The Nucleus (2024) 67:291–309
https://doi.org/10.1007/s13237-023-00439-1

REVIEW ARTICLE

The fungal infections and their inhibition by Zinc oxide nanoparticles: an alternative approach to encounter drug resistance

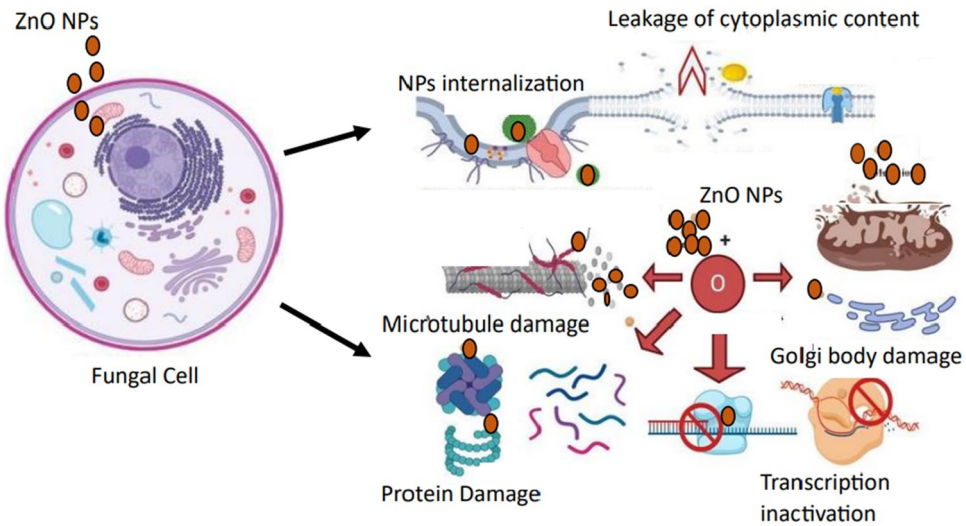
Pranjali Mahamuni-Badiger[1] · Vishrut Ghare[2] · Charushila Nikam[1] · Nilam Patil[3]

Received: 18 February 2023 / Accepted: 25 September 2023 / Published online: 17 October 2023

Abstract

Over the last few years, varieties of fungal infections have created a serious threat to human populations worldwide. Fungi are prone to develop infections mainly in immune-compromised individuals, like patients suffering from HIV/AIDS, diabetes, and hematological disorders. Alarmingly, this threat is continuously increasing with the use of the long-term therapeutic and prophylactic use of antifungal agents that have promoted the emergence of multi-drug resistant fungi. Therefore, there is a need to use alternative antifungal agents that will act even against multi-drug-resistant fungi. Among all nanoparticles zinc oxide nanoparticles (ZnO NPs) received much more attention worldwide due to their unique properties like diverse morphology, large surface area to volume ratio, biocompatibility, broad-spectrum antibacterial, antifungal activities, less sensitivity for the development of resistance towards micro-organisms and stay non-hazardous in the environment. This review aims to discuss the multiple drug resistance in fungi, the general properties of ZnO, their synthesis methods, their antifungal applications, their antifungal mechanism, and the nanotoxicity of ZnO. This review will offer a door for the use of ZnO NP-based materials as an alternative approach for treating multi-drug resistant fungal infections.

Graphical abstract



Keywords ZnO NPs · Antifungal activity · Biocompatibility · Antifungal agent · Multi-drug resistant

Corresponding Editor: Somnath Paul; Reviewer: Ishita Rehman.

Extended author information available on the last page of the article

Introduction

The emergence and spread of fungal infections posed a great threat worldwide to immune-suppressed individuals suffering from HIV, diabetes, COVID, diseases related to hematological disorders, transplantation, and chemotherapy [62]. Recently fungal infections, like mucormycotic, aspergillosis, and candidiasis have created severe threats that occurred in patients affected by COVID-19 or those getting recovered from the disease. The incidence rate of mucormycotic is in the range of 0.005–1.7 per million populations globally. According to some reports, the estimated prevalence rate in India is 140 per million population [46].

