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COMPARISON OF UV-PHOTOCATALYTIC AND INTRINSIC ANTIFUNGAL ACTIVITY OF TiO_2 NANOPARTICLES AGAINST *CANDIDA TROPICALIS*

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ABSTRACT

This study aimed to evaluate the antifungal activity of titanium dioxide nanoparticles (TiO_2 NPs) against Candida tropicalis ATCC 750 under dark and UV light activation conditions. Anatase-phase TiO_2 nanoparticles were applied to C. tropicalis suspensions at four concentrations (125, 250, 500, and 1000 µg/mL) and four exposure times (15, 30, 45, and 60 min). Experiments were performed under two conditions: a dark environment and UVA irradiation (365 nm, 15 cm distance). Antifungal activity was assessed by colony counting at the end of each exposure period, and both log reduction and percentage reduction values were calculated.

The combination of UV irradiation and TiO_2 nanoparticles demonstrated significantly higher antifungal activity compared to the dark condition. Under UV activation, complete elimination (100% mortality, >2.48 log reduction) was achieved at 45 minutes with 1000 µg/mL TiO_2 and at 60 minutes with 500 µg/mL. In contrast, under dark conditions the maximum reduction observed was 0.84 log (85.7%) at 1000 µg/mL after 60 minutes, and complete elimination was not obtained at any concentration. The UV-only group showed a limited antifungal effect, with only 0.27 log reduction (46.7%) after 60 minutes. Overall, the photocatalytic activity of the UV-activated TiO_2 system was approximately nine times stronger than the effect of UV irradiation alone.

These findings demonstrate that UV-activated TiO_2 nanoparticles exhibit strong photocatalytic antifungal activity against C. tropicalis and can achieve complete elimination at sufficiently high concentrations. Therefore, TiO_2 nanoparticles may represent a promising alternative antifungal strategy for infections caused by C. tropicalis, particularly in cases where antifungal resistance is increasing.

1. INTRODUCTION

Nanotechnology is a field that aims to impart new properties to materials by manipulating them at the atomic and molecular levels. This technology holds high potential in materials science, environmental applications, medicine and medical applications, and many other areas [1]. The unique surface structure and chemical reactivity of nanomaterials, particularly metal and metal nanoparticles, have contributed to the production of new antimicrobial agents for controlling resistant bacteria and fungi [2]. Titanium dioxide (TiO_2) nanoparticles, in particular, stand out as a material with high potential for use in various biological and antimicrobial applications due to their large surface-to-volume ratio, high aspect ratio, reactivity, and photocatalytic activity [2, 3].

TiO_2 nanoparticles exhibit three different crystal structures: anatase, rutile, and brookite. The brookite form is not widely used. However, the rutile form has a very strong UV light absorption capacity, which is why it is widely used in the cosmetics and paint industries. The anatase form is unique in terms of its photocatalytic activity with UV light and ROS production. It is frequently used in waste treatment and as an antimicrobial agent [1, 4]. The capacity of TiO_2, especially when exposed to UV light, to produce reactive oxygen species (ROS) enhances its antimicrobial properties. Numerous previous studies have

demonstrated the antibacterial and antifungal effects of TiO_2 NPs on bacteria and fungi under visible and UV light [5-10].

Fungal infections are quite common worldwide, and approximately 1.5 million people die each year [11]. Candidiasis is a fungal disease caused by various *Candida* species, which can occur in different parts of the body and produce many symptoms. Several factors, such as a weakened immune system, vitamin deficiency, intensive antibiotic use, obesity, pregnancy, and age, can contribute to the severity or fatality of these symptoms. *Candida* species are also the cause of bloodstream infections and deep infections frequently seen in hospitalized patients [12]. Finally, mucosal and superficial skin infections caused by *Candida* species and dermatophytes affect a significant portion of the human population.

Resistance to antifungal drugs is increasing, particularly in pathogenic fungal species such as *Candida,* complicating treatment processes and increasing the need for new and effective treatment strategies [13]. At this stage, many studies have reported the use of TiO_2 NPs as an alternative agent against fungi [1, 3, 8]. A review of the literature reveals that there are no studies testing the effects of anatase-form TiO_2 nanoparticles on *C. tropicalis* in visible and UV light environments in relation to time and concentration. This study focuses on how anatase-form TiO_2 NPs affect *C. tropicalis* at different concentrations and under different UV exposure times.

