Scientific Research Publishing

Advances in Nanoparticles, 2016, 5, 206-223
http://www.scirp.org/journal/anp
ISSN Online: 2169-0529
ISSN Print: 2169-0510

# The Interesting of Antifungal Effects of Novel *In Vitro* Fabrics of Stabilized ZnO Nanofluids

**Fatemeh Katouzian[1], Zahra Fakhroueian[2*], Soheila Moradi Bidhendi[3]**

[1]Department of Microbiology, Faculty of Advanced Sciences and Technology, Pharmaceutical Sciences (IAUPS), Tehran, Iran
[2]School of Chemical Engineering, College of Engineering, and IPE, University of Tehran, Tehran, Iran
[3]Microbiology Department, Razi Vaccine and Serum Research Institute, Karaj, Iran
Email: *fakhroueian@ut.ac.ir

**How to cite this paper:** Katouzian, F., Fakhroueian, Z. and Bidhendi, S.M. (2016) The Interesting of Antifungal Effects of Novel *In Vitro* Fabrics of Stabilized ZnO Nanofluids. *Advances in Nanoparticles*, **5**, 206-223.
http://dx.doi.org/10.4236/anp.2016.54022

**Received:** September 15, 2016
**Accepted:** November 12, 2016
**Published:** November 15, 2016



## Abstract

According to the extent of fungal infections, to be chronic these such diseases and recently the emerging issue of increased antibiotic resistance in fungal infections, most of scientists are going to find a proper way to replace antibacterial agent by significant semiconductor ZnO nanoparticles (NPs). They are well known to be one of the most important and special metal oxide nanoparticles in pharmaceutical against the most common fungi. ZnO nanoparticles were synthesized using sol-gel, hydrothermal and functionalized surface methods and formulated in water solutions as nanofluids. XRD, FTIR and SEM techniques and UV-Vis absorbance spectroscopy characterized their ZnO modified nanostructures. Also antimycotic potential according to generally tests such as: (MIC) minimum inhibitory concentration, (MFC) minimum fungicidal concentration and normally well diffusion method with standard strains fungi were performed. Among five common fungi strains using in this research, new various ZnO nanofluids showed noticeable results for dermatophyte fungi like *Trichophyton mentagrophytes*, *Microsporum gypseum*, *Microsporum canis*, *Candida albicans and Candid tropicalis* which had un growth zones in order 70, 40, 35, 30 and 30 mm in comparing with Clotrimazole reference reagent: 30, 25, 25, 18 and 20 mm by well method. The performance of MIC for ZnO nanofluids on fungi was determined to be equal to 0.35, 3.12, 6.25, 6.25 and 6.25 μgr/ml and MFC of nanoproducts showed the 1.5, 12.5, 25, 25 and 25 μgr/ml. Therefore, the designed ZnO nanofluids could reveal the most effect on fungi which cause dermal (ringworm), mucosal (thrush) and vaginal infections, so we are able to apply these surface high energetic ZnO water-based nanofluid formulations as in vitro nanomedicine and nanohygiene for the first time.

## Keywords

ZnO Nanofluids, Potential Medical Applications, Antifungal Activity,

Traditional Resistant Antibiotic, Surface Modification, Nanomedicine, Nanohygiene, MFC, Nanoformulation, Stabilized Water-Based Nanoparticle

## 1. Introduction

Infections caused by fungi has a wide range, depending on the location were classified to superficial cutaneous, mucosal, subcutaneous and systemic infection. Although systemic infections can be fetal, but the level of improvement cutaneous is often limited and curable. Fungi classified into two groups pathogenic and opportunistic. The first group affects healthy people and second group affects susceptible individuals [1]. Recently, serious searches for new antimicrobials have performed due to the appearance of various infectious morbidity and improvement in occurrence of drug resistance against some of pathogenic fungi and zinc oxide nanoparticles are the most promising and novel therapeutic agents for this event. It was found that, the upsurge in the recent research about the nanoparticles and interesting advantage application as antimicrobials are due to having individual physiochemical properties and biological toxicity against growth inhibitory of microbes and fungi [2]. Main difference parameters cause nanoparticles including 1 - 100 nm in diameter to show significantly chemical reactivity differently than their bulk structures, and their properties are able to change with the decrease in dimensions [3]. The surface of nanoparticles can be manipulated exclusively by physico-chemical, and optical reactions suitably for desired applications especially in the field of untifungi and nanomedicine [4]. Nanoparticles have unique large surface area, high surface-to-volume ratio, and energetic management atoms with small size dependent chemical and physical properties that have dimensions comparable to biological functional units containing exceptional shape and morphology, so they are being explored extensively currently. These characteristic make them as a fascination candidate for various application in both in vivo and in vitro antibacterial and antifungal in the field of nanomedicine researches. ZnO nanoparticles (NPs) and significant their nanofluids are using mainly in targeted drug delivery, imaging, sensing, antimicrobials to target highly pathogenic and great drug resistant microbes, cancer treatment and artificial implants in modern medicine science. Biocompatibility is an extensive potential for nanobiomedical application researches and most of the nanomaterials especially ZnO nanoparticles indicate this trait [5]. The antifungal applications of two nanometal oxide ZnO and MgO nanoparticles (NPs) with nearly average size of 30 and 50 nm, respectively are considered on some fungal infections [6]. R.V. Ravishankar *et al.* [2] also investigated that ZnO and CuO (NPs) owing to highly antiseptic and fungicides nature are being combined towards a diversity of pharmaceutical, medicinal substance and skin coatings. Recently, the scientists are going to find a proper way to replace old and traditional nanomedicine by novel materials such as effective ZnO nanoparticles and special nanofluids as new drugs, due the extent of fungal infections and increasing antibiotic resistant and lack of new control strategies [7]. ZnO NPs and their

novel water based nanofluids showed that could be potent pharmaceutical against the most common fungi, with high strong control the growth of fungi. One of the important and usual applications of ZnO nanoparticles is wallpapers in hospitals similar to fungicide and antimicrobials. Because the strong antifungal effect against bacterial pathogens and specific fungi, ZnO (NPs) is also an effective component in producing of dermatophytic lesions to form of antifungal creams, pomades, and lotions [8]. It was reported that superficial fungal infections (SFI) are prevalent skin diseases and affect millions of people worldwide [9]. Earlier most dermatophyte strains had relatively restricted geographical distribution but currently, dermatophytosis has become one of the most common human infectious diseases worldwide. It is obvious that, the environmental bacterial and fungal pollution contributes a major problem to human and animal health. Dermatophytes are fungi that require keratin for growth. These fungi can cause superficial infections of the skin, hair, and nails. Dermatophytoses are referred to as "tinea" infections. They are also named for the body site involved. Microsporum, Trichophyton, and Epider-mophyton species are the well-known pathogens in skin infections. Ring worm, athlete's foot, jock itch are also the main diseases cause by dermatophytes fungi [10]. Also, *Candida albicans* and *Candida tropicalis* are in group of nosocomial candidemia. Candidiasis is usually a very localized infection of the skin or mucosal membranes, including the oral cavity (thrush), the pharynx or esophagus, the gastrointestinal tract, the rectum, anus, perianal/perirectal or anorectal area (in men as well as women), the perineum, the urinary bladder, the fingernails or toenails (onychomycosis), and the genitalia (vagina, penis, etc.) [11] [12]. The patient people who have HIV/AIDS and lost their immune system can increase oral candidiasis infection more than healthy ones, especially for adult people with wearing dentures [13].

The present study was designed to conduct laboratory to evaluate in vitro inhibitory effect of ten various stabilized formulations of water-based zinc oxide nanofluids on the growth of five dermatophyte and systemic fungi for the first time and all the results were compared with clotrimazole as an antifungal medicine standard reagent. It is certainly, we can apply them in nanomedical soon, because these manufacturing are very novel technology in medicine industry.

