agent [32]. Mycosynthesis of TiO_2NPs [1, 2] and AgNPs [41-44] from the crude extract of different fungal strains has been utilized. Siddiqi and Husen [11] suggested that biosynthesis of metal nanoparticles via bioreduction of metal salts may be stabilized by organic molecules present in fungal and bacterial cells. When a fungus is exposed to metal salts such as Titanium, Silver or other metals, it produces enzymes and other metabolites to protect itself from unwanted foreign materials, and during this, the metal ions are reduced to metal nanoparticles [45].

In comparison with our results, *Aspergillus flavus* and other fungi isolated from soil produced spherical TiO_2 nanoparticles, but with larger sizes (62–74 nm) and had antimicrobial activity [10]. The reduction of ions by fungi occurs through the action of both reductase enzymes and electron shuttle quinones [46]. Furthermore, biosynthesis of nanoparticles results in a more consistent size distribution pattern, comparing to chemical or physical approaches. The production of AgNPs in the solution was visualized by gradual colour change into pale brown, reddish brown to dark brown, while, production of TiO_2NPs was noticed visually by white to orange precipitate. The concentration of the synthesized AgNPs stock dispersion was measured using the

AAS. Plasmon resonance peak obtained at 420 nm is well-indicated for various metal nanoparticles with sizes between 2-100 nm, and at this wavelength, the synthesized nanoparticles were highly dispersed [47].

TEM images of synthesized nanoparticles showed their spherical shapes and nearly uniform size. In this respect, *Fusarium oxysporum* fabricated spherical, extracellular TiO_2NPs, 6–13 nm size [14]. Also, from 20 fungal strains screened, only four belong to *Rhizopus arrhizus* (two strains), *Trichoderma gamsii* and *Aspergillus niger* were recorded to synthesize spherical shapes AgNPs ranging between 30-100 nm diam [43].

It is worth mentioning that, MIC values of our mycosynthesized TiO_2NPs are much lower than that synthesized chemically by Sol-Gel technique [48, 49]. Titanium dioxide nanoparticles are of interest for recent medical research due to their highly inhibitory effect against pathogenic bacteria that enter in ecosystem food chain [50]. TiO_2 nanoparticles had the ability to inhibit the growth of resistant strain of *E. coli* which causes nosocomial infections and resistant to most of the broad spectra antibiotics [51]. It has been found that growth inhibition is concentration-dependent and 100 ppm nanoparticle concentration is the most effective, bursts fungal cell wall, resulting in complete destruction [52]. It was suggested that, TiO_2 nanoparticles kill microorganisms by oxidization through electromagnetic attraction between microorganisms (negatively charged) and the metal oxide (positively charged) [51].

The obtained results revealed that AgNPs become more effective against pathogenic microorganisms when mixed with tioconazole, although, Prabhu and Poulose [53] observed that even least concentration of silver nanoparticles have a high antimicrobial effect. Also, 50% and 75% concentrations of AgNPs synthesized from *Fusarium oxysporum* showed the ability to inhibit the growth of some multidrug resistant bacteria and *candida* species [44]. The inhibitory effect of silver nanoparticles may be due to their action on the DNA and inactivation of cellular protein [54]. In addition, it was suggested that silver ions bind to functional proteins, resulting in protein denaturation and this was revealed when *E. coli* was treated with highly reactive metal oxide nanoparticles, a bacterial membrane exhibits a significant increase in permeability, leaving the bacterial cells incapable of properly

regulating transport through plasma membrane and causing cell death [54]. The advantage of Silver nanoparticles over Silver ions as antimicrobial agent is that it is effective at siginficantly lower concentrations than that of Silver ions. This small size range of silver nanoparticles adds to its antibacterial property, since it can easily penetrate bacterial cell membrane and thereafter damage the respiratory chain, affect the DNA, RNA, and division of the cell, and finally lead to cell death [37].

AgNPs harm the bacterial cell wall, modify membrane permeability and cause a fall in plasma membrane potential. Silver ions are liberated from the nanoparticle surface and come into the bacterial cell to produce reactive oxygen species (ROS), which damage bio-macromolecules [55].

The tested yeast species were recorded previously as the causal agents of vaginal and vulvovaginal candidiasis [39, 56, 57], Otomycosis [58], Chronic mucocutaneous candidiasis [59] and hospital-acquired infections [60]. Also, dermatophytic fungi are the main causal agents of Tinea capitis, Tinea pedis, Tinea unguim, Tinea barbae, Tinea faciei, Tinea cruris and other skin, hair and nail infections [61]. Recently, it was suggested that, Gram-negative bacteria are more sensitive than Gram-positive bacteria and this variation in sensitivity between Gram-negative and Gram-positive bacteria towards Titanium dioxide nanoparticles may be attributed the difference in their cell outer layer structure and their interaction with the charged TiO_2 nanoparticles [62]. This advantage of TiO_2NPs can be exerted in coating the medical devices, such as catheters to avoid the infection. The current results revealed that *C. albicans* AUMC 11588, *C. glabrata* AUMC 11592, *C. tropicalis* AUMC 11593, *Clavispora lusitaniae* AUMC 11591, *Pichia kudriavzevii* AUMC 11590 showed high sensitivity against mycosenthesized nanoparticles either singly or mixed with tioconazole. Although these strains exhibited high resistant to 8 antifungal drugs studied by Hassan *et al.* [39]. So, the present work could offer high valuable mycosynthesized nanoparticles with a potential application as antimicrobial agents against human pathogenic microorganisms. Therefore, addition of antimicrobial nanomaterials to cotton fabrics used in the manufacture of suture or wound bands could reduce the rate of infection in patients [63].

Conclusion: Application of fungi in biosynthesis of nanoparticles is simple,

cheap and eco-friendly way. Biologically active metabolites from fungi represent excellent scaffolds for this purpose. Results revealed that, mycelial extract of *Penicillium chrysogenum* AUMC 6092 played an important role in reduction and stabilization of TiO_2NPs and AgNPs. Furthermore, the mycosynthesized nanoparticles had valuable antimicrobial activity against Gram- negative and Gram-positive bacteria, human pathogenic *Candida* spp., in addition to some strains of dermatophytic fungi. Of these, some *Candida* strains were recorded as multidrug resistant strains. Consequently, these nanoparticles are candidated for application singly or in combination with Tioconazole as broad spectrum antimicrobial agents. The application of NPs is based mainly on their size, where smaller- size NPs are more effective. The new antimicrobial agents are of a great importance in controlling some multidrug resistant pathogenic microorganisms and consequently, reduce mortality and morbidity rate of the infectious diseases.

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Figure legends:

Fig. 1. UV-Vis absorption spectrum of the TiNPs shows absorption band between 290-490 nm after 7 days of incubation in dark.

Fig. 2. UV-Vis absorption spectrum of the AgNPs shows absorption band at 415 nm after 7 days of incubation in dark.

Fig 3. Transmission electron microscopy (TEM) photographs of TiNPs.

Fig 4. Transmission electron microscopy (TEM) photographs of AgNPs.

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