2. MATERIALS AND METHODS

2.1. Preparation of TiO_2 Nanoparticle Solution

TiO_2 NPs were purchased from Thermo Fisher Scientific (Titanium (IV) oxide, NanoArcTM, anatase, nanopowder, 99.9%, 32 nm, metals basis) and used without further purification. The stock solution was prepared at a concentration of 10 mg/ml with ultra-pure water and sonicated for 60 minutes. Working concentrations (1000-500-250-125 µg/ml) were obtained from the stock solution using appropriate dilutions.

2.2. Preparation of Microorganism Culture

The stock *C. tropicalis* culture was thawed from the deep freezer, inoculated into Sabouraud dextrose broth, and incubated overnight 30 °C. [14]. The *C.tropicalis* ATCC 750 culture was subcultured on Sabouraud dextrose agar (Difco Laboratories, Detroit, Mich.) prior to the experiment. The culture was suspended in sterile physiological water and adjusted to the 0.5 McFarland standard (between 1×10^6 and 5×10^6 CFU/mL). This culture density was diluted to a suitable dilution ratio (10^{-3}) for analysis and plate counting.

2.3. Time-kill curves analysis

The time-kill curve test was performed according to the Clinical and Laboratory Standards Institute (CLSI) guideline. Müller Hinton broth medium was added to 24-well cell plates to a total volume of 1 ml, followed by the specified concentration of TiO_2 (125-250-500-1000 µg/ml), and then *C. tropicalis* culture was added. Wells containing only yeast and medium, without TiO_2 NPs, were used as negative controls. Wells were designed to collect samples at 15, 30, 45, and 60 minutes to determine time-dependent inhibition rates. One of the plates designed in this way was kept in the dark, while a similar plate was kept under UVA (365 nm, 15 cm distance, 16 solar UV lamps (model F8T5BLB with 8 W rating and peak irradiance in 28.7 cm × 37 cm array.) and the catalytic activity of TiO_2 under UV was evaluated. The UV control group, which contained no TiO_2 NPs but was only kept under UV for the specified periods, was also included in the study.

CFU calculations were performed using the colony counting method. For this purpose, 100 µL samples were taken from the relevant wells at each time point (15, 30, 45, 60 minutes) and diluted in series with PBS (10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}). One hundred microliters were taken from each dilution and spread onto MHA plates using a Drigalski spreader. The plates were incubated at 35°C for 24 hours. After incubation, plates containing 30-300 colonies were evaluated, and colony counting was performed. The number of viable yeast cells was calculated using the following formula (a). In the study, a dilution factor of 10^2 (100) and an inoculation volume of 0.1 mL were applied. Antifungal activity was calculated as the log reduction value using the following formula (b). The percentage reduction in the yeast population was

calculated using the following formula (c). The time required to reduce the population by 1 log (90%) (D-value) was calculated using the following formula (d). All experiments were conducted in triplicate.

(a) CFU/mL = (Colony Count × Dilution Factor) / Inoculum Volume (mL)
(b) Log Reduction = $\log_{10}(N_0) - \log_{10}(N_t)$
(c) % Reduction = $[(N_0 - N_t) / N_0] \times 100$
(d) D-value = t(time) / Log Reduction

(N_0: Initial CFU/mL value (T=0, negative control), N_t: CFU/mL value after t time)

2.4. Statistical Analyses

In our study, the antifungal activity of TiO_2 nanoparticles (NP) on *C. tropicalis* ATCC 750 strain under dark and UVA conditions was evaluated through colony count experiments performed with three independent replicates (n = 3). Raw CFU (colony-forming unit) data for each concentration (125, 250, 500, and 1000 µg/mL) and time point (15, 30, 45, and 60 minutes) were analysed as the mean ± standard deviation (SD) across three replicates. A baseline CFU value of 300 CFU obtained from the negative control group was used. To assess the effect of concentration, a one-way analysis of variance (One-Way ANOVA, F(3,8)) was performed at each time point. Differences between dark and UVA conditions were tested using an independent samples t-test [t(4)] for each concentration-time combination. Pairwise comparisons between concentrations at 60 minutes were also performed using the t-test. Statistical significance thresholds were set at: *p < 0.05, **p < 0.01, ***p < 0.001.

