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RESEARCH ARTICLE

The Effect of Silver and Titanium Dioxide Nanoparticles on Fungi Isolated from Patients in Babylon City

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Abstract

The samples of skin, hair and nail were collected from 50 dermatophytosis patients who are admitting to Marjan Teaching Hospital in Hilla-Babylon province during the period from August 2018 to September 2018. Out of the dermatophytosis patients, there are 32 males and 18 females, the patients age ranges from 15 to >45 years. Potato Dextrose Agar and dermatophyte test medium agar had used for isolation of fungi. For identification of isolated fungi had used microscopic examination after staining the sample with methylene blue. According to the results of this study, the males are 64.3% while females are 35.7% with dermatophytosis patients. The most age groups are between (31-45) years with dermatophytosis recorded (46%). From the result found most cases present in skin 52%. In order to determine the minimum inhibitory concentration of silver nanoparticles, microdilution method was used in 96-well microplates. The concentrations of 1000, 500, 250, 125, 62.5, 31.25, 15.625, 7.81and 3.9 ppm were prepared from silver nanoparticles. From the result was found the IC-50 and IC-90 of SNPs for *Trichophyton* strains were estimated at 283.78 and 848.76 ppm, respectively. The IC-50 and IC-90 of SNPs for *Candida albicans* were estimated at 5.77 and 25.99 ppm, respectively. For *A. niger*, the IC 50 and IC 90 of SNPs were 69.49 and 632.08 ppm, respectively. The IC 50 and IC 90 of SNOs for *Mucor* were 25.53 and 354.75 ppm, respectively. While In order to determine the minimum inhibitory concentration of titanium dioxide nanoparticles, microdilution method was used in 96-well microplates. The concentrations of 32, 16, 8, 4, 2, 1, 0.5, 0.25and 0.125 mg/ml were prepared from titanium nanoparticles. From the result was found the IC 50 and IC 90 of TiO_2 nanoparticles for *Trichophyton* strain were estimated at 6.89 and 23.63 mg/ml, respectively. The IC 50 and IC 90 of TiO_2 nanoparticles for *C. albicans* were 3.49 and 19.5 mg/ml, respectively. The IC 50 and IC 90 of TiO_2 nanoparticles for *A. niger* were 8.82 and 23.67 mg/ml, respectively. The IC 50 and IC 90 of TiO_2 nanoparticles for *Mucor* were 8.82 and 23.67 mg/ml, respectively.

Keywords: *Dermatophytes, silver nanoparticles, Titanium dioxide nanoparticles, Minimum Inhibitory Concentration, Aspergillus niger.*

Introduction

Superficial mycoses (SM) or dermatomycoses include all the cutaneous and cutaneo-mucous fungal infections. The causative fungi are divided into two large groups: [1] yeasts (genera Candida, Trichosporon and Malassezia) and [2] filamentous fungi, basically dermatophytes that include the genera Microsporum, Trichophyton and Epidermophyton [1]. Dermatophytosis is mainly confined to the non-living superficial cornified layers because its fungal agents are not able to penetrate into the deeper tissue or organ of a healthy host. However, this infection also depends on the fungi, immune status of the host, and site of infection [2].

Therefore, the prevalence of superficial mycosis is near about 20 -25% worldwide and dermatophytes being the leading cause of superficial fungal infection [3]. The dermatophytic infection spreads easily by direct contact with the infected humans and animals or through fomites [4]. With the evolution of biomedical nanomaterials, new antimicrobial was developed due to the physiochemical properties of NPs [5].NPs usually ranging in dimension from 1-100 nanometers (nm) so they have unique properties from bulk particle that has the possibility of controlling and deal with the structures at molecular and atomic level.

The percentage of atoms at the surface of NPs more than the total number of atoms at the surface of bulk particles so the surface-to-volume ratios of NPs become large. The physiochemical properties of NPs are varied in nature and they have highly viable in biomedical field and antimicrobial agent [6]. The antimicrobial activity and physicochemical properties of NPs against biological molecules were related to their small size, high surface area to volume ratio, solubility, shape, surface coatings and charge.

As well as the biological molecules sizes are similar to the NPs structures that lead to easy penetration of NPs inside the microorganisms [7]. Silver nanoparticles (AgNPs) are the particles with size of 2-100 nm, which contain 20-15,000 silver atoms. These particles are used in medicine, dental cements, treatment of wounds and burns, water purification, and textile engineering. Several studies have been carried out concerning the antimicrobial properties of AgNPs against various pathogens such as viruses, fungi, and some bacterial species.

Most of which have confirmed the antimicrobial properties of AgNPs. The mechanisms of action of AgNPs referred to their accumulation on the membrane of microorganisms, formation of pores, change in permeability of cell wall, and inhibition of respiration process. In addition, it has been shown that AgNPs can greatly inhibit cellular respiration, DNA replication, and cell division, which result in the loss of cell viability and lead to cell death [8]. Titanium dioxide NPs (TiO2 NPs) is a promising material, used in many applications due to its high photo catalytic activity, dielectric properties, high stability and low cost [9].

