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A Comparative Study of the Anti-Fungal Activity of Zinc Oxide and Titanium Dioxide Nano and Bulk Particles with Anti-Fungals against Fungi Isolated from Infected Skin and Dandruff Flakes.

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Research Article

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ABSTRACT

The anti-fungal activity of Zinc oxide and Titanium dioxide nano-particles was assessed by treating eight fungal cultures - *Aspergillus niger, Trichophyton, Fonsecaea*, *Aspergillus flavus, Rhizopus oryzae*, *Fusarium*, *Ramichloridium schulzeri* and *Cladosporium*, isolated from infected skin and dandruff flakes with the nanoparticles and analysing the extent of growth inhibition on agar and in broth media. The anti-fungal activity of these nano-particles was also compared to that of their respective bulk-particular forms, as well as to two commonly used anti-fungals, namely Amphotericin-B and Miconazole. The nano-particles were found to be more effective than the bulk-particles and almost equally efficient as Amphotericin-B, however Miconazole was found to be a better anti-fungal at an equal concentration. Zinc oxide nano-particles were better anti-fungals than Titanium dioxide, thus its anti-fungal activity at different concentrations was assessed to identify the concentration that shows similar anti-fungal activity as 3µg/ml of Miconazole. The reason for performing this study was to investigate the possibility of replacing presently used anti-fungal drugs with nano-particles in topical applications to treat mycosis.

INTRODUCTION

The increased incidence of localised and systemic fungal diseases, especially due to a rise of new and fatal immunodeficiency diseases as well as immune-suppressive therapy, has led to an extensive rise in the use of anti-fungal drugs obtained either naturally (from other microbes) or synthesised artificially. However, this increased use is accompanied not only by undesirable side effects but also the development of resistance by the target fungi. An enormous impediment to the discovery of new, efficient and safe anti-fungal medications is the realisation of the threat to human life by fungi, only recently [1]. Thus, there is a need to find new alternatives that the fungi are less likely to develop resistance to and which show minimal, preferably negligible side-effects. “The areas addressed include mechanisms underlying this resistance, improved methods to detect resistance when it occurs, alternate options for the treatment of infections caused by resistant organisms, and strategies to prevent and control the emergence and spread of resistance” [1]. The causes of the development of resistance to anti-fungals just as in the case of anti-bacterials are numerous, such as a change in the structure of the target site of action, production of drug degrading enzymes, decrease in the permeability of the cell wall or cell membrane of fungi to the anti-fungals, genetic modifications, antigenic variations, etc [1,2] . In the given study, the effectiveness of zinc oxide and Titanium dioxide nano-particles as anti-fungal agents, and their possible use in therapy has been studied. These nano-particles have already found use in many topical applications such as

sunscreens and body lotions without too many undesired reactions; therefore their use for the treatment of skin infections has been gaining much importance.

Amphotericin B is at present, the most widely used anti-fungal, originally obtained from Streptomyces nodusus, administered intravenously to treat systemic mycosis. Its toxicity to the kidneys has limited its use. It is effective against fungi like Candida spp., Cryptococcus, Histoplasma and Aspergillus. It is also used to treat leishmaniasis. Amphotericin B binds to the fungal cell wall component, ergosterol, causing a change in membrane permeability. It may also simulate macrophages by oxidative mechanisms [3]. "Amphotericin B-induced nephrotoxicity is manifested as azotaemia, renal tubular acidosis, impaired renal concentrating ability and electrolyte abnormalities like hypokalaemia and sodium and magnesium wasting. All these abnormalities occur to varying degrees in almost all patients receiving the drug. Upon withdrawal of therapy renal function gradually returns to baseline, although in some instances permanent damage is sustained, especially when the cumulative dose exceeds 5 g" [4]. It is also not recommended for pregnant women. Miconazole is usually used to treat tinea pedis, tinea corpois, tinea cruris, tinea unguium and vaginal candidiasis. It is a broad spectrum anti-fungal drug that inhibits not just dermatophytes and yeast, but can also be used as an antibiotic against Gram-positive bacteria [5]. It is especially effective against Candida albicans at a concentration of 10µg/ml. It interacts with 14-α demethylase, leading to the inhibition of ergosterol synthesis, increasing cellular permeability [6]. "Miconazole may also inhibit endogenous respiration, interact with membrane phospholipids, inhibit the transformation of yeasts to mycelial forms, inhibit purine uptake, and impair triglyceride and/or phospholipid biosynthesis" [6]. Some patients have been found exhibit hypersensitivity towards it. Its use during pregnancy should be carefully monitored.