## 2. Materials and Experimental Details

### 2.1. Synthesis and Formulation of New Soluble ZnO Nanofluids

ZnO nanoparticles (NPs) were prepared by chemical hydrolysis as sol-gel process, wet-chemical procedures, and followed by hydrothermal method [14]. The surface of synthezied ZnO NPs were functionalized to hydrophilic and modified by specific reagents, and carried out in aqueous media and formulated using various nonionic surfactants including different HLB, ethylene glycol solvent (EG), polyvinyl pyrrolidone (PVP), oily herbal fatty acids, organosilane, water soluble solvents and suitable pH reagents. We could synthesis of 10 novel nanoformulations in this study containing synthezied ZnO NPs, and experimented through 5 various fungal strains.

### 2.2. Introducing of 10 Novel Various ZnO Nanoformulations with Their Numeric Codes

Sample 1—with ZnO 48 code, the synthezied ZnO NPs powder was soluble in mixture of water and alcohol, and dimethyldichloro silane in EG solvent which is amphiphilic and soluble in water.

Sample 2—with ZnO 51 code, ZnO NPs powder modified by Soya fatty acid was dissolved in water and 1ml organosilane, and pH was adjusted by citric acid.

Sample 3—with ZnO 391code, ZnO NPs powder was functionalized with multiwall CNT (carbon nano tubes), then dissolved in mixture of distilled water, ethylbanzene solvent, and LA2EO (lauryl alcohol 2-EO) as nonionic surfactant.

Sample 4—with ZnO 45 code, ZnO NPs powder was capped with green Soya fatty acid, then dissolved in nano PVP polymer and PEG 4000 mixed in liquid distilled water.

Sample 5—Synthesis of ZnO/PVP nanocomposites, 0.008 mole zinc acetate dihydrate was hydrolyzed by 0.09 mole liquid KOH. Then the 2 - 4 gram PVP polymer was added and refluxed during 6 - 8 h. The supernatant product was evaporated and washed with mixture of water and alcohol solution, then it was dried for 6 hours at 80˚C in oven to obtain the precursor.

Sample 6—with ZnO 175C code, 0.01 gr ZnO NPs powder modified with CNT was dissolved in mixture of LA2EO as emulsifier for water-oil emulsion, cyclohexane and vinyl acetate solvents. Then ZnO/PVP nanocomposites containing alcohol was added.

Sample 7—with ZnO 95 code, in this nano formulation, 0.02 gr ZnO NPs powder was added to 2 ml Triton-X-100 and 3 ml LA3-EO (lauryl alcohol 3-EO) in 2-butanol alcohol. Hydroxy propyl cellulose as stabilizer containing CaO NPs in liquid water were added and mixed completely.

Sample 8—with ZnO 175P code, this nanoproduct is similar to the sample of No.6, but we used a mixture of 2 ml Span 80 as emulsifier, solubilizer and stabilizer in 1 ml organosilane as dispersing agents in liquid water.

Sample 9—with ZnO 109 P3 code, it was fabricated from ZnO and $TiO_2$ NPs along with a small amount of sodium dodecyl sulfate (SDS) as anionic surfactant and PEG 4000 in alcohol solvent contains acidic pH value.

Sample 10—with ZnO 176D code, the mixture of zinc nitrate and zinc chloride were hydrolyzed with $H_2O_2$ solution included polyvinyl alcohol (PVA) stabilizer and LA3EO nonionic hydrophilic surfactant. The obtained black product was washed several times and annealed at 400˚C for 6 hr. 0.05 gr ZnO NPs product was dissolved in ethylbanzene and vinyl acetate solvents contained acidic pH value through liquid water along with 1 ml Span 80. This nanoformulation could mix strongly in order to obtain homogeneous ZnO water solution nanofluid.

### 2.3. Fungi Strains and Culture Media, and Typical Bacteriological Tests for Considering of Ten New Water-Based ZnO Nanofluids with Dermatophytes and *Candida* Spp.

Table 1 and Table 2 illustrate the significant biological reactions against several fungi

**Table 1.** Evaluation of ten ZnO nanofluids and nanoformulations as antifungal drugs against *Candida* species activity.

| Well method (mm) / MIC (µgr/ml) MFC (µgr/ml) Microbial strains | ZnO48 | ZnO51 | ZnO391 | ZnO45 | ZnO/PVP | ZnO175C | ZnO95 | ZnO175P | ZnO109P3 | ZnO176D | CL 300 µgr/ml |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *Candida albicans* PTCC5027 | 18 | 30 | 20 | 20 | 25 | - | - | 25 | - | 20 | 18 mm |
| | 50 | 6.25 | 25 | 25 | 12.5 | - | - | 12.5 | - | 25 | |
| | 100 | 25 | 100 | 100 | 50 | - | - | 50 | - | 100 | |
| *Candida tropicalis* PTCC5028 | - | 20 | 20 | 15 | 15 | 15 | 15 | 30 | 20 | 20 | 20 mm |
| | - | 25 | 25 | 50 | 50 | 50 | 50 | 6.25 | 25 | 25 | |
| | - | 100 | 100 | 100 | 100 | 100 | 100 | 25 | 100 | 100 | |

**Table 2.** Evaluation of ten ZnO nanofluids as antifungal drugs against typical dermatophytes species activity.

| Well method (mm) / MIC (µgr/ml) MFC (µgr/ml) Microbial strains | ZnO48 | ZnO51 | ZnO391 | ZnO45 | ZnO/PVP | ZnO175c | ZnO95 | ZnO175P | ZnO109P3 | ZnO176D | CL 300 µgr/ml |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *Microsporum canis* PTCC5069 | 25 | 25 | - | 35 | 30 | 30 | 20 | 20 | 25 | 30 | 25 mm |
| | 12.5 | 12.5 | - | 6.25 | 6.25 | 6.25 | 25 | 25 | 12.5 | 6.25 | |
| | 50 | 50 | - | 25 | 25 | 25 | 100 | 100 | 50 | 25 | |
| *Trichophyton mentagrophytes* PTCC5054 | 20 | 30 | 50 | 40 | 30 | 70 | 40 | 40 | 30 | 30 | 30 mm |
| | 25 | 6.25 | 1.56 | 3.12 | 6.25 | 0.75 | 3.12 | 3.12 | 6.25 | 6.25 | |
| | 100 | 25 | 6.25 | 12.5 | 25 | 3.12 | 12.5 | 12.5 | 25 | 25 | |
| *Microsporum gypseum* PTCC5070 | 35 | 20 | 20 | 28 | - | 35 | - | 40 | 15 | 10 | 25 mm |
| | 6.25 | 25 | 25 | 6.25 | - | 6.25 | - | 3.12 | 50 | 100 | |
| | 50 | 100 | 100 | 25 | - | 25 | - | 12.5 | 100 | 100 | |

related to Clotrimazole (300 µg/ml) as standard reference by well diffusion, MFC, and MIC methods. Therefore, for evaluation of antifungal activity, five strains obtained from the Persian Type Culture Collection (PTCC), Tehran, Iran, including Microsporum gypseum (PTCC 5070), Microsporum canis (PTCC 5069), *Trichophyton mentagrophytes* (PTCC 5054), *Candida albicans* (PTCC5027) and *Candida tropicalis* (PTCC5028) were provided. All culture were applied in this project such as: Sabouraud dextrose agar (SDA, Merck), Sabouraud dextrose broth (SDB, Merck), Dermatophyte selective agar (DTM agar, Merck).

### 2.3.1. Well Diffusion Method

All fungi were cultured in medium for fresh culture, after time growth, then starting to do antimicrobial methods. ZnO nanofluids were tested in vitro for their antifungal activities against strains by the agar diffusion technique [14]. The tested samples were dissolved in water to prepare chemicals of stock solutions. The suspension of pathogenic fungi were cultured on Sabouraud dextrose agar and DTM agar media (Merck),

respectively in Petri dishes with an inner diameter 9 cm to provide thin agar plates after solidification of thickness 3.4 - 3.5 mm. After solidification, hollows of 5 mm diameter wells were cut from the agar using a sterile cork-borer, and 0.1 ml of each of the tested solutions were poured into the wells. The Petri dishes were incubated at 5˚C - 8˚C for 2 - 3 h to permit good diffusion and then incubated for 24 - 48 h for yeast and 5 - 7 days for dermatophytes fungi at 28˚C. After incubation the diameter of inhibition zone (mm) was measured. Clotrimazole were purchased from Padtan Teb Company and used in a concentration of 300 µg/ml as standard antifungal references [15].