3. RESULTS and DISCUSSION

The antifungal efficacy of TiO_2 nanoparticles at different concentrations against *C.tropicalis* ATCC 750 strain was evaluated using the colony count method in the presence of darkness and UV light. The effect of TiO_2 nanoparticles on *C.tropicalis* under dark conditions is shown in Figure 1 and Table 1. When examining the negative control group, no significant change in colony count was observed over 60 minutes (300 colonies), whereas a decrease in colony count was detected in the groups treated with TiO_2 NPs, depending on the dose and time.

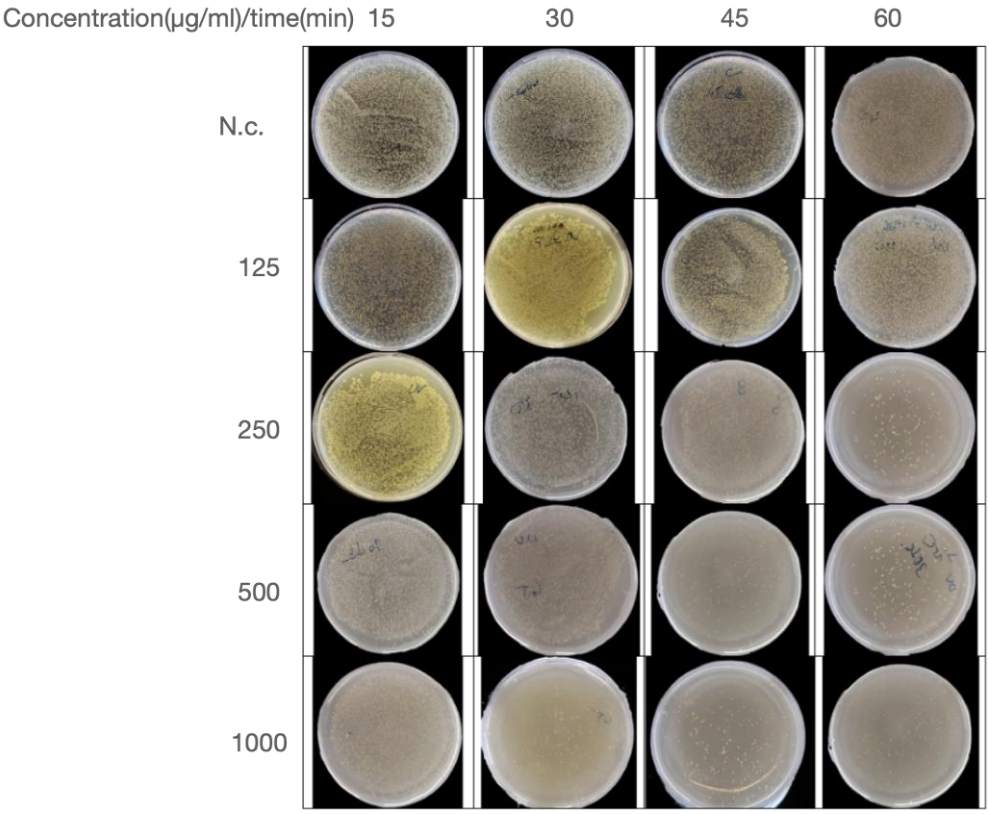

Figure 1. Petri dish images in a dark environment, with TiO_2 NPs applied at different doses and durations

Table 1. Number of colonies in a dark environment, with TiO_2 NPs applied at different doses and durations

***C. tropicalis* - TiO_2 NP dark condition (CFU, mean ± SD)**				
TiO_2 (µg/mL)	**15 min.**	**30 min.**	**45 min.**	**60 min.**
125	300.0 ± 0.0	288.3 ± 7.6	270.0 ± 5.0	205.0 ± 5.0
250	300.0 ± 0.0	198.3 ± 7.6	258.0 ± 50.3	135.0 ± 5.0
500	231.3 ± 10.3	175.0 ± 5.0	117.3 ± 6.4	95.0 ± 10.0
1000	216.7 ± 12.6	163.3 ± 10.4	64.0 ± 8.9	38.3 ± 4.5
Negative control	300.0 ± 0.0	300.0 ± 0.0	300.0 ± 0.0	300.0 ± 0.0

It has been observed that the antifungal efficacy of TiO_2 NPs increases significantly with UV activation (Figure 2 and Table 2). At a concentration of 500 µg/mL for 60 minutes of exposure, and at a concentration of 1000 µg/mL for 45 to 60 minutes of exposure, no colonies were observed in the Petri dish.