TiO2 NPs have different chemical, magnetic, optical and structural properties and they have more toxicity effects than its bulk particles so it was used in pharmaceutical products, catheters to prevent urinary tract infections, cosmetics, dental implants and packaging. Photo activity of TiO2 NPs was effective against Gram-positive bacteria, Gram-negative bacteria, fungi, and bacteriophage. The studies have shown that the cell membrane that is exposed to TiO2 NPs will destroy followed by cell wall damage leading to the cell death. In comparison to the conventional antibiotics, nanostructured antimicrobial is regarded assistant factor in reducing the toxicity and lowering the cost more stable for long-term storage so NPs can resist high pH and temperature without being inactivated [10].

Methods and Materials

In this study, silver and TiO_2 nanoparticles were not prepared at first. Silver nanoparticles with a concentration of 2000 ppm with size 20 nm from Pars Co., TiO_2 nanoparticles with size 12-20 were purchased from Pishgaman Company of Mashhad. Different concentrations of nanoparticles were prepared for silver nanoparticles concentrations of 1000, 500, 250, 125, 62.5, 31.25, 15.62, 7.81, 3.9, 1.95 ppm was prepared and for titanium dioxide concentrations of 32, 16, 8, 4, 2, 1, 0.5, 0.25, 0.125 were prepared.

Minimum inhibitory concentrations (MIC), they were examined on pathogenic fungi, *Trichophyton Candida albicans, Aspergillus niger* and *Mucor*. For preparation of culture media and culturing of fungi were carried out according to the protocol, then, preparation of different doses of nanoparticles, fungi cultivation, determined the MIC.

RPMI Medium

Roswell Park Memorial Institute (RPMI) composed from:

- RPMI powder 10.43 g.
- MOPs (N-Morpholino Propanesulfoni Acid) buffer 34.53 g.
- Glutamine.
- Sodium hydroxide (NaOH) 1N.

Preparation of Silver and Titanium Dioxide Nanoparticles

The silver nanoparticle solution from Parshia Company was prepared in a particle size of 20 nm and at a concentration of 2000 mg / L in 1 liter and stored in dark place at a temperature of 25 °C. Titanium Oxide Nanopowder (TiO_2, anatase, 99+%, 10-25 nm) from Pioneer Company of Iranian Nanomaterials.

Titanium Dioxide Nanoparticles (TiO_2) Coating

The CMC-coated TiO_2 NPs were prepared by dissolving 0.2% (w/v) of CMC in deionized

(DI) water using a magnetic stirrer for 12h. After complete dissolution, TiO_2 NPs were added to each solution and dispersed under sonication for 3 min.

Results

Age Distribution

In this study, age distribution of dermatophytosis patients including 17 (34%), 23 (46%) and 10 (20%) between the ages from 15 to 30 years, from 31 to 45 years and above 45 years respectively, as in Figure (1a).

Site of Infection

The site of infection for 50 patients included in this study explained in Figure (1b). Dermatophytosis patients were classified depends on the place of lesion into skin 26 (52%), hair 14 (28%), and nail 10 (20%).

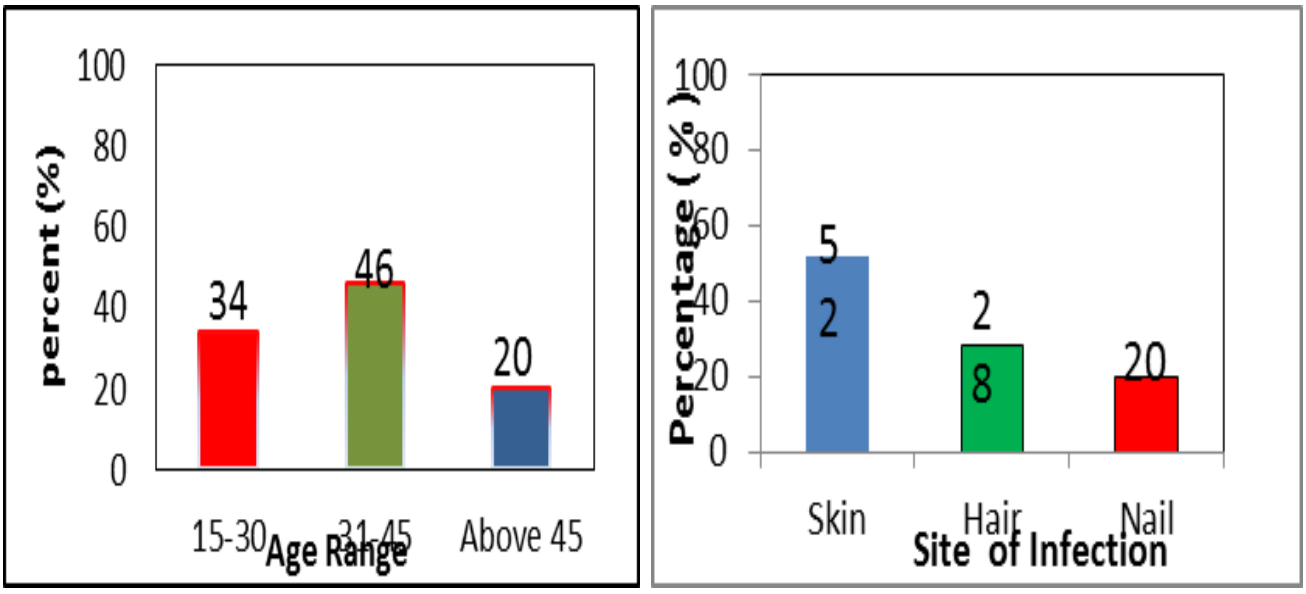

Figure 1: Age Distribution b- Site of Infection for Dermatophytosis Patients

Results of MIC of Silver Nanoparticles in Vitro

For this study, program SPSS (1) were used for MIC analysis.