#### 2.3.2. Determination of Minimum Inhibitory Concentration Method (MIC)

The aim of broth methods is to determine the lowest concentration of the assayed antimicrobial agent (minimal inhibitory concentration, MIC) that, under defined test conditions, inhibits the visible growth of the fungi being investigated. MIC values are used to determine susceptibilities to drugs and also to evaluate the activity of new antimicrobial agents. For broth dilution, often determined in 13 tubes format, fungi are inoculated into a liquid growth medium in the presence of different concentrations of ZnO nanofluid. Growth is assessed after incubation for a defined period of time for *Candida* spp. (24 - 48 hrs.) and dermatophytes species (4 - 7 days) and then the last tube of non-growth are MIC value [16].

#### 2.3.3. Determination of Minimum Fungicidal Concentration Method (MFC)

Turbidity indicates growth of the microorganism and the MIC is the lowest concentration where no growth is visually observed. To determine the MFC, the dilution representing the MIC and at least two of the more concentrated test product dilutions are plated for 24 - 48 hrs for *Candida* species and 4 - 7 days for dermatophytes species, then enumerated to determine viable CFU/ml. The MFC is the lowest concentration that demonstrates a pre-determined reduction (such as 99.9%) in CFU/ml when compared to the MIC dilution [17]. **Table 1** show all of these very significant results for *Candida* spp. and **Table 2** indicate dermatophytes species activities.

#### 2.3.4. The Presentation of Two Tables Due to Determination of Ten New ZnO Nanofluids against Five Microbial Strain Fungal Pathogens

**Table 1** illustrates the significant biological reactions against several fungi related to Clotrimazole (300 µg/ml) as reference. For the evaluation of antifungal activity, the results showed that among of ten different nanoformulation of ZnO nanofluids, sample 2 (ZnO51), displays the best effect on *C. albicans* in well method with inhibition zone 30 mm, MIC 6.25 µgr/ml, and MFC 25 µgr/ml. So, the sample 8 (ZnO175P), illustrates the best effect on *C. trupicalis* in well method with inhibition zone 30 mm, MIC 6.25 µgr/ml, and MFC 25 µgr/ml in comparing with Clotimazole (18 and 20 mm as references ) respectively.

**Table 2** exhibits that among of various ten nanoformulation of ZnO nanofluids, sample 4 (ZnO45), demonstrates the best effect on *M. canis* in well method with inhibition zone 35 mm, MIC 6.25 µgr/ml, and MFC 25 µgr/ml. Sample 6 (ZnO175C), introduced the best effect on *T. mentagrophytes* in well method by inhibition zone 70 mm,

MIC 0.75 μgr/ml, and MFC 3.12 μgr/ml. The sample 8 (ZnO175P) registers the best effect on *T. mentagrophytes* and Microsporum gypseum in well method with inhibition zone 40 mm, MIC 3.12 μgr/ml, and MFC 12.5 μgr/ml in comparing with control (25, 30, and 25 respectively).

## 3. Results and Discussion

### 3.1. Characterization of ZnO Nanofluids

#### 3.1.1. SEM Pictures of Synthezied ZnO Nanoparticles (NPs) and Some Their Nanofluids

SEM images of ZnO/PVP NPs in Figure 1, and various stable nanofluids 4 and 6 sample solutions containing different morphologies were showed in Figure 2 and Figure 3.

ZnO NPs as interesting fine nanospherical were located among the green fatty acid layers indicated in Figure 4.

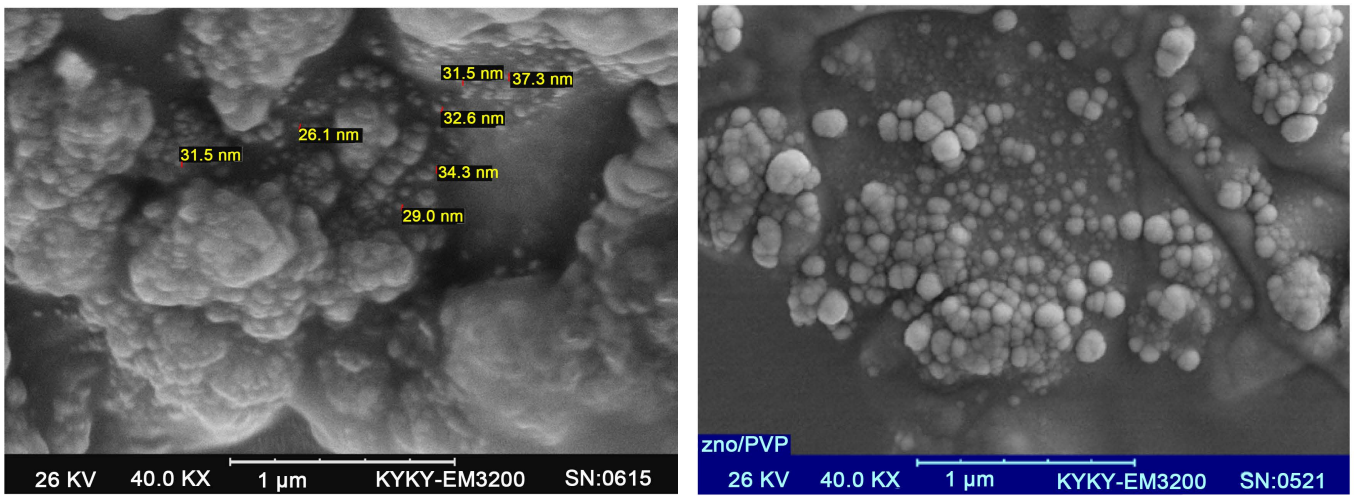



Figure 1. SEM images of ZnO/PVP nanocomposites as spherical nanoparticles.

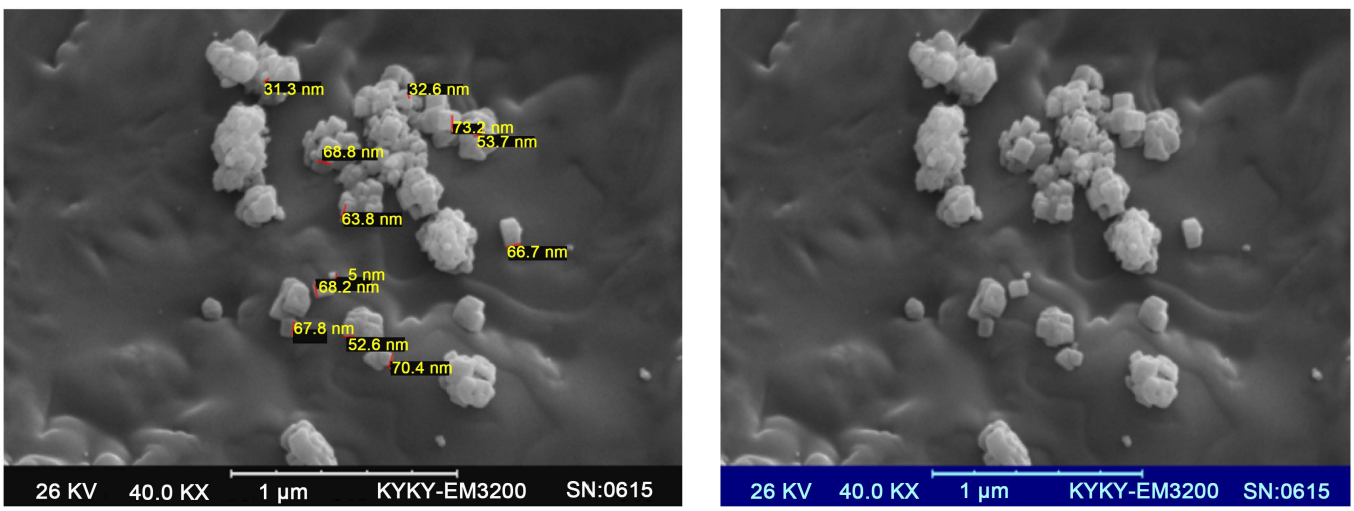



Figure 2. SEM images of sample 4 (ZnO 45) nanofluid, according to interesting nano cubic formation.