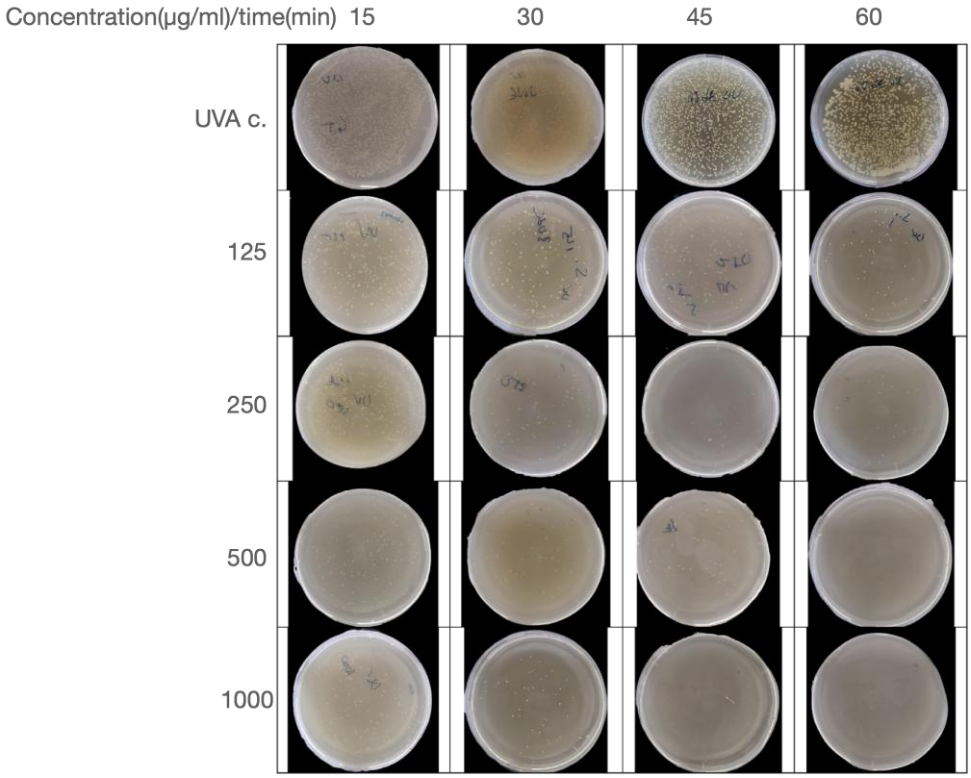

Figure 2. Petri dish images under UVA with TiO_2 NPs applied at different doses and durations

Table 2. Number of colonies under UVA + TiO_2 NPs applied at different doses and durations

***C. tropicalis* - TiO_2 NP + UVA 365 nm (CFU, mean ± SD)**				
TiO_2 (µg/mL)	**15 min.**	**30 min.**	**45 min.**	**60 min.**
125	131.7 ± 7.6	104.7 ± 5.0	94.3 ± 4.0	85.3 ± 4.6
250	122.3 ± 8.7	94.3 ± 6.0	80.0 ± 4.0	71.7 ± 3.5
500	82.3 ± 6.7	66.0 ± 6.6	74.3 ± 2.1	0 ± 0
1000	63.3 ± 6.1	45.0 ± 0.0	0 ± 0	0 ± 0
UV control	300.0 ± 0.0	300.0 ± 0.0	216.7 ± 15.3	158.3 ± 7.6

The log reduction value is a standard measurement method indicating the extent to which an antifungal agent reduces the microorganism population [15]. When log reduction values were examined in the dark, they were observed to increase in parallel with the increase in TiO_2 concentration. The value of 0.53 at a concentration of 125 µg/mL was found to be 0.84 log at a concentration of 1000 µg/mL. With UV activation, this value reached its highest (>2.48) log value, particularly at a concentration of 500 µg/mL with no reproduction observed and 60 minutes of exposure, and at a concentration of 1000 µg/mL with 45-60 minutes of exposure. A reduction of 0.27 log was observed in the control group treated with UVA alone. This value demonstrates that UV treatment alone is low in efficacy and that it gains 9 times more efficacy when applied with TiO_2 NPs (Table 3).