Titanium dioxide nano-particles have a size ranging between 1nm and 150 nm. Their properties depend upon the method of preparation and the conditions under which they are synthesised, such as the pH. In general, these nano-particles form aggregates and agglomerates [7]. Titanium nano-particles have diverse applications in the paint, plastic, paper, rubber, food, ceramic, chemical, electronic and cosmetic industries. Titanium dioxide nano-particles are more preferably used as UV blockers than the bulk forms, in sunscreens and plastics, due to their property of absorbing UV light and stability to photo-degradation. Such sunscreens are found to be quite safe for people of all ages and babies [8]. The latest use is in the field of proteomics, to modify and study naturally occurring proteins [9].

Zinc oxide nano-particles possess excellent durability and heat resistance [10]. One of the most important uses of zinc oxide nano-materials is in the preparation of medically as well as cosmetically useful substances. Zinc oxide is more commonly used than titanium dioxide nano-particles in sunscreens, since it is a better blocker of ultraviolet light, and also causes lesser side effects. "Zinc oxide nano-particles also have good biocompatibility to human cells" [10]. These nano-particles are an important source of zinc, which is involved in various essential biochemical reactions in the body, since they function as enzyme co-factors [11] .They also help in maintaining a healthy immune system [10]. Zinc oxide nano-particles are also relatively good antimicrobial agents, whose activity increases with a reduction in the particle size [10].

MATERIALS AND METHODS

Isolation and identification of fungi from skin

The infected portions of the skin of the diseased persons were first wiped with a cotton swab dipped in alcohol, and were allowed to dry. The infected skin was scraped with a sterile scalpel or a sterile slide, to obtain an adequate amount of the sample [12]. The samples were transferred to the microbiology laboratory in sealed envelopes, and cultured within 24 h of collection on Potato dextrose agar medium. The plates were then incubated at RT for 5 days, and the plates were observed for the growth of fungal colonies. The fungal colonies thus obtained on the agar plates after primary isolation, were sub cultured on Potato dextrose agar slants in order to obtain pure cultures. The pure cultures were stained using lactophenol cotton blue solution/ dye, and observed under the microscope. Based on the observed colony morphology and the microscopic characteristics, the fungi were identified.

Synthesis of Zinc oxide nano-particles

0.2M (1.756gm) of Zinc Acetate was dissolved in 40ml of Dimethyl Sulphoxide, and the mixture was stirred on a magnetic stirrer for about 30 minutes. In another flask, 1.2M (1.344gm) Potassium hydroxide was prepared in 10ml of Ethyl alcohol. The Potassium hydroxide - Ethanol mixture was added drop wise to

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the Zinc acetate, followed by stirring for 5 minutes on a magnetic stirrer.0.24ml of Thioglycerol was then added and the mixture was stirred for an hour until it turned milky white in colour. The solution was centrifuged at 3000rpm for 10 minutes; the pellet was collected and washed thrice with methanol and was then suspended in methanol [13].

Synthesis of Titanium dioxide nano-particles

To 20ml of Titanium trichloride solution, 60 ml of 0.1N Ammonium Hydroxide solution (0.4ml of Ammonia + 59.6ml of distilled water) was added. The solution was stirred for 48 h on a magnetic stirrer till a white colour solution of Titanium dioxide is formed. The solution was centrifuged at 4000rpm for 10 minutes. The pellet thus obtained was collected, dried and then suspended in isopropanol.

Preparation of the agar plates and measurement of anti-fungal activity

Potato dextrose agar medium was used to carry out the antifungal activity of ZnO nano and bulk particles and TiO_2 nano and bulk particles. Control plates (without ZnO and TiO_2) were used to measure the zone diameter of growth. The media was incorporated with ZnO and TiO_2 nano and bulk particles at a concentration of 12µg/ml and then sterilized to know the effect of heat on the activity of the nanoparticles. In another set of media the particles were incorporated after sterilization to study the antifungal activity. Different plates were inoculated with the fungi isolated from skin and dandruff and incubated for 24h, 48h and 72h respectively at RT. The antifungal activity was estimated by measuring the zone diameter of growth.