Other human pathogenic fungi can develop different skin, mucosal, and invasive infections. They can affect human beings effectively due to their unique growth parameters like tolerance to high temperature (37 °C), speedy growth, invasion capacity, and escaping ability from the host immune system [103]. Most of the common genera responsible for the development of lethal infections are *Mucor, Candida, Cryptococcus, Aspergillus, Coccidioides,* and *Rhizopus*. Also, fungal infections are very critical to handle as the interaction of fungal toxins creates severe situations [6, 46]. The ample use of antibiotics to treat fungal infections is resulting in the development of drug-resistant fungi. Also, lack of efficiency, selectivity, efficacy, toxicity, and resistance of antifungal agents causes failure of antifungal treatment. So there is a need for the use of alternative agents to treat fungal infections [20].

In this regard, nanoparticles play an essential role in the research areas of modern technologies around the world [64, 84]. Due to their unique properties, such as high surface area-to-volume ratio, which modify their chemical and physical properties, magnetic properties, electrical properties, optical properties, and mechanical properties, nanoparticles perform specific and specific functions [77]. Among all nanoparticles ZnO nanoparticles (ZnO NPs) are majorly reported for their antibacterial, antifungal, and antibiofilm activities with less toxicity to human cells [55]. ZnO NPs possess better biocompatibility as compared with other nanoparticles. Also, Zinc oxide NPs have been listed as "Generally Recognized as Safe" by the FDA (Food and Drug Administration) of the United States [23, 50, 83]. ZnO NPs are grouped as II-VI semiconductors with wide band gap energy of 3.3 eV and high excitation energy of 60 eV. ZnO NPs found in wurtzite, zinc blend, and rock-salt structures. ZnO NPs are reported for their intense anti-bacterial, anti-diabetic, anti-fungal, anti-cancerous, and other diverse biomedical applications [11, 73]. Out of these phases wurtzite phase is comparatively more stable thermodynamically at ambient conditions. According to some reports, the total worldwide production of ZnO NPs is at third rank after silica and titanium dioxides [64, 65]. Versatile applications of ZnO NPs are shown in Fig. 1 [66].

Despite the widespread use of ZnO NPs, the development of antifungal nanomaterials for the treatment of fungal infections has been limited. This review aims to highlight the

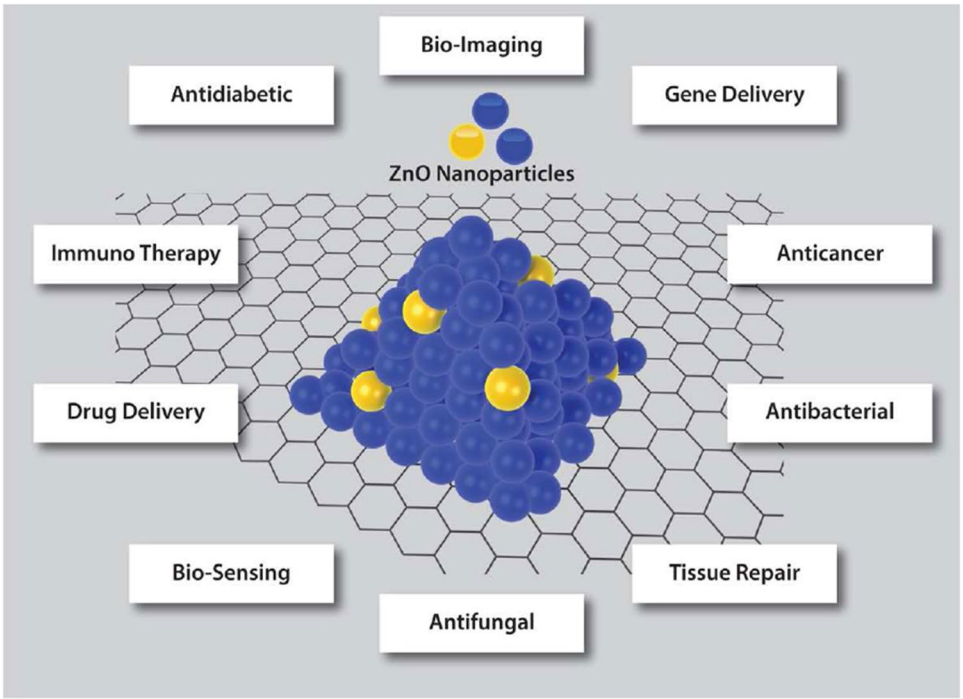


Fig. 1 Different applications of ZnO NPs in biomedical field [66]

various characteristics of ZnO NPs that play an important in the inhibition of fungi and their critical role in the antifungal action [50]. It is hoped that this review will serve as a resource for the researchers who are actively working on the development of ZnO-based antifungals.