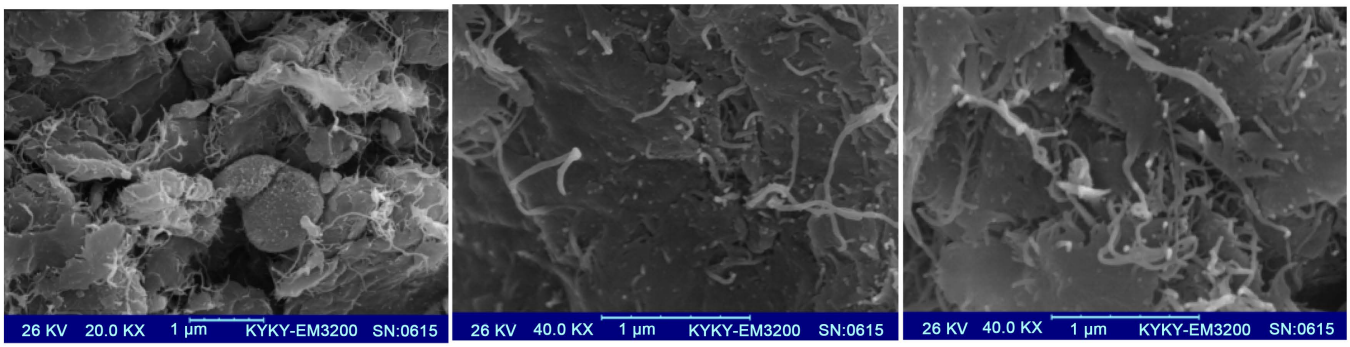



**Figure 3.** SEM images of sample 6 (ZnO175C) indicate homogeneous morphology for multiwall carbon nanotubes ((MWNTs)) which are coated on ZnO NPs (typical surface modification).

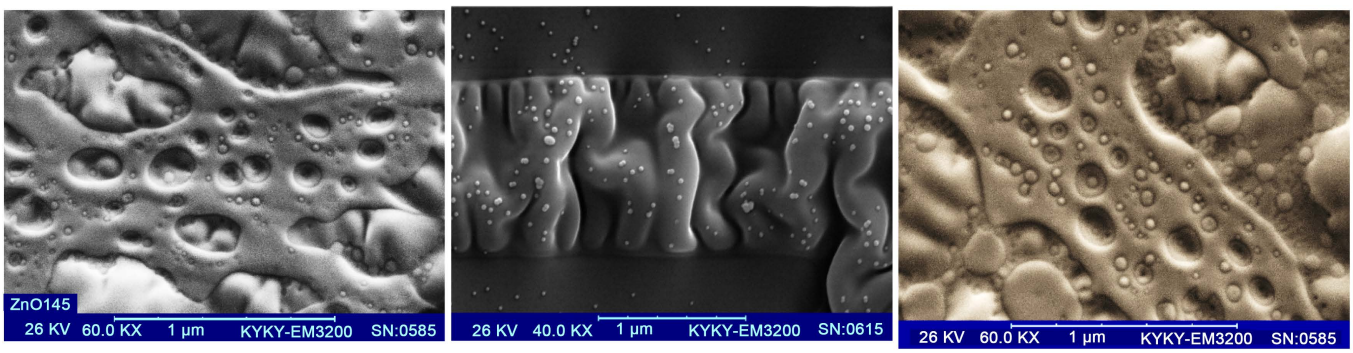



**Figure 4.** SEM images of ZnO NPs immersed into Soya fatty acid layers, and dispersed in sample 4 (ZnO45) nanofluid solution as fine spherical forms.

**Figure 5** reveals typical surface morphology and structure pictures for uniformly spherical ZnO NPs in Sample 1-with ZnO 48 code, and Sample 7-with ZnO 95 code nanofluids.

### 3.1.2. XRD Pattern of ZnO NPs and ZnO Nanofluids Solutions

**Figure 6** illustrates XRD pattern analyses of ZnO nanoparticles powder (a), and ZnO NPs dispersed in nanofluids (b, c), which the pattern of ZnO NPs is compatible with JCPDS card number 36 - 1451, recording the crystallographic structure of the nanoparticles as typical pure hexagonal phase with Wurtzite structure including space group p63 mc.

### 3.1.3. FTIR Spectrum of Hexagonal Structure of ZnO NPs and Surface Modified in Nanofluids

FTIR spectroscopy (Bruker Co. model Tensor 27) for ZnO NPs (a), ZnO NPs immersed in green fatty acids (b), and ZnO NPs dispersed in water-based fluid (c) were determined in **Figure 7**. A sharp peak at 493.50 $cm^{-1}$ can be assigned to the Zn-O stretching bond in ZnO NPs (a) and two low frequency at 619 - 857 $cm^{-1}$ can be attributed to hydride Zn-H bending modes. FTIR spectroscopy (b) shows a very sharp peak at 1357.12 $cm^{-1}$ for –COO groups and also water –OH stretching band (at 3303.31 $cm^{-1}$) of carboxylic acid group derivative of Soya fatty acid during surface modification process of

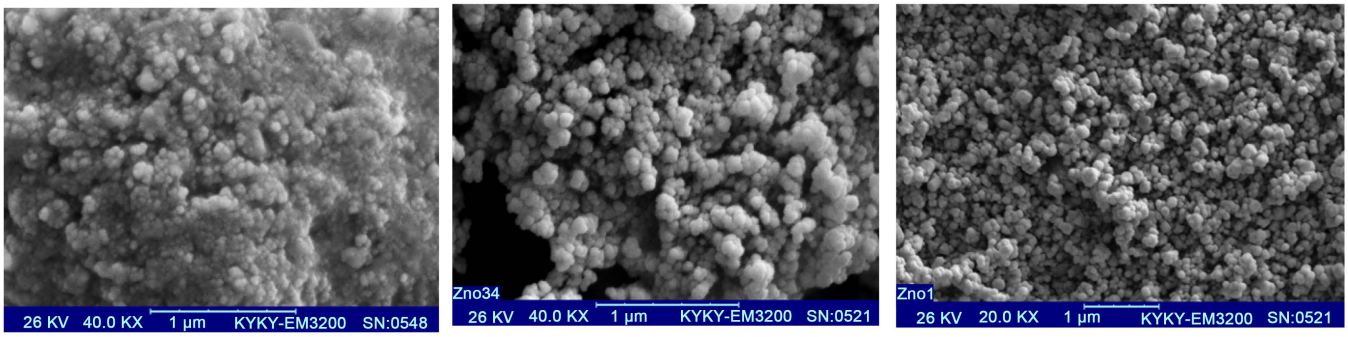



**Figure 5.** SEM images of typical ZnO NPs powder as homogeneous nanospherical.

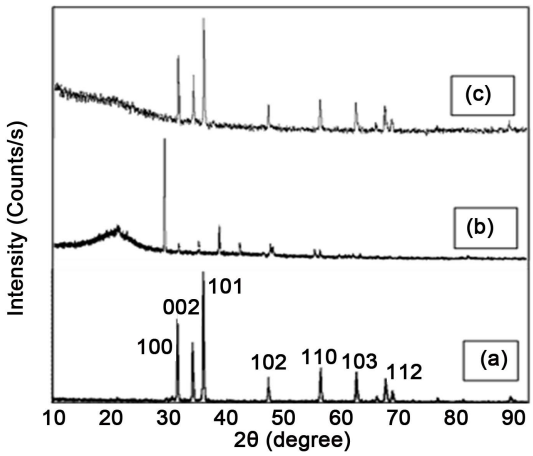



**Figure 6.** XRD curves of ZnO NPs powder as nanospherical form (a) without any surface modification, and (b) is belong to the ZnO NPs coated with green fatty acids, and (c) can point to fine ZnO NPs modified with multiwall carbon nanotubes.