Preparation of the broth flasks and measurement of anti-fungal activity

Sabouraud broth was used to measure the dry and wet weight of the fungal cultures. The media was incorporated with ZnO and TiO_2 nano and bulk particles before and after sterilization at a concentration of 12µg/ml and control broth was used to measure the wet and dry weights of the cultures. The flasks were incubated at RT for 5 days. The anti-fungal activity of zinc oxide nano particles was compared to that of commercially available anti-fungal agents- Amphotericin-B and Miconazole to assess the effectiveness of the nano particles. The anti-fungal activity of different concentrations of the Zinc oxide nano particles were measured to estimate the approximate concentration that shows a similar anti-fungal activity to 3µg/ml of Miconazole.

RESULTS

The antifungal activity of ZnO and TiO_2 nanoparticles was estimated by measuring the zone diameter of growth. The ZnO nanoparticles were effective against the fungi. From Table 1 we interpret that the effect of nanoparticles was high when added after sterilization of the media .*Aspergillus niger, Fonseceae, Rhizopus oryzae* showed mat growth after 72h of incubation in the control plates. The zone diameter of growth reduced drastically in the plates incorporated with ZnO nanoparticles after sterilization. Against *Trichophyton, Aspergillus flavus* there was not much effect after 48h, 72h but about 90% reduction in growth was observed after 24h. ZnO nanoparticles were also effective against *Fusarium, Ramichloridium schulzeri, Cladosporium* there was about 40-50% reduction in growth after 72h. From Table 2 it is clear that the bulk ZnO particles were not very effective against the fungi tested in comparison with the ZnO nanoparticles.

Sabourauds broth was used to measure the dry and wet weights of the fungal cultures. Table 3 shows a reduction in wet weight and dry weight of the cultures in the media incorporated with ZnO nanoparticles. Though there is reduction in weight of *A.niger, Trichophyton, A.flavus* and *Rhizopus oryzae* in the medium incorporated with ZnO nanoparticles before sterilization it was comparatively less than the nanoparticles incorporated after sterilization. Against *Ramichloridium schulzeri* and *Cladosporium* there was more than 75% reduction in weight. This shows that the ZnO nanoparticles were able to effectively inhibit the growth of the fungal cultures. From Table 4 we can infer that ZnO nanoparticles are more effective than the bulk particles.

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Table 1: Diameter of the colony of different fungi on Potato dextrose agar with Zinc oxide nano-particles (12µg/ml)

Organisms	Diameter of the colony (cm)								
	Control			Before sterilization			After sterilization		
	24h	48h	72h	24h	48h	72h	24h	48h	72h
Aspergillus niger	1	1.3	mat	0.6	1	mat	0.4	0.9	5.7
Trichophyton	6.3	mat	mat	0.8	mat	mat	0.3	mat	mat
Fonsecaea	0.3	1.4	mat	0.2	1	mat	0.2	0.4	0.9
Aspergillus flavus	1.6	mat	mat	1.3	mat	mat	0.8	mat	mat
Rhizopus oryzae	5.7	mat	mat	1.4	2.7	mat	1.1	1.4	6
Fusarium	0.7	4.2	4.7	0.4	1.5	2.8	0.4	1	1.8
Ramichloridium schulzeri	0.5	1.8	2.5	0.5	1.3	1.6	0.5	0.9	1
Cladosporium	0.7	1	1.2	0.4	0.8	1	0.4	0.6	1

Table 2: Diameter of the colony of different fungi on Potato dextrose agar with Zinc oxide bulk-particles (12µg/ml)