Multiple drug resistance in fungi

Fungi are eukaryotic ubiquitous microorganisms living in various types of habitats. Some fungi are considered normal flora in different parts of the body including skin, genitourinary tract, mouth, and gastrointestinal tract, and play significant roles in human health [44]. These normal floras play an important role in human health, but when the immune system is weakened, they can act as a pathogen that can cause invasive fungal infection (IFI) that can affect multiple organs. IFI is a type of fungal infection that is responsible for an estimated 1.5–1.7 billion deaths each year, with superficial infections being the most costly [14, 17].

Multidrug resistance in pathogens increases the risk of mortality and mortality-related morbidity which are often termed superbugs. Antifungal-resistant organisms evolve due to stable genetic alterations in the fungal species in response to specific anti-fungal antibiotics that elevate the therapeutic failure [29]. Standardized in vitro antifungal susceptibility testing (AFST) assays provide the specification of drugs and microorganism, pharmacodynamics response in animals, and clinical study. MDR can be divided into primary and secondary resistance [69].

Primary resistance is when organisms have never been exposed to the drug in a specific host. Secondary host acquired resistance is resistance that develops after exposure to the drug, which is further classified as intrinsic and extensive resistance. Intrinsic resistance is the inability of microorganisms to be sensitized to certain first line drugs used to treat infections based on clinical evidences of patients [79]. For example, *Candida* spp. to fluconazole. On the other hand, extrinsic resistance refers to the ability of XDRs to sustain at least ½ inhibitory effects on antimicrobial drugs. This occurs when the patient is treated with first line drugs. For example, XDR-TB resistance against fluroquinolone [9]. Clinical resistance is the situation where the pathogen is only inhibited by antimicrobial concentration that is greater than the safety dosages [117].

Fungi from *Candida, Aspergillus, Cryptococcus*, and P*neumocystis* are the most common pathogens causing infections. Antibiotics used to treat such infections are azoles (fluconazole, itraconazole, voriconazole, posaconazole etc.), echinocandis (caspofungin, micafungin, and anidulafungin) and, polymers like amphotericin B (AMB) [14]. Failure of azole therapy has been found in patients suffering from cryptococcal meningitis during the time of azole therapy. This failure can be due to host, drug, and fungal aspects. Some hosts suffering from abdominal and liver abscesses can resist absorption and distribution of antifungal agents at the site of infection and exposure to antifungal agents develops survival even in the presence of antifungal drugs that promotes resistance. Resistance to antifungal agents can be acquired in which fungi show more resistance during antifungal therapy or can be intrinsic like *candida krusei* which exhibits resistance to azole. For example, Fluconazole [39]. Recently identified causative agent of candidiasis is multi-drug resistant *C. auris* has been developed significant increase in different countries like India and South Africa. High rates of fluconazole resistance in *C. globrata* (2.8–6.8%) *C. parapsilosis* (0.6–4.6%) and *C. tropicalis* (1.1–9.2%) were reported along with *C. krusei* and *C. auris* [9].

Antifungal resistance to triazole has been observed over the past 20 years in clinical isolates and environmental isolates around the world. Antifungal resistance has been observed in A. spergillus, the causal agent of Aspergillosis, with notable differences in resistance between geographic regions and patient cohorts, with more frequent resistance observed in high-risk patients [58]. Some of fungi and drugs to which they are resistant are given in Table 1.

The identification of resistance depends upon susceptibility testing of microorganisms, detecting MICs against particular antimicrobials that when they are compared with clinical reports give susceptibility or resistance. There are various methods used for antifungal susceptibility testing broth microdilution, disc diffusion, azole agar screening, gradient diffusion, and employment of automated machines [80].