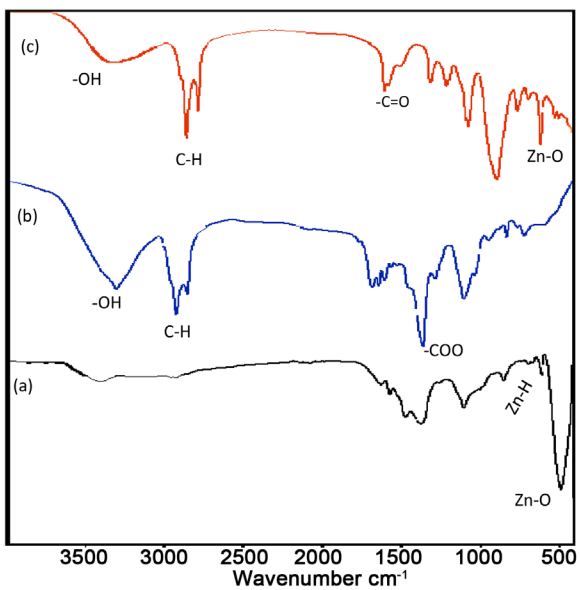



**Figure 7.** FTIR spectrum for (a) ZnO NPs;(b) ZnO NPs immersed in green fatty acids and (c) sample 6 (ZnO 175C), which ZnO NPs incorporated on multiwall CNT.

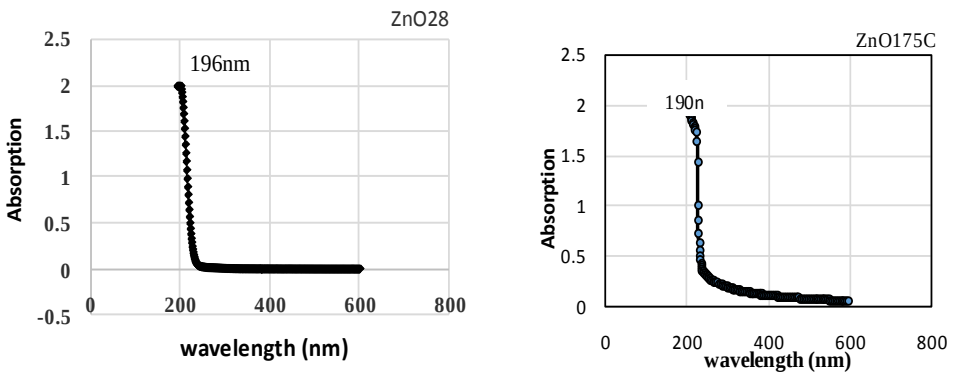
ZnO NPs [14]. In sample 6 (ZnO 175C) (c) it was found that the broad band at 3300.79 $cm^{-1}$ can be attributed to –OH stretching vibration modes and very low frequency at 720. 06 $cm^{-1}$ and the other at 1465. 25 $cm^{-1}$ is also for typical multiwalled carbon nanotubes (MWNTs). In addition, a group of peaks in the 1600 - 1741.24 $cm^{-1}$ range constitutes the G-band due to a symmetry carbon structure, and one peak around 1373.22 $cm^{-1}$, which called the D-band, is assigned to the presence of disorder in graphitic materials were seen in figure (c) spectroscopy. The C=O groups of pure PVP show a prominent peak at 1663.25 $cm^{-1}$ in FTIR spectrum which are able to have interaction between PVP and metal nanoparticles.

### 3.1.4. UV-Vis Spectrophotometer of ZnO NPs as Powder

The absorption spectrum of ZnO nanostructures as powder and nanofluids appeared around of 190 - 196 $cm^{-1}$ with max curves in the area of low wavelengths due to the many active sites of the surface defects, which are potential surface luminescence centres. UV-Vis emission peaks can show very fine particle sizes in nanofluids containing high band gaps and various nanostructural defects. **Figure 8** indicates different kinds of UV-Vis spectroscopies for two ZnO nanoproducts.

## 3.2. Result of Antifungal Activities Derivative of Table 1 and Table 2

According to the results of tests and **Table 1** and **Table 2**, on various fungi in well method showed that samples 1, 2, 4, 6, 5, 3, 8, 9, 10, and 7 had the greatest effect on the fungus *Trichophyton mentagrophytes* (Ringworm disease). The best and highest results on other fungi such as *Trichophyton mentagrophytes*, *M. gypseum*, *M. canis*, *C. albicans*, *Candida tropicalis* with inhibition control to clotrimazole 70, 40, 35, 30, 30 mm were measured (for diseases such as: alopecia, cutaneous and mucosal infections). In the MIC test results on fungi showed that nanofluids with codes 1, 2, 4, 6, 5, 3, 8, 9, and 10, the largest number of nanofluids zinc oxide on *Trichophyton mentagrophytes* indi-




(a) (b)

**Figure 8.** The UV-Vis (Perkin Elmer model lambda 35) absorption spectrum of ZnO nanoproducts are shown of two ZnO NPs (a), and sample 6 (ZnO 175C) nanofluid (b). It can be detected that the absorption edge appeared toward strong-sharp peaks at 196 and 190 nm in the ultraviolent region. The band gap energy will be actually more than the 3.37 eV in the bulk structure, and UV exciton emission were also shifted to blue shifts compared with ZnO bulk.

cated antimicrobial effects. The best and highest effects were on *T. mentagrophytes*, *M. gypseum*, *M. canis*, *C. albicans*, *C. trupicalis* rate respectively 0.75, 3.12, 6.25, 6.25, 6.25 μgr/ml. In the MFC test results on fungi showed that nanofluids 1, 2, 4, 6, 5, 3, 8, 9, 10, and 7 the largest number of nanofluids zinc oxide on *Trichophyton mentagrophytes* had presented antimicrobial effects. In this regard, we can compare obtained results with Emana El Disney and colleagues from Egypt in 2013, whom they published one paper about the antifungal activity of zinc oxide nanoparticles on dermatophytes spp and *Candida albicans* with concentration 5 - 40 mg/ml and in diameter 57 nm. Inhibition zones were measured in well method for *Trichophyton mentagrophytes*, Microsporum canis and *Candida albicans* in order 22, 23, 30 mm (but we could achieved to 70, 40 and 30 mm in this study). Given that the infection is shared between humans and animals are important economically and antibiotic resistance in animals can be seen, it can be concluded that zinc oxide nanoparticles with proper water-based formulation as anti-infective nanofluid agents can be used for dermal medical applications [18].

### 3.3. Statistical Analysis

The results of determining the antimicrobial effect of zinc oxide nanofluids on different fungi, all data related to the average obtained in each experiment were analysed. Statistical analysis using statistical parametric test ANOVA and Tukey & Duncan and 95% respectively and the $P < 0.05$ as a statistical index is intended. According to the standard deviation of the data antimicrobial according to the control significant differences exist and this shows that nanofluids had good antimicrobial effects on more fungi in this research. **Figure 9** indicates the statistical chart for various ZnO nanofluids comparison with control sample.