Organisms	Diameter of the colony (cm)								
	Control			Before sterilization			After sterilization		
	24h	48h	72h	24h	48h	72h	24h	48h	72h
Aspergillus niger	1	1.5	mat	0.7	0.9	mat	0.3	0.7	7.2
Trichophyton	6.3	mat	mat	0.7	0.8	mat	0.4	1.7	mat
Fonsecaea	1.5	mat	mat	0.7	1.5	mat	0.5	0.7	mat
Aspergillus flavus	1.8	mat	mat	0.9	2	mat	0.8	1.8	mat
Rhizopus oryzae	7	mat	mat	1.4	2.7	8.3	1.1	1.4	8
Fusarium	0.9	1.4	4	0.9	2	2.6	0.9	1.9	2.6
Ramichloridium schulzeri	0.8	1.3	2.2	0.5	1.1	2	0.3	0.9	1.1
Cladosporium	0.4	1	1.5	0.2	0.8	1.2	0.2	0.8	0.9

Table 3: Weight of the culture of different fungi in Sabourauds broth with Zinc oxide nano-particles (12µg/ml)

Organisms	Weight of the colony (gm)					
	Control		Before sterilization		After sterilization	
	Wet weight	Dry weight	Wet weight	Dry weight	Wet weight	Dry weight
Aspergillus niger	4.31	2.23	3.42	1.96	2.4	0.96
Trichophyton	6.27	4.71	5.77	4.11	4.73	2.77
Fonsecaea	3.48	1.94	2.96	1.52	2.32	1.09
Aspergillus flavus	3.79	2.42	2.31	1.82	2.19	1.64
Rhizopus oryzae	4.45	2.75	3.69	1.49	3.48	1.41
Fusarium	3.68	1.8	2.67	1.53	2.37	1.13
Ramichloridium schulzeri	4.32	2.45	3.28	1.94	2.49	0.34
Cladosporium	2.93	0.92	2.74	0.7	2.24	0.68

Table 4: Weight of the culture of different fungi in Sabourauds broth with Zinc oxide bulk-particles (12µg/ml)

Organisms	Weight of the colony (gm.)					
	Control		Before sterilization		After sterilization	
	Wet weight	Dry weight	Wet weight	Dry weight	Wet weight	Dry weight
Aspergillus niger	4.35	2.29	3.84	2.46	3.02	1.99
Trichophyton	6.2	4.83	5.96	3.28	5.73	2.77
Fonsecaea	3.74	2.93	3.19	2.03	3.01	1.74
Aspergillus flavus	3.82	2.67	2.88	1.8	2.4	1.21
Rhizopus oryzae	4.38	2.72	4.26	2.31	3.49	1.44
Fusarium	3.56	1.76	2.45	1.17	2.34	0.98
Ramichloridium schulzeri	4.41	3.25	3.54	2.21	3.23	1.64
Cladosporium	3.05	1.86	2.73	0.64	2.62	0.5

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Table 5: Diameter of the colony of different fungi on Potato dextrose agar with Titanium dioxide nano-particles (12µg/ml)

Organisms	Diameter of the colony (cm)								
	Control			Before sterilization			After sterilization		
	24h	48h	72h	24h	48h	72h	24h	48h	72h
Aspergillus niger	0.9	2.2	mat	0.5	1.7	mat	0.4	1.3	2
Trichophyton	3.7	mat	mat	1.5	mat	mat	0.3	mat (no spores)	mat (no spores)
Fonsecaea	0.3	1.4	mat	0.2	1	mat	0.2	0.9	mat
Aspergillus flavus	1.6	mat	mat	1.3	mat	mat	0.8	mat	mat
Rhizopus oryzae	7	mat	mat	2	6.8	mat	1.4	2.7	6.4
Fusarium	0.7	4.2	4.7	0.4	1.5	2.8	0.4	1.5	2.8
Ramichloridium schulzeri	0.5	1.8	2.5	0.5	1.3	1.6	0.4	0.9	1.1
Cladosporium	0.3	0.9	1.2	0.2	0.7	0.8	0.2	0.5	0.6

Table 6: Weight of the culture of different fungi in Sabourauds broth with Titanium dioxide nano-particles (12µg/ml)