Table 1: Absorption of wells in the presence of different concentrations of SNPs (ppm)

Groups	0	3.9	7.8	15.6	31.25	62.5	125	250	500
Trichophyton	1.187 ±0.005	1.07 ±0.009	0.819 ±0.001	0.702 ±0.001	0.637 ±0.014	0.599 ±0.003	0.146 ±0.001	0.002 ±0.004	-0.027 ±0.009
C. albicans	0.831 ±0.008	0.550 ±0.001	0.532 ±0.001	0.263 ±0.001	0.101 ±0.011	0.001 ±0.008	-0.012 ±0.001	-0.014 ±0.004	-0.027 ±0.001
A. niger	0.982 ±0.003	0.748 ±0.010	0.690 ±0.009	0.605 ±0.006	0.538 ±0.002	0.324 ±0.005	0.288 ±0.007	0.030 ±0.004	-0.003 ±0.001
Mucor	1.025 ±0.027	0.776 ±0.007	0.506 ±0.005	0.477 ±0.008	0.415 ±0.006	0.352 ±0.006	0.240 ±0.001	0.020 ±0.004	-0.002 ±0.001

Table 2: % Inhibition in the presence of different concentrations of SNPs (ppm)

Groups	3.9	7.8	15.6	31.25	62.5	125	250	500
Trichophyton	9.59	12.13	21.73	27.05	30.16	67.29	79.09	81.47
C. albicans	46.06	48.11	78.79	97.26	100	100	100	100
A. niger	21.93	27.67	36.07	42.69	63.83	67.39	92.88	96.15
Mucor	20.68	46.76	49.56	55.55	61.64	72.46	93.72	95.84

The Strains of *Trichophyton* isolated from patients were cultured in a laboratory medium. The fungi isolated from the patients had a high pathogenicity. The IC-50 and IC-90 of SNPs for *Trichophyton* strain was estimated at 283.78 and 848.76 ppm, respectively (figure 2). As well as IC-50 and IC-90 of SNPs for *C. albicans* was estimated at 5.77 and 25.99 ppm, respectively (Figure 3).

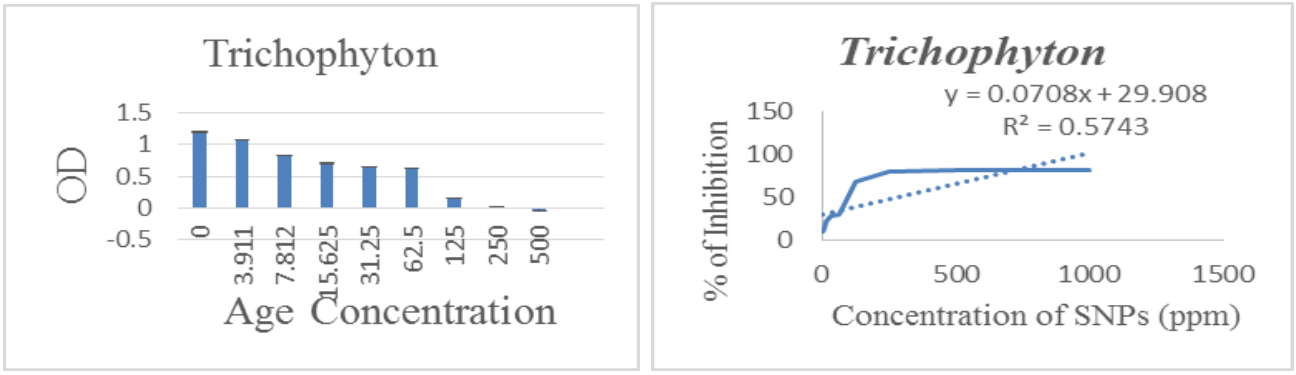

Figure 2: Anti-fungal effect of different concentration of SNPs against *Trichophyton*

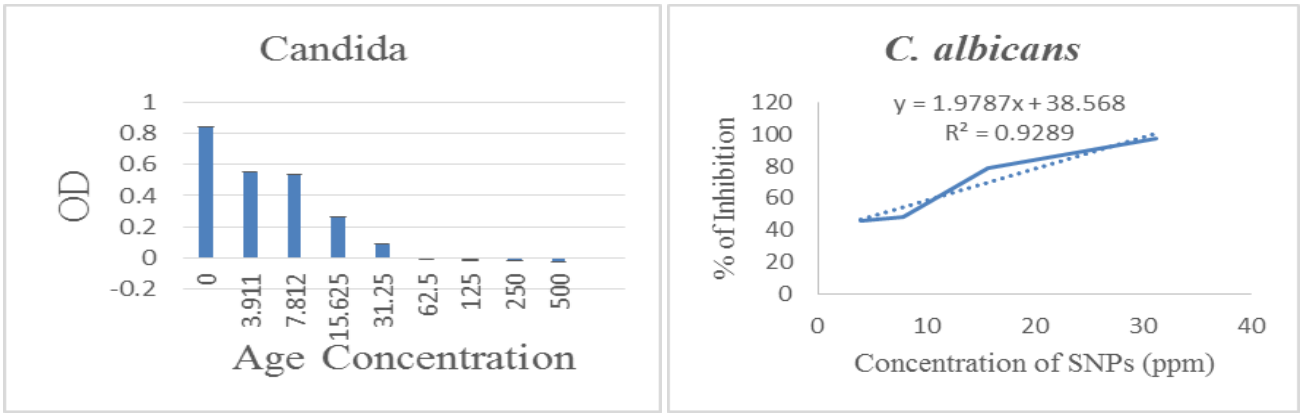

Figure 3: Anti-fungal effect of different concentration of SNPs against *C. albicans*