### 3.4. The Presentation of a Few Important Pictures from Antifungal Well Method Experimental

There are a few pictures based on laboratory data in **Figures 10-13** containing the re-

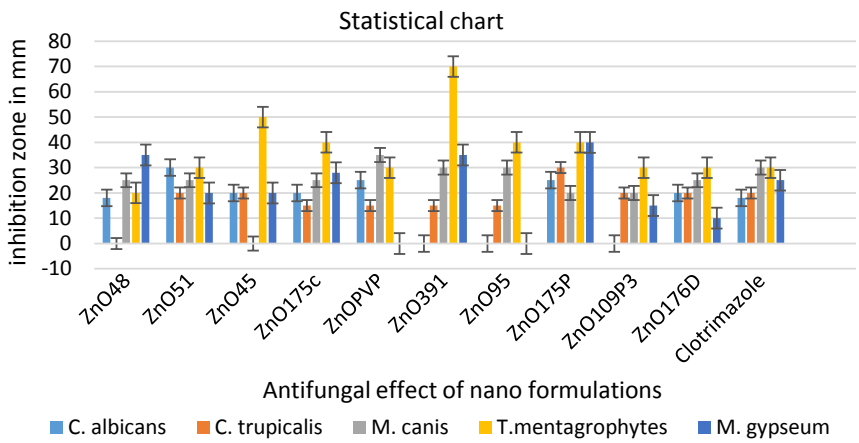
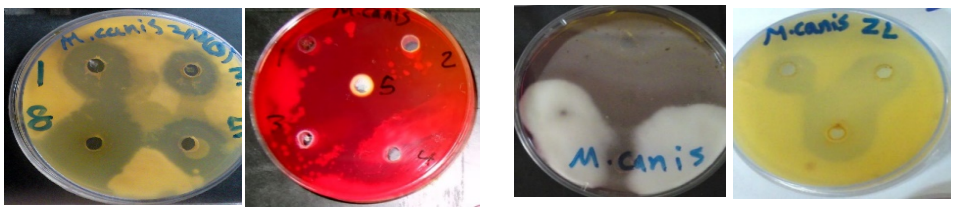



**Figure 9.** Overview of a chart with data which show antifungal effects of ten different formulation of ZnO nanofluids (samples 1, 2, 4, 6, 5, 3, 7, 8, 9, and 10) comparing with control.

(a) (b)

**Figure 10.** The best result of well method for (a) sample 3 (ZnO391) and (b) sample 4 (ZnO45) pictures showing inhibition zone on *C. trupicalis* (20, 15 mm) and *C. albicans* (20, 20 mm) in order with highest concentration 80 μgr/ml.

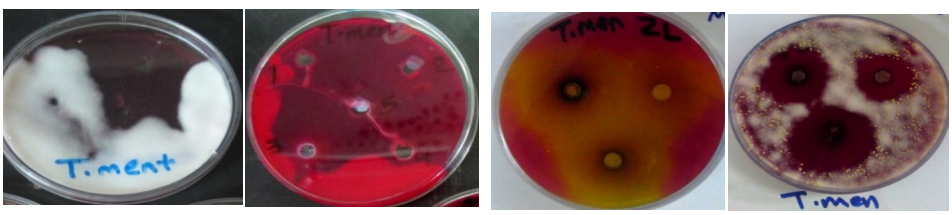


(a) (b) (c) (d)

**Figure 11.** The best result of well method for (a) sample 4 (ZnO45) and (b) samples 7 (ZnO 95), 8 (ZnO175P), 9 (ZnO109P3), and 10 (ZnO 176D); (c) sample 6 (ZnO175C) and (d) sample 2 (ZnO51), pictures showing inhibition zone on *M. canis* contain highest concentration 80 μgr/ml.

(a) (b) (c)

**Figure 12.** The best result of well method for (a) sample 8 (ZnO 175P); (b) sample 6 (ZnO175C) and (c) sample 2 (ZnO51) pictures showing inhibition zone (40, 35, 20 respectively) on *M. gypseum* contain highest concentration 80 μgr/ml.



(a) (b) (c) (d)

**Figure 13.** The best result of well method for (a) sample 6 (ZnO 175C); (b) sample 7 (ZnO 95), 8 (ZnO175P), 9 (ZnO109P3), 10 (ZnO176D), (c) sample 2 (ZnO51); and (d) sample 5 (ZnO/PVP) pictures showing inhibition zone (70, 40, 40, 30, 30, 30, 30 respectively) on particularly for *T. mentagrophytes* trichophytosis including highest concentration 80 μgr/ml.

sults of well method with different concentration of ZnO nanofluids (10, 30, 50, 80 μgr/ml). The pictures show desirable antifungal of ZnO samples with highest concentration (80 μgr/ml) on different fungi with Clotrimazole as control, Figure 10 determined antifungal effect of ZnO nanofluids on *C. trupicalis* and *C. albicans* with inhibition zone in order 20, 20 mm, Figure 11 examined antifungal effect of ZnO nanofluids with codes ZnO95, ZnO175P, ZnO109P3, ZnO176D, ZnO175C and ZnO51 on *M. canis* contain inhibition zone in order 20, 20, 25, 30, 30, 25 mm, Figure 3 represented antifungal effect of codes ZnO175P, ZnO175C, and ZnO51 with inhibition zone in order 40, 30, 20 mm on *M. gypseum*, Figure 12 showed antifungal efficacy of samples with codes ZnO175C, ZnO 95, ZnO175P, ZnO109P3, ZnO176D, ZnO51, and ZnO/PVP including inhibition zone in order 70, 40, 40, 30, 30, 30, 30 mm on *T. mentagrophytes*. Overall, between all ZnO nanoformulations containing sample 6 (ZnO175C), and finally sample 8 (ZnO175P) have displayed interesting and significant properties through the effective reactions on fungi, particularly on *T. mentagrophyte.*

According to the unique derived results of this study, we can give a few suggestions relating to the use of new ZnO nanoproducts as nanoformulations which have great novelty in nanomedicine.

a) It should suggest, ZnO nanofluid products can prepare for the prevention of genital infections and use as the typical of hygienic gels.

b) In order to prevent the spread of microorganisms in hospitals, they can be in the context of medical fabrics for all personnel recipients of these nanoformulations.

c) Regarding to the more studying on fungi wall, to synthesize new formulation of ZnO nanofluids could gain a greater effect on the resistant microorganisms.

d) Because proper response of ZnO nanofluids, they have been demonstrated for skin diseases in vitro, and can be also focused on other fungi pathogenic and special variations in clinical appearance research in the near future.

Based on ten different formulation of zinc oxide NPs and nanofluids on five common and problematic pathogenic fungal species. It was found that, the effect of nanofluids on dermal fungi including *Trichophyton mentagrophytes* were more efficient and higher than the others replied. *T. mentagrophytes* can cause a series of infections that affect the feet, face and body. There are, this fungal skin diseases in humans and livestock animals on the skin with hair or without hair, so for better results in the nanoformulation in vivo condition should work on a solution or ointment to prevent and treat skin lesions animal or human and application for human more sensitive to the subject. As a fungicide for washing the fungus lesions and crust and its treatment, or as a disinfectant in places like swimming pools and gyms because there is the possibility of outbreak ringworm infections in such public places.

## 4. Distribution of Fungal Species in the Environment

The extent of fungi in nature is very high.They are commensals that turn pathogenic or opportunistic after alteration of the host immune system. AIDS is one of the most important contributing factors for the increasing number of the patients with fungal in-