Table 3. Log reduction values

***C. tropicalis* - TiO_2 dark condition - Log Reduction (mean ± SD)**				
Concentration/time(min)	**15**	**30**	**45**	**60**
125	0.000 ± 0.000	0.017 ± 0.012	0.046 ± 0.008	0.165 ± 0.011
250	0.000 ± 0.000	0.180 ± 0.017	0.072 ± 0.091	0.347 ± 0.016
500	0.113 ± 0.019	0.234 ± 0.012	0.408 ± 0.024	0.501 ± 0.046
1000	0.142 ± 0.025	0.265 ± 0.027	0.674 ± 0.063	0.896 ± 0.051
***C. tropicalis* - TiO_2 and UVA 365 nm - Log Reduction (mean ± SD)**				
Concentration/time(min)	**15**	**30**	**45**	**60**
125	0.358 ± 0.025	0.458 ± 0.021	0.503 ± 0.019	0.546 ± 0.024
250	0.390 ± 0.031	0.503 ± 0.028	0.574 ± 0.022	0.622 ± 0.021
500	0.563 ± 0.036	0.659 ± 0.043	0.606 ± 0.012	>2.48 ± 0.000
1000	0.677 ± 0.041	0.824 ± 0.000	>2.48 ± 0.000	>2.48 ± 0.000
UV control	0.000	0.000	0.141	0.278

When comparing the percentage reduction values at the end of the total duration, the greatest difference (39.9%) between the groups exposed to UV light and the dark groups was observed at a concentration of 125 µg/mL. Between the UV and dark groups, the difference in percentage reduction values decreased as the concentration increased. In the dark environment, a total reduction of 87.2% was observed at the highest concentration and duration; in the groups exposed to UV and TiO_2, a 100% reduction was observed at similar duration and concentration (Table 4).

The independent samples t-test analysis revealed statistically significant differences between dark and UVA conditions at each concentration and time point. At the 125 µg/mL dose, highly significant differences were observed at all time points [$t(4) \geq 30.45$, $p < 0.001$]; at this concentration, UVA caused approximately twice as much CFU reduction compared to the dark condition. At the 250 µg/mL dose, a relatively lower significance value was obtained at 45 minutes [$t(4) = 6.11$, $p < 0.01$]; this was thought to be related to the high intra-replication variability (SD = ±50.3) observed in the dark condition at that time point. In all other comparisons, a high level of significance was maintained [$p < 0.001$]. The comparison at 60 minutes at a dose of 500 µg/mL is particularly noteworthy: while 95.0 ± 10.0 CFU were observed under dark conditions, complete elimination was achieved under UVA conditions, and this difference was found to be highly statistically significant [$t(4) = 16.45$, $p < 0.001$]. At a dose of 1000 µg/mL, the comparison at 45 minutes [$t(4) = 12.47$, $p < 0.001$] and the comparison at 60 minutes [$t(4) = 14.72$, $p < 0.001$] clearly demonstrate the superior antifungal activity of UVA. The limited activity of UV control (without TiO_2) (0.278 log at 60 minutes) supports that the observed strong antifungal effect can be attributed directly to the photocatalytic mechanism—namely, the production of reactive oxygen species (ROS) triggered by UV light on the TiO_2 surface.

Table 4. Percentage of decrease rates

TiO_2 dark condition - Percentage Reduction (mean ± SD)				
Concentration/time (min)	**15**	**30**	**45**	**60**
125 µg/mL	0.0 ± 0.0%	3.9 ± 2.5%	10.0 ± 1.7%	31.7 ± 1.7%
250 µg/mL	0.0 ± 0.0%	33.9 ± 2.5%	14.0 ± 16.8%	55.0 ± 1.7%
500 µg/mL	22.9 ± 3.4%	41.7 ± 1.7%	60.9 ± 2.1%	68.3 ± 3.3%
1000 µg/mL	27.8 ± 4.2%	45.6 ± 3.5%	78.7 ± 3.0%	87.2 ± 1.5%
Negative control	0.0 ± 0.0%	0.0 ± 0.0%	0.0 ± 0.0%	0.0 ± 0.0%
TiO_2 and UVA 365 nm - Percentage Reduction (mean ± SD)				
Concentration/time (min)	**15**	**30**	**45**	**60**
125 µg/mL	56.1 ± 2.5%	65.1 ± 1.7%	68.6 ± 1.3%	71.6 ± 1.5%
250 µg/mL	59.2 ± 2.9%	68.6 ± 2.0%	73.3 ± 1.3%	76.1 ± 1.2%
500 µg/mL	72.6 ± 2.2%	78.0 ± 2.2%	75.2 ± 0.7%	100.0 ± 0.0%
1000 µg/mL	78.9 ± 2.0%	85.0 ± 0.0%	100.0 ± 0.0%	100.0 ± 0.0%
Only UV control	0.0 ± 0.0%	0.0 ± 0.0%	27.8 ± 5.1%	47.2 ± 2.5%