For *A. niger*, the IC 50 and IC 90 of SNPs were 69.49 and 632.08 ppm, respectively (Figure 4). As well as the IC 50 and IC 90 of SNOs for *Mucor* were 25.53 and 354.75 ppm, respectively (Figure 5).

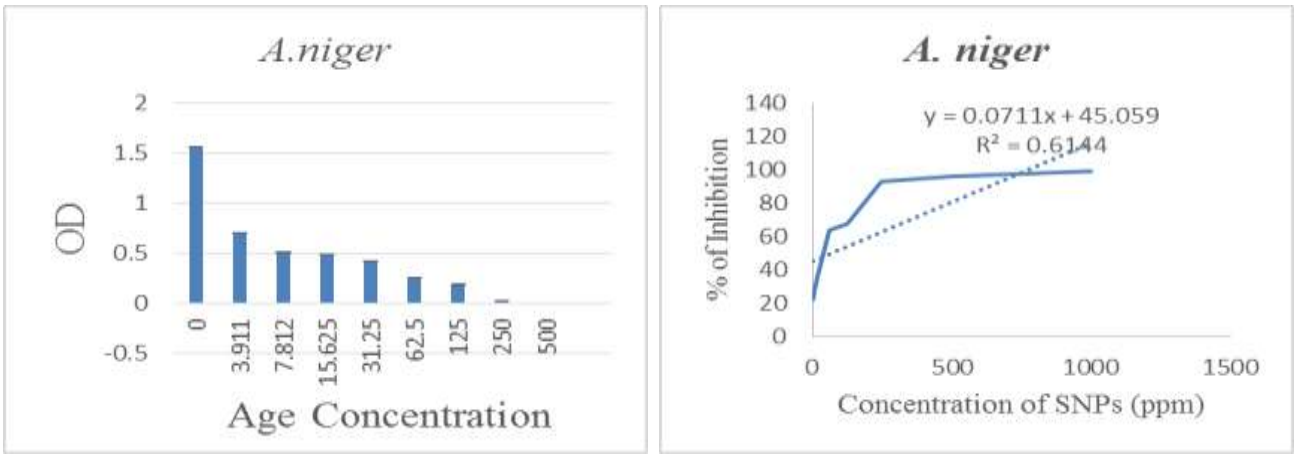

Figure 4: Anti-fungal effect of different concentration of SNPs against *A. niger*