Organisms	Weight of the colony (gm)					
	Control		Before sterilization		After sterilization	
	Wet weight	Dry weight	Wet weight	Dry weight	Wet weight	Dry weight
Aspergillus niger	4.19	2.23	3.67	1.99	3.26	0.99
Trichophyton	6.27	4.02	5.88	3.92	3.77	1.9
Fonsecaea	3.4	2.61	2.95	1.26	2.33	1.08
Aspergillus flavus	3.75	2.28	3.58	2.17	3.55	2.15
Rhizopus oryzae	4.43	2.53	4.3	2.05	3.21	1.5
Fusarium	3.57	2.22	2.67	1.26	2.47	1.15
Ramichloridium schulzeri	4.1	2.85	3.09	1.83	3.05	1.7
Cladosporium	3	1.84	2.6	1.33	2.55	1.14

Table 7: Diameter of the colony of different fungi on Potato dextrose agar with Titanium dioxide bulk-particles (12µg/ml)

Organisms	Diameter of the colony (cm)								
	Control			Before sterilization			After sterilization		
	24Hrs	48Hrs	72Hrs	24Hrs	48Hrs	72Hrs	24Hrs	48Hrs	72Hrs
Aspergillus niger	2.6	mat	mat	1.1	mat	mat	0.8	mat	mat
Trichophyton	mat	mat	mat	mat	mat	mat	8.1	mat	mat
Fonsecaea	0.4	mat	mat	0.3	mat	mat	0.2	4.1	5.6
Aspergillus flavus	2	mat	mat	1.9	mat	mat	0.7	mat	mat
Rhizopus oryzae	7	mat	mat	1.7	mat	mat	0.7	mat	mat
Fusarium	0.6	3.3	4	0.2	1.5	1.8	0.2	0.8	1.1
Ramichloridium schulzeri	0.8	1.5	2	0.6	1.1	1.3	0.5	0.9	0.9
Cladosporium	0.6	1.3	1.5	0.5	1.1	1.4	0.3	0.9	1

All the fungi isolated and characterised were inhibited by Titanium dioxide nano particles, especially *Trichophyton*. From Table 5 we can infer that *Aspergillus species, Fonseca* and *Rhizopus oryzae*, showed mat growth in control plates after 48 h. Their growths was inhibited by TiO_2 nanoparticles after 48h. All the fungi were inhibited by the nano particles incorporated into the medium after sterilization rather than before. The maximum inhibition was observed in the cases of *Fusarium*, *Cladosporium* and *Rhizopus oryzae*. *Trichophyton* was best inhibited by titanium dioxide nano particles as compared to zinc oxide nano particles and the bulk particles. *Trichophyton* did not sporulate much, although the mycelial growth was observed. From table 7 we can infer that the titanium dioxide particles were not as effective as nanoparticles against the fungi, except against *Fusarium*, *Cladosporium* and *Ramichloridium schulzeri.* The fungi were inhibited by the bulk particles incorporated into the medium after sterilization rather than before. The broth medium, similar results were observed as in the case of growth in solid media. Most of the organisms showed a reduction in growth by half, while *Ramichloridium schulzeri* exhibited an almost four times reduction in growth and *Aspergillus species* and *Cladosporium* did not show a very great reduction in growth as compared to the control.

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Table 8: Weight of the culture of different fungi in Sabourauds broth with Titanium dioxide bulk-particles (12µg/ml)

Organisms	Weight of the colony (gm) Control Wet weight	Control Dry weight	Before sterilization Wet weight	Before sterilization Dry weight	After sterilization Wet weight	After sterilization Dry weight
Aspergillus niger	4.38	2.84	3.61	2.65	3.48	2.46
Trichophyton	6.35	4.83	5.97	3.86	5.84	3.78
Fonsecaea	3.8	2.37	3.75	2.34	3.27	1.5
Aspergillus flavus	3.75	2.28	3.58	2.17	3.55	2.15
Rhizopus oryzae	4.43	2.53	4.3	2.05	3.27	1.5
Fusarium	3.57	2.23	2.67	2	2.47	1.15
Ramichloridium schulzeri	4.25	2.5	3.39	1.97	3.19	1.77
Cladosporium	3.2	1.82	2.6	1.32	2.54	1.21

The antimicrobial activity of zinc oxide nano particles, which showed the maximum anti-fungal activity when compared to the bulk particles and titanium dioxide nano particles, was compared to that of Amphotericin-B and Miconazole -the commonly used anti - fungal drugs, used for chemotherapy of mycosis. In Table 9, 10 we observe that the fungi were most inhibited by miconazole, and the least by zinc oxide nano particles at a concentration of 3µg/ml. Amphotericin-B showed an almost equal inhibition as zinc oxide nano particles, except in the case of *Fonseca* and *Fusarium. Ramichlorium schulzeri* and *Cladosporium* were slightly more inhibited by Amphotericin-B.