fections (nissapatorn 2003, singh 2003) [19] [20]. The expanding population of immune deficiency patients that use intravenous catheters and increasing use of broad spectrum antibiotics are factors that contribute to the increase of these infections (Ortega 2011) [21]. Nosocomial candidemia can be produced by *Candida albicans* and *Candida tropicalis*, because they are abundant in most of the hospital equipment and medical devices. Furthermore, removal of the infected device and their management are challenge now because the kinds of infection sources can be cause of death in patients. Candidiasis is usually a very localized infection of the skin or mucosal membranes, including the oral cavity (thrush), the pharynx or esophagus, the gastrointestinal tract, the rectum, anus, perianal/perirectal or anorectal area (in men as well as women), the perineum, the urinary bladder, the fingernails or toenails (onychomycosis), and the genitalia (vagina, penis, etc.).Certainly, medicines and health problems can cause more yeast to grow, particularly in warm, moist body areas. This can effect sometimes dangerous symptoms. Invasive candidiasis (IC) or candidemia is a leading cause of mycosis-associated mortality in the United States. More than 90% of invasive infections are caused by *Candida albicans* and *Candida tropicalis* (pfaller 2007) [22]. Vulvovaginal candidiasis (VVC) is an infection most commonly due to *Candida albicans* (Nviriesy, 2008) [23]. An estimated 70% to 75% of healthy adult women have at least one episode of VVC during their lifetimes (Sobel *et al.*, 1998) [24]. Prevalence rates are higher in women treated with broad spectrum antibiotics, pregnant women, diabetic women (De Leon *et al.*, 2002) [25], and women with HIV/AIDS (Duerr *et al.*, 2003) [26]. Currently an increase in the number of yeast that is resistant to the antifungal drugs is recognized worldwide. Dermatophytosis (tinea or ringworm) of the scalp, glabrous skin, and nails is caused by a closely related group of fungi known as dermatophytes which have the ability to utilize keratin as a nutrient source. Dermatophytes are, by far, the most prevalent of the 3 major classes of superficial infections. Less frequently, superficial skin infections are caused by nondermatophyte fungi and *Candida* species. *Trichophyton, Microsporum* and *Epidermophyton* are species of dermatophytes commonly invade human keratin. The infection may spread from person to person (anthropophilic), animal to person (zoophilic), or soil to person (geophilic). The tinea infections are prevalent globally but they are common in tropics and may reach epidemic portions in geographical areas with higher humidity, overpopulation and poor hygienic living conditions (Peerapur *et al.* 2004) [27]. The spreading , frequency and the causative agents involved vary from place to place depending upon the climatic, socioeconomic conditions and the population density (Das *et al.* 2009) [28]. The disease process in dermatophytosis is distinctive for two reasons: Firstly, no living tissue is invaded the keratinized stratum corneum is simply colonised. However, the presence of the fungus and its metabolic products usually arouse an allergic and inflammatory eczematous response in the host. The type and severity of the host response is often related to the species and strain of dermatophyte causing the infection. Secondly, the dermatophytes are the only fungi that have evolved a dependency on human or animal infection for the survival and dissemination of their species. The skin lesions and the clinical course

of the disease usually vary according to the host and pathogenesis of the causative dermatophyte. *T. mentagrophytes* a zoophilic isolate, is more often associated with inflammatory lesions of the scalp, the glabrous skin, the nails, and the beard region (Schieke, 2012) [29]. It is further classified as anthropophilic or zoophilic. The differential diagnosis of dermatophytoses consist of eczema, psoriasis, atopic dermatitis, contact dermatitis, seborrhoeic dermatitis, etc (Barry I, 2003) [30].

In immunocompromised patients, it is more difficult to diagnose dermatophytosis due to the atypical clinical presentation (Odom R.B, 1994) [31]. The treatment of dermatophytoses would be more effective when the selection of antifungal agent is based on the identity of the causative agent. Both topical and systemic therapies are commonly. The resistance to many of the traditional antifungal and antibacterial agents now in use has emerged. Hence, there is an inevitable and urgent medical need for antibiotics with novel antimicrobial mechanisms (Whitesides, G. M. 2003) [32]. The active oxygen generation, active nano-surface properties, oxygen species released on the nano-surface of ZnO NPs, the generation of $H_2O_2$ on these large surface area of ZnO NPs, presence of fewer $Zn^{+2}$ ions and hydrogen bonding with amino acids of protein and cell membranes in media solution, also strong diffusion of ZnO NPs, energetic surface by modifications, structural defects and oxygen vacancies containing wide band gaps due to many trapping states among the layers of transition states can be proposed as a new mechanism in this proof (Fakhroueian Z. 2013) [33]. It was found that, it can promote the biocidal properties and bactericidal efficacy of ZnO nanoparticles and nanofluids with decreasing particle size.

According to data in this research with good bacteriological study in comparing with control and the importance of fungal diseases in Iran. Between five strains were worked on them, candidiasis by *C. albicans* which cause important diseases, especially in women and children can be annoying. Thrush mouth baby, diaper rash and vaginitis have a high incidence of disease in such groups. Nano Zinc oxide is a safe material for treatment this fungi with dermal application as cream. On the other hand, ringworm is which caused by *T. mentagrophytes* and *M. canis* in domestic animals like cow, cat and dog ,specially *T. mentagrophytes* is also the most frequent cause of an acute inflammatory condition distinguished by pustule, blister and vesicle formation. In animals, *T. mentagrophytes* infection can appear as ringworm; an inflamed ring with flakey or crusting skin with or without hair loss. To wash the skin of animals with ZnO nanofluids can be useful to prevent getting this such diseases, because *T. mentagrophytes* is zoonotic, it can be spread from animals to humans through direct contact. It can cause a series of clinical infections in human that affect the feet, face and body. The most well-known dermatophytosis (tinea) infections such as tinea pedis (feet), tinea corporis (trunk and arms), tinea cruris (groin), tinea manum (hands), tinea capitis (scalp), tinea barbae (beard area), tinea unguim (or onychomycosis) (nail), (Weitzman. I. 1995) [34].

To suggest for treatment, ZnO nanofluids can add to formulation of Clotrimazole or other fungal drug and shampoo for healing better and faster.

## 5. Conclusion

We investigated about the removal of several usual fungal infections such as five comment dermatophytes and *Candida* with ten novel nanoformulation of ZnO nanoparticles which were fabricated by sol-gel, wet-chemical, hydrothermal, and formulated using ZnO nanoparticles powder in water-based solution methods. These results suggest that these nanoformulation of ZnO nanofluids could be applied as effective fungicide in treatment of disease and have great capacity to be added to the ointment or prepare lotions in order to reduce these fungal infection diseases, with access to new technology in fabrication of nanomedicine fields. Also, ZnO nanofluids that were synthezied and formulated as new fungicides agents in this study emerge for the first time and we succeed to provide a valuable package that prevents and eliminates the harmful fungal infections for future nanomedical industry.

## References

[1] Zaini, F., Mehbod, A.S.A. and Emami, M. (1999) Comprehensive Medical Mycology. 1st Edition, Tehran University Publication, 1-17 [Book in Persian].

[2] Ravishankar Rai, V. and Jamuna Bai, A. (2011) Nanoparticles and Their Potential Application as Antimicrobials, Science against Microbial Pathogens: Communicating Current Research and Technological Advances. In: Méndez-Vilas, A., Ed., *Formatex, Microbiology Series*, No. 3, Vol. 1, Spain, 197-209.

[3] Neuberger, T., Schopf, B., Hofann, H., Hofmann, M. and von Rechenberg, B. (2005) Super Paramagnetic Nanoparticles for Biomedical Applications: Possibilities and Limitations of a New Drug Delivery System. *Journal of Magnetism and Magnetic Materials*, **293**, 483-496. http://dx.doi.org/10.1016/j.jmmm.2005.01.064

[4] Buzea, C., Pacheco, I.I. and Robbie, K. (2007) Nanomaterials and Nanoparticles: Sources and Toxicity. *Biointerphases*, **2**, MR17-MR71. http://dx.doi.org/10.1116/1.2815690

[5] Patolsky, F., Zheng, G. and. Lieber, C.M. (2006) Nanowire Sensors for Medicine and the Life Sciences. *Nanomedicine* (Lond), **1**, 51-65. http://dx.doi.org/10.2217/17435889.1.1.51

[6] Wani, A.H. and Shah, M.A. (2012) A Unique and Profound Effect of MgO and ZnO Nanoparticles on Some Plant Pathogenic Fungi. *Journal of Applied Pharmaceutical Science*, **2**, 40-44.

[7] Neeran Obied, J. (2015) Antifungal Activity of Zinc Oxide Nanoparticles on *Aspergillus fumigatus* Fungus & *Candida albicans* Yeast. *Journal of Natural Sciences Research*, **5**, 23-27.