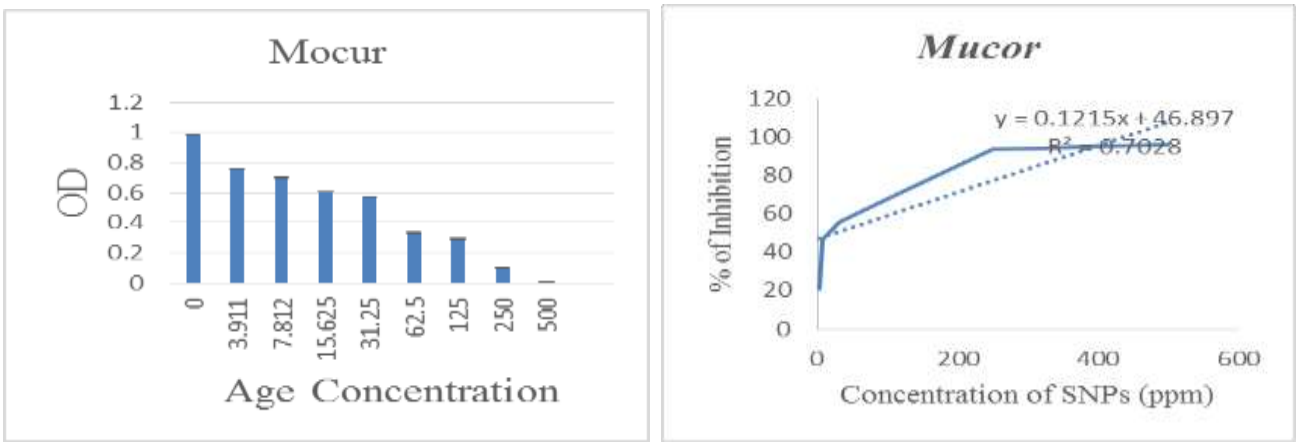

Figure 5: Anti-fungal effect of different concentration of SNPs against *Mucor*

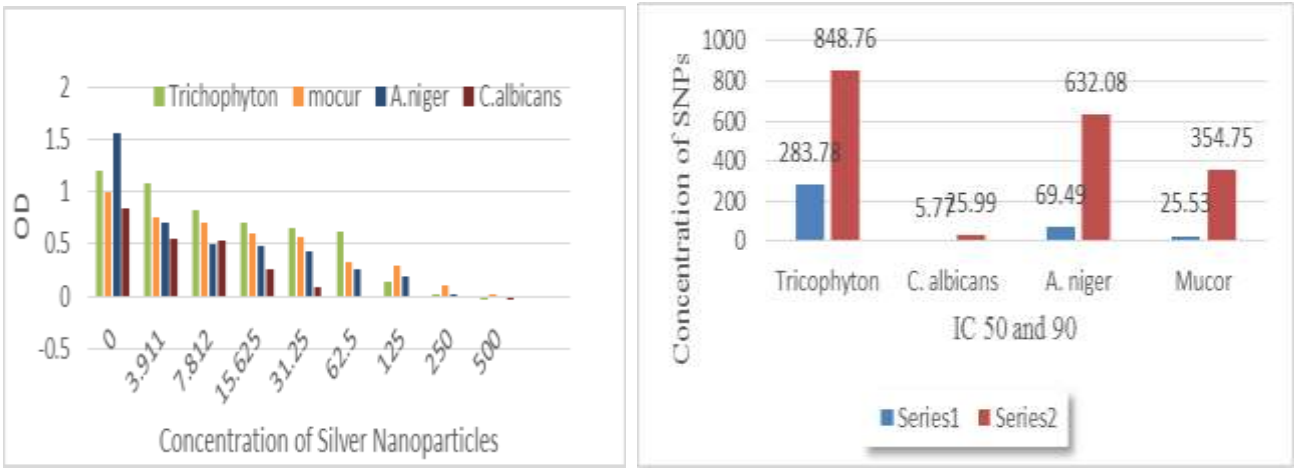

Figure 6: Comparison of inhibitory levels of fungi in different concentrations of SNPs

Results of MIC of Titanium dioxide Nanoparticles in Vitro

For this study, program SPSS (1) were used for MIC analysis.

Table 3: Absorption of wells in the presence of different concentrations of TiO_2 (mg/ml)

Groups	0	0.125	0.250	0.500	1	2	4	8	16	32
Trichophyton	1.221 ±0.002	1.089 ±0.003	0.934 ±0.013	0.703 ±0.005	0.657 ±0.003	0.431 ±0.009	0.311 ±0.009	0.215 ±0.006	0.129 ±0.009	0.032 ±0.004
C. albicans	0.872 ±0.008	0.824 ±0.015	0.756 ±0.016	0.712 ±0.005	0.669 ±0.008	0.622 ±0.010	0.346 ±0.005	0.133 ±0.004	-0.003 ±0.002	-0.021 ±0.001
A. niger	1.331 ±0.002	1.250 ±0.002	0.906 ±0.008	0.727 ±0.006	0.648 ±0.010	0.629 ±0.007	0.567 ±0.008	0.383 ±0.002	0.104 ±0.006	0.023 ±0.002
Mucor	0.930 ±0.011	0.858 ±0.002	0.820 ±0.001	0.745 ±0.004	0.734 ±0.003	0.690 ±0.006	0.559 ±0.008	0.257 ±0.001	0.056 ±0.005	0.008 ±0.001

Strains of *Trichophyton* isolated from patients were cultured in a laboratory environment. The fungi isolated from the patients had a high pathogenicity. IC 50 and IC 90 of TiO_2 nanoparticles for *Trichophyton* strain were estimated at 6.89 and 23.63 mg/ml (Figure 7). As well as The IC 50 and IC 90 of TiO_2 nanoparticles for *C. albicans* were 3.49 and 19.51 (Figure 8).

Table 4: percentage Inhibition in the presence of different concentrations of TiO_2 (mg/ml)

Groups	0.125	0.250	0.500	1	2	4	8	16	32
Trichophyton	0	9.19	30.86	35.18	56.38	67.63	76.64	84.71	93.81
C. albicans	18.48	26.54	31.75	36.84	42.41	75.12	100	100	100
A. niger	0	11.39	28.82	36.51	38.07	44.40	62.32	89.48	97.37
Mucor	17.71	22.19	31.05	32.35	37.54	53.01	88.66	91.71	100

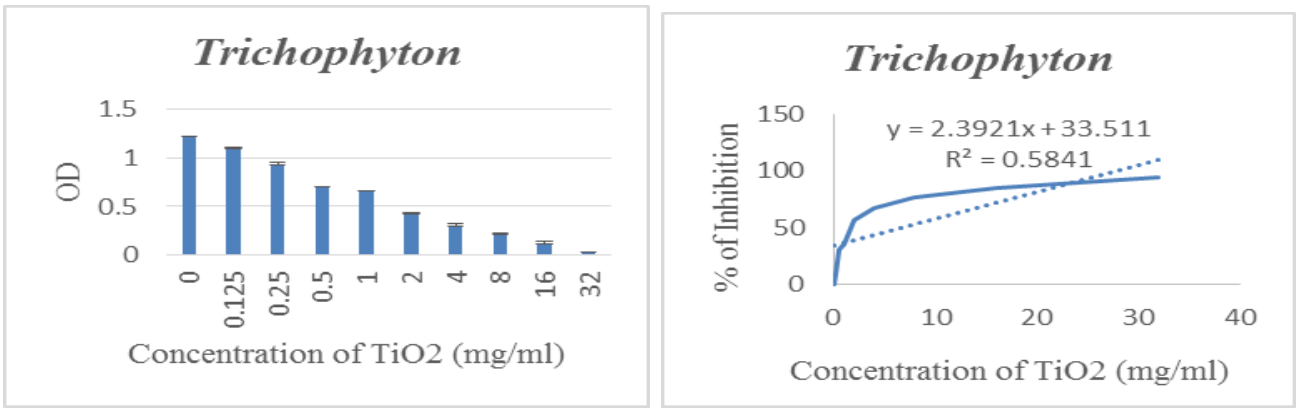

Figure 7: Anti-fungal effect of different concentration of TiO_2 against *Trichophyton*