Since the antimicrobial activity of Zinc oxide nano particles and Miconazole showed the maximum anti-fungal activity when compared to the bulk particles, Titanium dioxide nano particles, and that of Amphotericin-B. Miconazole was the better anti-fungal drug, it was used as a standard to find out the concentration of Zinc oxide nano particles that showed an equal inhibition of growth of the test organisms. Thus, the concentration of Zinc Oxide nanoparticles that showed almost an equal inhibition against the test organisms, as Miconazole at3µg/ml was studied. Fusarium and Cladosporium required the least concentration of the nano particles (15µg/ml), whereas Trichophyton required the maximum concentration of zinc oxide nano particles to exhibit an inhibition equal to 3µg/ml of Miconazole (30 µg/ml).

Table 9: Weight of the culture of different fungi in Sabourauds broth with Zinc oxide nano-particles (3µg/ml), Amphotericin-B (3µg/ml) and Miconazole (3µg/ml)

Organisms	Weight of the colony (gm) Control Wet weight	Control Dry weight	ZnO nano particles (3µg/ml) Wet weight	ZnO nano particles (3µg/ml) Dry weight	Amphotericin-B (3µg/ml) Wet weight	Amphotericin-B (3µg/ml) Dry weight	Miconazole (3µg/ml) Wet weight	Miconazole (3µg/ml) Dry weight
Aspergillus niger	4.16	2.85	4.13	2.8	4.05	2.78	1.97	0.53
Trichophyton	6.27	4.02	6.27	4	5.66	3.93	1.51	0.16
Fonsecaea	3.7	2.84	3.7	2.82	2.72	1.71	1.54	0.19
Aspergillus flavus	3.81	2.55	3.81	2.54	3.81	2.52	1.35	0.8
Rhizopus oryzae	4.43	2.52	4.41	2.35	4.32	2.26	1.29	0.83
Fusarium	3.2	2.23	3.2	2.22	2.13	1.09	1.52	0.87
Ramichloridium schulzeri	3.94	2.09	3.93	2.08	2.94	1.67	1.33	0.71
Cladosporium	3.19	2.5	3.18	2.48	2.88	1.98	1.71	0.82

Table 10: Weight of the culture of different fungi in Sabourauds broth with Zinc oxide nano-particles and Miconazole (3µg/ml)

Organisms	Weight of the colony (gm) Control Wet weight	Control Dry weight	ZnO nano particles Wet weight	ZnO nano particles Dry weight	Miconazole (3µg/ml) Wet weight	Miconazole (3µg/ml) Dry weight
Aspergillus niger	4.17	2.23	(18µg/ml)1.84	0.47	1.99	0.58
Trichophyton	6.27	4.02	(30µg/ml)1.37	0.1	1.51	0.16
Fonsecaea	3.68	2.74	(18µg/ml)1.52	0.12	1.54	0.19
Aspergillus flavus	3.83	2.55	(18µg/ml)1.35	1.83	1.35	1.81
Rhizopus oryzae	4.43	2.57	(24µg/ml)1.16	0.49	1.25	0.63
Fusarium	3.25	2.23	(15µg/ml)1.37	0.72	1.52	0.87
Ramichloridium schulzeri	3.94	2.09	(21µg/ml)1.3	0.67	1.33	0.71
Cladosporium	3.19	2.5	(15µg/ml)1.64	0.8	1.71	0.82