C.tropicalis is a significant causative agent of invasive candidiasis, particularly in Latin America and Asia, and stands out among *Candida* species other than *C. albicans*. The recent increase in azole-resistant *C.tropicalis* isolates necessitates the development of alternative treatment strategies [16-18]. In our study, we found that TiO_2 nanoparticles exhibit dose- and time-dependent antifungal activity against *C.tropicalis*, and that this activity is significantly enhanced by UVA (365 nm) irradiation. With UV photoactivation, complete elimination was achieved in 45 minutes at a TiO_2 concentration of 1000 µg/mL and in 60 minutes at a concentration of 500 µg/mL. These findings demonstrate that TiO_2 NPs have a very high photocatalytic antifungal potential. Ahmad and his team studied TiO_2 NPs (in anatase form) similar to ours against *C. albicans* and reported that a 90-hour exposure period at a concentration of 100 µg/mL resulted in 65% cell death and that this effect was dose-dependent (50-100-150 µg/mL). Furthermore, it was determined that the anatase form exhibits higher activity in comparison to the rutile form [1]. The findings of the present study suggest that antifungal activity is significantly more effective at concentrations and durations where complete elimination is observed. In a study by Kermani et al. (2020), the antifungal activity of TiO_2 NPs against various *Candida* species, including *C. albicans*, *C.tropicalis*, *C. glabrata*, *C. krusei*, and *C. parapsilosis*, was reported. It was observed that *C.tropicalis* exhibited higher minimum inhibitory concentrations (MICs) (256 µg/mL) and minimum fungicidal concentrations (MFCs) (512 µg/mL) in comparison to other *Candida* species [8]. In the present study, limited activity was observed at concentrations ranging from 125 to 250 µg/mL. However, a 68.3% reduction in activity was observed at a concentration of 500 µg/mL, which is consistent with the findings of the aforementioned study. While an 85% elimination was observed at the highest concentration, complete elimination with UV catalytic activity (500-1000 µg/mL) is consistent with the results reported in the literature. The antifungal mechanism of TiO_2 NPs is based on the direct interaction of nanoparticles with the cell wall and membrane, penetration into the cell, and the production of reactive oxygen species (ROS) [19]. It has been observed that titanium dioxide (TiO_2) in the anatase phase exhibits superior photocatalytic activity in comparison to its rutile form, thereby enhancing its antifungal efficacy [20]. This phenomenon elucidates the elevated elimination rate of TiO_2 NP in the anatase form has a high elimination rate in a short time.

In the literature, the photocatalytic activity of TiO_2 with a broad bandgap of 3.2 eV activated under UV light is attributed to its role in generating numerous reactive oxygen species (ROS), such as singlet oxygen ($^1O_2$), superoxide anion radical ($O_2^{\bullet-}$), hydroxyl radical ($^{\bullet}OH$), and perhydroxyl radical ($HO_2^{\bullet}$). These ROS molecules trigger intracellular reactions, leading to cell damage and death [2, 21]. In our study, under UV light, TiO_2 application achieved complete elimination (>2.48 log reduction) in 45