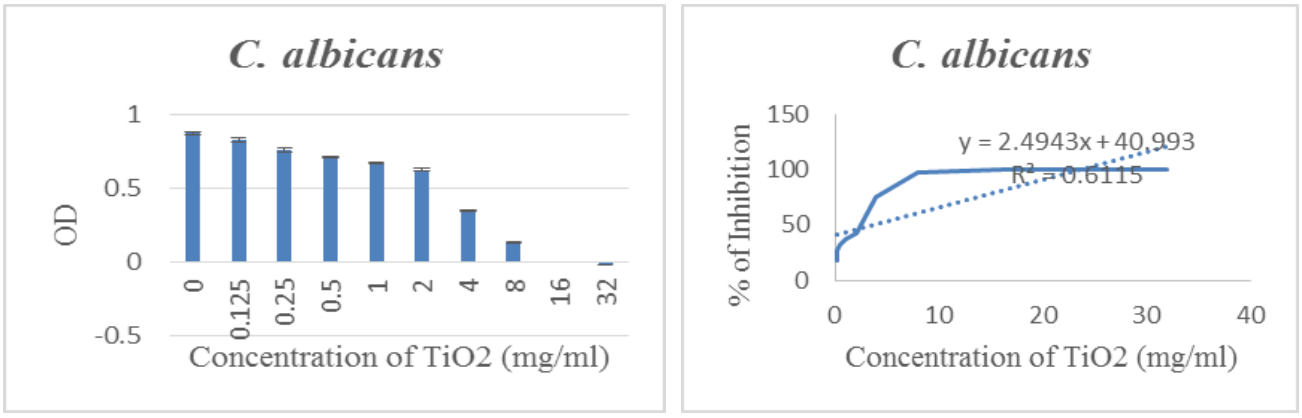

Figure 8: Anti-fungal effect of different concentration of TiO_2 against *C. albicans*

The IC 50 and IC 90 of TiO_2 nanoparticles for *A. niger* were 8.82 and 23.67 (Figure 9). As well as The IC 50 and IC 90 of TiO_2 nanoparticles for *Mucor* were 8.82 and 23.67 (Figure 10).

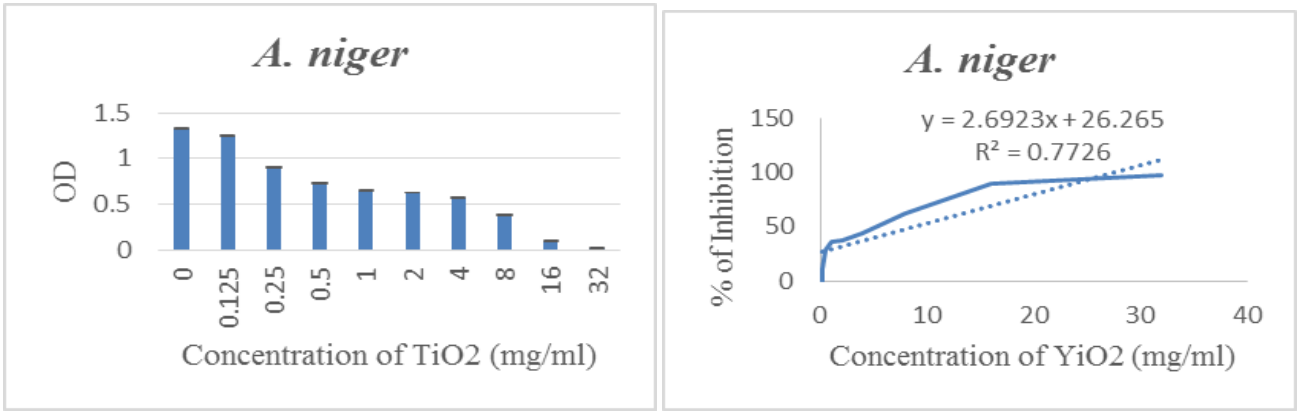

Figure 9: Anti-fungal effect of different concentration of TiO_2 against *A. niger*