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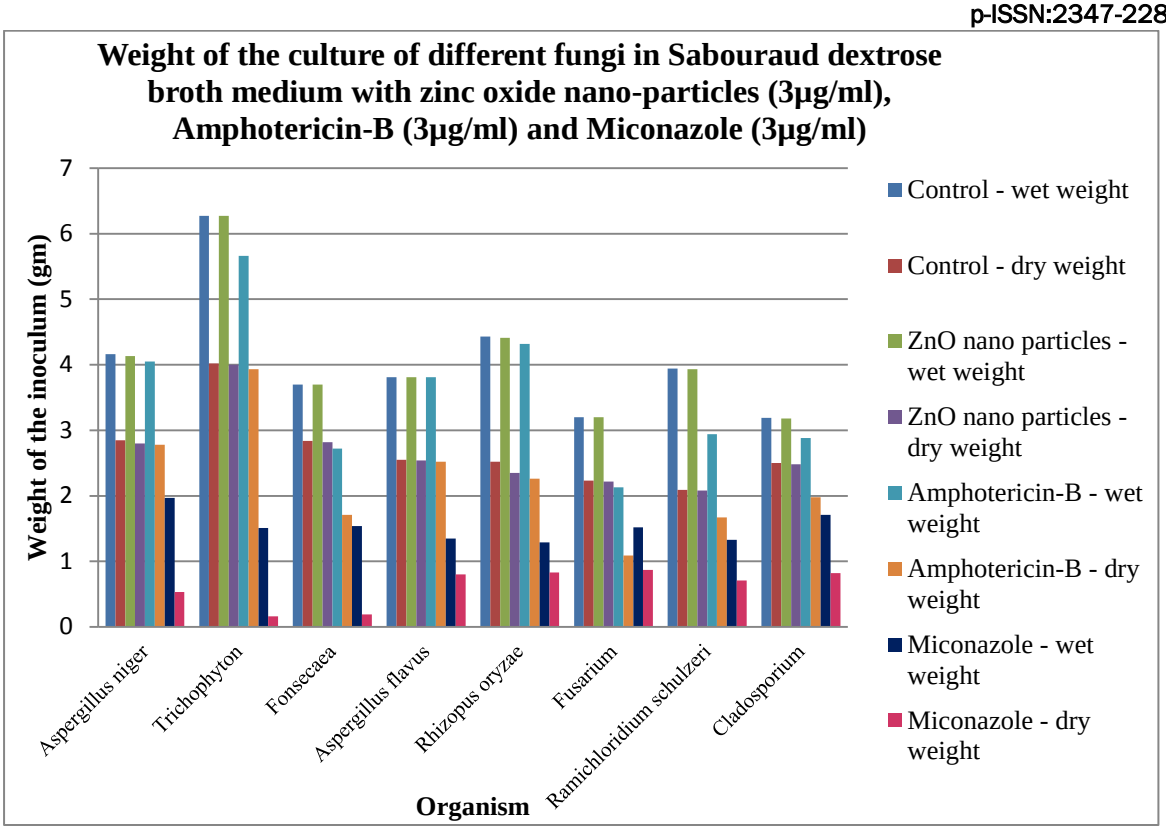

Both Zinc oxide and Titanium dioxide nano-particles exhibited a reasonable amount of anti-fungal activity, at a concentration of 12µg/ml, against all the fungi that were tested in the study, though zinc oxide proved to be the better anti-fungal, except in the case of *Trichophyton*, which was more susceptible to titanium dioxide nano-particles, both in the solid and liquid media. When compared to their bulk forms, the nano-particles were found to be better at inhibiting fungal growth. The anti-fungal activity of nano and the bulk particles of zinc oxide and titanium dioxide were good, when they were incorporated into the medium after sterilization, rather than before, where they showed poor activity.

CONCLUSION

Nano-particles are better anti-fungal agents than the corresponding bulk material of the same composition, due to their greater surface area [8]. Nano-particles exhibit non-specific activity, thus the chances of the development of resistance against them is very low. Zinc nano-particles denature the proteins and nucleic acids within bacterial cells, inhibiting the replication. Its inhibitory activity is perhaps the same in the case of fungi. The anti-fungal activity of zinc nano-particles increases with an increase in its concentration in the medium as was observed in this paper, though the concentration required varies in the case of different fungi. Zinc oxide nano-particles are relatively safe and do not cause serious side-effects in comparison to anti-fungal drugs like Amphotericin B and Miconazole which lead to severe side-effects like renal damage, skin damage, etc. due to which their use has been restricted, especially in the case of pregnant women, young children, the immune-compromised and the elderly [4]. Therefore along with further research, these nano particles could be incorporated into antifungal medicines, which may prove to be a great boon to mankind.

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