[8] Martínez-Flores, E., Negrete, J. and Torres Villasenor, G. (2003) Structure and Properties of Zn-Al-Cu alloy Reinforced with Alumina Particles. *Materials & Design*, **24**, 281-286. http://dx.doi.org/10.1016/S0261-3069(03)00028-1

[9] Pierard, G.E., Arrese J.E. and Pierard-Franchimont, C. (1996) Treatment and Prophylaxis of Tinea Infections. *Drugs*, **52**, 209-224. http://dx.doi.org/10.2165/00003495-199652020-00005

[10] Bhatia, V.K. and Sharma, P.C. (2014) Epidemiological Studies on Dermatophytosis in Human Patients in Himachal Pradesh, India. *Springerplus*, **3**, 134-140. http://dx.doi.org/10.1186/2193-1801-3-134

[11] Samia, A.C.S., Dayal, S. and Burda, C. (2006) Quantum Dot-Based Energy Transfer: Perspectives and Potential for Applications in Photodynamic Therapy. *Photochemistry and Photobiology*, **82**, 617-625. http://dx.doi.org/10.1562/2005-05-11-IR-525

[12] Pfaller, M.A. and Diekema, D.J. (2007) Epidemiology of Invasive Candidiasis: A Persistent public Health Problem. *Clinical Microbiology Reviews*, **20**, 133-163. http://dx.doi.org/10.1128/CMR.00029-06

[13] Pappas, P.G., Kauffman, C.A., Andes, D., Benjamin Jr., D.K., Calandra, T.F., Edwards Jr., J.E., Filler, S.G., Fisher, J.F, Kullberg, B.J, Ostrosky-Zeichner, L., Reboli, A.C., Rex, J.H., Walsh, T.J. and Sobel, J.D. (2009) Clinical Practice Guidelines for the Management of Candidiasis: 2009 Update by the Infectious Diseases Society of America. *Clinical Infectious Diseases*, **48**, 503-535. http://dx.doi.org/10.1086/596757

[14] Fakhroueian, Z., Dehshiri, A.M., Katouzian, F. and Esmaeilzadeh, P. (2014) *In Vitro* Cytotoxic Effects of Modified Zinc Oxide Quantum Dots on Breast Cancer Cell Lines (MCF7), Colon Cancer Cell Lines (HT29) and Various Fungi. *Journal of Nanoparticle Research*, **16**, 2483 (1-14).

[15] Magaldi, S, Mata-Essayag, S., Hartung de Capriles, C., Perez, C., Colella, M.T., Olaizola, C. and Ontiveros, Y. (2004) Well Diffusion for Antifungal Susceptibility Testing. *International Journal of Infectious Diseases*, **8**, 39-45. http://dx.doi.org/10.1016/j.ijid.2003.03.002

[16] Ashrafi, F. (2011) Practical Microbiology. 2nd Edition, Tehran Ahsan Publication, 190-199 [Book in Persian].

[17] Majoros, L., Kardos, G., Szabó, B. and Sipiczki, M. (2005) Caspofungin Susceptibility Testing of *Candida* inconspicua: Correlation of Different Methods with the Minimal Fungicidal Concentration. *Antimicrob Agents Chemother*, **49**, 3486-3489. http://dx.doi.org/10.1128/AAC.49.8.3486-3488.2005

[18] EL-Diasty, E.M., Ahmed, M.A, Okasha, N., F. Mansour, S., I.EL-Dek, S., Abed EL-Khalek, H.M. and Youssif, M.H. (2013) Antifungal Activity of Zinc Oxide Nanoparticles Against Dermatophytic Lesions of Cattle. *Romanian Journal Biophysics*, **23**, 191-202.

[19] Nissapatorn, V., Lee, C., Fatt, Q.K. and Abdullah, K.A. (2003) AIDS-Related Opportunistic Infections in Hospital Kuala Lumpur. *Japanese Journal of Infectious Diseases* (*JJID*), **56**, 187-192.

[20] Singh, A., Bairy, I. and Shivananda, P.G. (2003) Spectrum of Opportunistic Infections in AIDS Cases. *Indian Journal of Medical Science*, **57**, 16-21.

[21] Ortega, M., Marco, F., Soriano, A., Almela, M., Martinez, J.A., Lopez, J., Pitart, C. and Mensa, J. (2011) *Candida* Species Bloodstream Infection: Epidemiology and Outcome in a Single Institution from 1991 to 2008. *Journal of Hospital Infection*, **77**, 157-161. http://dx.doi.org/10.1016/j.jhin.2010.09.026

[22] Pfaller, M.A. and Diekema, D.J. (2007) Epidemiology of Invasive Candidiasis: A Persistent Public Health Problem. *Clinical Microbiology Reviews*, **20**, 133-163. http://dx.doi.org/10.1128/CMR.00029-06

[23] Nyiriesy, P. (2008) Vulvovaginal Candidiasis and Bacterial Vaginosis. *Infectious Disease Clinics of North America*, **22**, 637-652. http://dx.doi.org/10.1016/j.idc.2008.05.002

[24] Sobel, J.D., Faro, S., Force, R.W., Foxman, B., Ledger, W.J., Nyirjesy, P.R., Reed, B.D. and Summers, P.R. (1998) Vulvovaginal Candidiasis: Epidemiologic, Diagnostic, and Therapeutic Considerations. *American Journal of Obstetrics & Gynecology*, **178**, 203-211. http://dx.doi.org/10.1016/S0002-9378(98)80001-X

[25] De Leon, E.M., Jacober, S.J., Sobel, J.D. and Foxman, B. (2002) Prevalence and Risk Factors for Vaginal *Candida* Colonization in Women with Type 1 and Type 2 Diabetes. *BMC Infectious Diseases*, **2**, 1. http://dx.doi.org/10.1186/1471-2334-2-1

[26] Duerr, A., Heilig, C.M., Meikle, S.F., Cu-Uvin, S., Klein, R.S., Rompalo, A. and Sobel, J.D. (2003) Incident and Persistent Vulvovaginal Candidiasis among Human Immunodeficiency

Virus-Infected Women: Risk Factors and Severity. *Obstetrics & Gynecology*, **101**, 548-556. http://dx.doi.org/10.1097/00006250-200303000-00022

[27] Peerapur, B.V., Inamdar, A.C., Pushpa, P.V. and Srikant, B. (2004) Clinicomycological Study of Dermatophytosis in Bijapur. *Indian Journal of Medical Microbiology*, **22**, 273-274. http://www.ijmm.org/text.asp?2004/22/4/273/12826

[28] Das, K., Basak, S. and Ray, S. (2009) A Study on Superficial Fungal Infection from West Bengal: A Brief Report. *Journal of Life Sciences*, **1**, 51-55. http://www.bhj.org/journal/20044602april/index.htm

[29] Schieke, S.M. and Garg, A. (2012) Chapter 188: Superficial Fungal Infection. In: Wolff, K., Goldsmith, L.A., Katz, S.I., Gilchrest, B., Paller, A.S. and Leffell, D.J., Eds., *Fitzpatrick's Dermatology in General Medicine*, 8th Edition, McGraw-Hill, New York, 2277-2297.

[30] Hainer, B.L. (2003) Dermatophyte Infections. *American Family Physician*, **67**, 101-108. www.aafp.org/afp

[31] Odom, R.B. (1994) Common Superficial Fungal Infections in Immunosuppressed Patients. *Journal of the American Academy of Dermatology*, **31**, 56-59. http://dx.doi.org/10.1016/S0190-9622(08)81269-8

[32] Whitesides, G.M. (2003) The "Right" Size in Nanobiotechnology. *Nature Biotechnology*, **21**, 1161-1165. http://dx.doi.org/10.1038/nbt872

[33] Fakhroueian, Z., Harsini, F.M., Chalabian, F., Katouzian, F., Shafiekhani, A. and Esmaeilzadeh, P. (2013) Influence of Modified ZnO Quantum Dots and Nanostructures as New Antibacterial. *Advances in Nanoparticles*, **2**, 247-258. http://dx.doi.org/10.4236/anp.2013.23035

[34] Weitzman, I. and Summerbell, R.C. (1995) The Dermatophytes. *Clinical Microbiology Reviews*, **8**, 240-259.