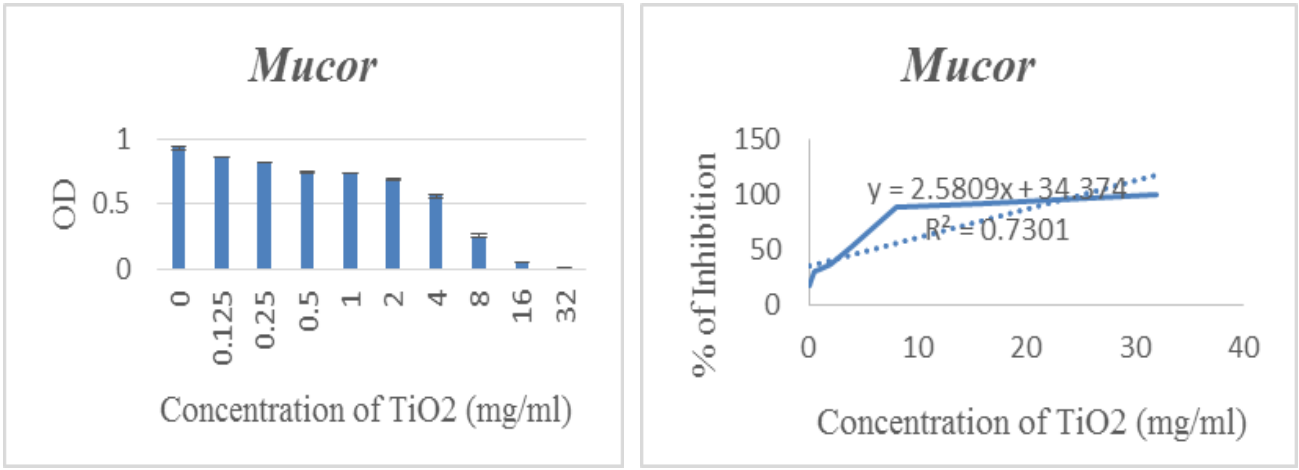

Figure 10: Anti-fungal effect of different concentration of TiO_2 against *Mucor*

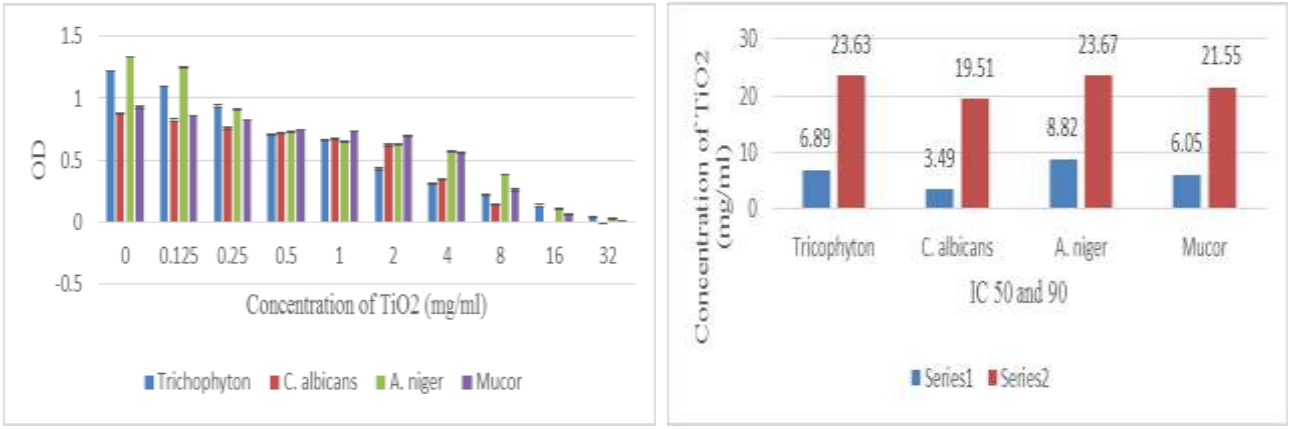

Figure 11: Comparison of inhibitory levels of fungi in different concentrations of TiO_2

Discussions

Age Distribution

From the result found most common age group was 31-45 years (46%), followed by 15-30 years (34%) and finally above 45 years (20%). In this study the result matched with study of (Bhatia et al., 2014) that was found that the most common age group was 21-50 years (64%) approximately [11].

As well as the results was matched with study of (Singla et al, 2013), who was found that the results revealed that the most common age group was 21-30 years (34%), followed by 31-40 years (27%) approximately [12]. A higher incidence of dermatophytosis was 31-45 years (46%), this predominance may be due to the individuals in this group are often most active because of their involvement in the outdoor activities such as studies and jobs [11].

Site of Infection

From the obtained result it is found that most cases present in skin (52%) followed by hair (28%) and less cases in nail (20%). In this research the result matched with study of (Konda, et al., 2017) that was found that most common dermatophyte isolated from skin scrapings [14]. As well as the results was matched with study of (Abed Ali, et al., 2017) who was found that the results revealed that 65% of the patients were infected with skin lesions followed by nail lesions (22.5%) and scalp lesions (12.5%) [15].While this finding did not agree with that of (Bakheshwain et al., 2011) who reported that nail samples (9%) were the most common dermatophyte isolated, followed by skin samples (7.5%) [13].

The most cases of dermatophytosis appear in skin may be regard to the fact that the prevalent fungal agents causing skin infection varies due to climate of the geographical region and may shift to another due to social demographic factors such as migration and health condition of individuals. Besides that, the increase in the immune suppressive conditions such as AIDS and the use of chemotherapies may account for the isolation of the NDM as the most common cause of skin infections [16].

The Minimum Inhibitory Concentration (MIC) of Silver Nanoparticles in Vitro

This research showed that the IC-50 and IC-90 of SNPs for *Trichophyton* strain was estimated at 283.78 and 848.76 ppm, respectively. Other study indicated that the results of MIC_{50} effects on *Trichophyton mentagrophytes* were 2±0.13 ug/ ml. However, the MIC_{100} of the tested antifungal Ag-NPs were relatively required higher concentrations, 5±1.0 ug/ml were required for MIC100 for inhibition the growth of *Trichophyton mentagrophytes* [17]. As well as the study of (ouf et al., 2016) matched approximately with other study who was found that the MIC50 and MIC100 of AgNPs for *Trichophyton rubrum* recorded far lower values compared with that recorded by fluconazole [18].