minutes at 1000 µg/mL, while at 500 µg/mL, this time was 60 minutes. When calculating the decimal reduction time (D value), which indicates the time required for a unit logarithmic decrease, it was found that approximately 36.4 minutes were required for a 1 log decrease at a concentration of 1000 µg/mL, and 45.2 minutes at 500 µg/mL. These values indicate that TiO_2 NPs provide highly effective photocatalytic inactivation against *C.tropicalis*. When UV control alone was used, only a 0.27 log reduction (46.7% decrease) was detected within 60 minutes, indicating that UV light alone has limited efficacy against C.tropicalis. The observed synergistic effect can be explained by the initiation of photocatalytic reactions on the surface of TiO_2 by UV light. It has been documented that the ROS produced result in cell death by leading to lipid peroxidation of the fungal cell wall, disruption of membrane integrity, protein oxidation, and DNA damage, suggesting a similar mechanism in the present study [22]. In the research conducted by Nadtochenko and colleagues, it was determined that *E. coli* cells in direct contact with TiO_2 under normal light conditions experienced damage to their cell membranes through a process of oxidation, without the concomitant production of ROS. Utilising the ATR-FTIR technique, the researchers detected the formation of peroxidation products as a consequence of photocatalysis of *E. coli* cells and the lysis of *E. coli* under light irradiation after approximately one hour in the presence of TiO_2 [23]. The antifungal activities of different derivatives of TiO_2 NP against various *Candida* species have been demonstrated in numerous studies. [24-27]. The complete elimination results obtained in our study were significantly higher than the efficacy values reported in these studies. In the study conducted by Thabet and colleagues, after exposure to UV photocatalysis for one hour, only 0.1% of yeast cells (*S. cerevisiae*) remained viable. Exposure to both UV-A and TiO_2 resulted in a rapid and significant decrease in the percentage of cells during the first 30 minutes. It was noted that 100% and 90% of the cells treated with non-activated TiO_2 and photocatalysis, respectively, were still able to grow within a similar time frame [28]. This and similar studies show that the thicker and more complex cell wall structures of yeast cells cause differences in antifungal activity levels. [29]. The multi-layered structure of the yeast cell wall, containing β-glucan, chitin and mannoprotein, forms a protective barrier against ROS penetration. However, the findings of this study demonstrate that this barrier can be overcome and complete elimination achieved at high concentrations (500-1000 µg/mL) and with sufficient UV exposure time (45-60 minutes). Contrary to studies reporting analogous results, it has been reported that TiO_2 NPs, a mixture of rutile and anatase forms, exhibited 90% and 70-90% inhibition on *C. glabrata* and infected macrophage cells, respectively. This was also achieved in the dark, and the activity was found to be effective even at low doses. Furthermore, it was reported that this effect was achieved through TiO_2 activity rather than ROS production [30]. In the present study, the application of 1000 µg/mL of TiO_2 np's in a dark environment resulted in an 85.7% reduction in cells at the conclusion of the entire period, which is consistent with the findings of this study. The high inhibition rate under dark conditions indicates that unphotocatalysed TiO_2 nanoparticles also exhibit antifungal activity, whereas photocatalysed nanoparticles demonstrate these inhibition values at lower exposure times and concentrations. However, photocatalytically active TiO_2 nanoparticles may cause the formation of reactive oxygen species and photo-cytotoxicity in human cells under UVA irradiation [31]. Therefore, the clinical application potential of the detected antifungal effect requires detailed human safety testing prior to application.

4. CONCLUSION

In conclusion, this study demonstrated that anatase-phase TiO_2 nanoparticles exhibit dose- and time-dependent antifungal activity against *C. tropicalis*, and that this activity is significantly enhanced by UVA photoactivation. Under dark conditions, a reduction of approximately 87.2% was achieved at the highest tested concentration and 60 min exposure time, but complete elimination was not achieved under any conditions. In contrast, UVA-activated TiO_2 NPs provided complete inhibition at a concentration of 1000 µg/mL within 45 minutes and at a concentration of 500 µg/mL within 60 minutes. The limited efficacy of the control group exposed only to UVA light confirmed that the observed antifungal effect could be attributed primarily to the formation of photocatalytic reactive oxygen species on the TiO_2 surface rather than UV irradiation. Statistical analysis revealed significant differences between concentration groups and between dark and UVA conditions at all time points for all tested parameters. These results indicate that UVA-activated anatase TiO_2 NPs represent a promising alternative antifungal strategy, particularly given the increasing prevalence of Candida species resistant to traditional

antifungal agents. Future studies determining MIC and MFC concentrations and incorporating in vivo infection models will further elucidate the clinical translation potential of this approach.

Statement of Research and Publication Ethics

The study is complied with research and publication ethics.

Artificial Intelligence (AI) Contribution Statement

This manuscript was entirely written, edited, analyzed, and prepared without the assistance of any artificial intelligence (AI) tools. All content, including text, data analysis, and figures, was solely generated by the author.

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