The values ranged 16 and 32 µg/ml, as well as indicated by the lower values of MIC50 and MIC100; all the dermatophytes species were more significantly sensitive to AgNPs as compared with fluconazole. Other study remembered the biosynthesized silver particles were highly potent against filamentous *Trichophyton* dermatophytes [19].This research showed that *C. albicans* strains isolated from patientsIC-50 and IC-90 of SNPs for them was estimated at 5.77 and 25.99 ppm, respectively.

Other study was found that The MIC50 of Ag-NPs on *Candida albicans,* was in 2±0.10 ug/ml. the MIC100 of the tested antifungal Ag-NPs was relatively required higher concentrations, 4±2.0 ug/ml for antifungal effect against *Candida albicans* [17].The difference in the MIC values (µg ml) of AgNPs between the fungal species has been documented for Candida, being 2-4 for *Candida albicans*, the MIC of *Candida albicans* was 0.4-1.6 as reported by [20].

The presented research demonstrate that *A. niger*, the IC 50 and IC 90 of SNPs were 69.49 and 632.08 ppm, respectively. Other studies found that MIC50 value of nano-Ag were 0.5mg/mL for *Aspergillus niger*, MIC_{90} values of nano-Ag were1 mg/mL for *Aspergillus niger* [21]. Other study found that the colloidal solutions containing up to 35 ppm AgNPs could inhibit the growth of *Aspergillus niger* [8].

The effect of silver nanoparticles on studied fungi samples can be illustrated by (Robles-Martínez et al., 2019) who was remembered that antimycotic activity is attributed to the

fact that particles stick to fungus membrane shows antimycotic activity, altering its permeability and thereby modifying cell viability or probably particles penetrate the membrane and modify respiratory capacity, stopping their cell division and thus causing cell death. Another possibility is that nano-particles can release silver ions that interact with thiol groups of the enzymes, inactivating them or generating free radicals with high reactive capacity and high cytotoxic activity.

Although AgNPs show antimycotic activity, Silver is a weak acid that tends to react with bases, these nanoparticles can react with sulfur and phosphorus which are weak bases and are found in most cells, such as deoxyribonucleic acid (DNA), causing their destruction and leading to cell death. These problems probably can be avoided by lower silver AgNPs concentrations and using antifungal associations [22].AL-Janabi et al., 2018 was explained that the Antifungal activity of Ag-NPs is often related to its ability to form complexes with various DNA bases and with electron donors of the respiratory chain in fungal cells. In dermatophytes, Ag-NPs decrease the activity of keratinase enzyme and ergosterol synthesis and also increase mycelium permeability [23].

The Minimum Inhibitory Concentration (MIC) of Titanium dioxide Nanoparticles in Vitro

This study showed that IC 50 and IC 90 of TiO_2 nanoparticles for *Trichophyton* strain were estimated at 6.89 and 23.63 mg/ml. the study of (George et al., 2014) who was found that the anti-fungal activity of nanoparticles and the bulk particles of titanium dioxide were good, in the case of *Trichophyton*, which was more susceptible to titanium dioxide nano-particles, both in the solid and liquid media (24). While The study of (AKHTAR et al.,2016) who reported that *Trichophyton mentagrophytes* did not show a very great reduction in growth as compared to the control [25].This work found that The IC 50 and IC 90 of TiO_2 nanoparticles for *C. albicans* were 3.49 and 19.51.

The study of (Ewerton Mima et al., 2013) who was found that minimum inhibitory concentration and minimum fungicidal concentration values of silver-doped nanocrystalline TiO2 nanoparticles (TiO2: Ag) for *Candida albicans* were 15.63 µg/mL and 31.25 µg/mL, respectively [26]. While the study of (Haghighi et al., 2013) Synthesized TiO2 nanoparticles and EDTA suppressed *Candida albicans* biofilms at the concentration of 5.14, 8.09 µg/ml for fluconazole susceptible strain and 5.35, 11. 33 µg/ml for fluconazole resistant strain [27].

This study demonstrate that The IC 50 and IC 90 of TiO_2 nanoparticles for *A. niger* were 8.82 and 23.67. The study of (Kądziołka et al., 2018) who was found that nitrogen modified titanium dioxide (N-TiO2) has a stronger antifungal activity against *P. chrysogenum* and *Aspergillus niger* than P 25 [28].The effect of titanium dioxide nanoparticles on studied fungi samples can be illustrated by (Durairaj et al., 2015) who was remembered that the metal oxides carry positive charge, while the microorganisms carry the negative charges and this causes electromagnetic attraction between microorganisms and the metal oxides, which leads to oxidation and finally death of microorganisms [29].

Conclusion

All age groups could infect with Dermatophytosis and the age range (31-45) years was found mostly in Dermatophytosis patients. Most Dermatophytosis patient's appeared infection of skin followed by hair then nail. The study appeared highly sensitive for most samples of Dermatophytosis for silver nanoparticles (AgNPs). The study showed high resistance for most samples of Dermatophytosis for titanium dioxide nanoparticles (TiO_2NPs).

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