Table 1 Common drug resistant fungi and drugs to which they are resistant [19]

Sr. no.	Name of fungi	Resistant drugs	Disease
1	*Candida* spp.	Fluconazole and echinocandins	Candidiasis
2	*Cryptococcus neoformans*	Fluconazole	Cryptococcosis
3	*Aspergillus* spp.	Azole	Aspergillosis
4	*Scopulariopsis*	Amphotericin B, flucytosine and azole	Onychomycosis

Ideal Properties of ZnO NPs suitable for treating fungal infections

Nano-based products are increasingly being used in various fields that offer solutions such as fungal infections, and treatments with the potential to cure diseases and improve patient's quality of life. NP-based products significantly overcome the major drawbacks associated with conventional therapies. Of all nanomaterials, ZnO nanomaterials are at the forefront that is the most suitable nano therapeutic agent to control many fungal diseases. In order to be used in the treatment of diseases, nanoparticles must possess certain ideal properties that make their use convenient. The following features make ZnO nano-materials more preferred [51, 102]. These features can be, (i) ZnO NPs can be gained with desired morphology easily by changing synthesis parameters in such a way that they can get easily penetrated through multiple layers of skin to treat deep tissue fungal infections [33]. The size and shape of ZnO NPs are important factors in determining degree of antifungal activities. The smaller the ZnO NPs, the easier it is to penetrate the cell or the biofilm matrix of the fungal cells. The shape of ZnO NPs also influences antifungal activities during morphological interaction. ZnO NPs with sharp edges, sharp spikes, sharp pillars or sharp projections on its surface can rapidly puncture and break fungal cells due to high local pressure [42], (ii) Functionalization of ZnO NPs with wide range of materials is possible that can improve or control the action of ZnO NPs. Functionalization can add quality properties like non-toxicity, biocompatibility, and increased intensity of antifungal infections [93] and, (iii) ZnO NPs are biocompatible and minimizes the chances of immunological responses [46].

Various aspects of synthesis methods for ZnO NPs synthesis and different morphologies

Zinc oxide can be obtained in a wide range of structures that are dependent on the synthesis method used. Various synthesis methods for the production of ZnO NPs are available including: vapour deposition, precipitation, hydrothermal synthesis, the sol–gel process, precipitation from microemulsions and mechano-chemical processes, that produce final product with particles in different sizes, shapes and spatial structure [57]. The synthesis methods can be divided as shown in Fig. 3 [113]. The selection of synthesis method mainly depends, as synthesis approach determines the morphologies and sizes of nanoparticles. ZnO NPs show highest range of properties and structures

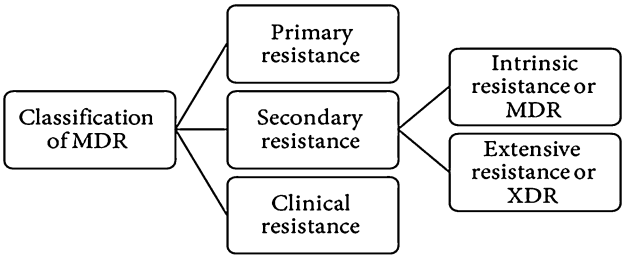


Fig. 2 Different types of multidrug resistance in fungi [104]

among other nano-metal oxides (Fig. 2). Examples of ZnO NPs morphology are given in Fig. 4.

It occurs in a dimensional (1D) structure that includes nanorods, helixes, ribbons [30], tubes [17], belts [40], wires, springs [105] and needles [110]. Two dimensional structures include nanoplates and nano pellets [44], while three-dimensional structure consists of flower, snowflakes, dandelion, coniferous urchin etc. [87]. Nanostructures have been synthesized by using physical, chemical, and biological methods; however, chemical method provides better control of morphologies and size [140]. Co-precipitation method is widely used method to produce ZnO NPs, in which reduction of zinc salt solution with the help of a reducing agent occurs that limits growth of particles to a specific dimension. The process of co-precipitation can be controlled by adjusting parameters like pH, temperature and reaction time [54]. Purwaninsih et al., prepared ZnO by co-precipitation method, using zinc acetate dihydrate, ammonia, and hydrochloric acid and observed the effect of heating time on size and morphology of particles, which concluded that size of particles decreases with increase in heating time with pseudo-spherical shape [90]. Katiyar et al., reported synthesis of cauliflower like ZnO by co-precipitation method by using LiOH [49]. Another simple synthesis method is the sol–gel method. Hasnidawani et al., studied characteristics of ZnO nanoparticles prepared by sol–gel method in which they used zinc acetate dihydrate, ethanol, NaOH and distilled water as a reagent and obtained rod shaped nano ZnO NPS within range of 81.28–84.98 nm [35].

Arrays of ZnO nanorods in another investigation were synthesized on ZnO seed layer by using hydrothermal method, which exhibited the best UV induced photocatalytic degradation of *Escherichia coli* bacteria. Furthermore, they also proved as strong photon-induced antibacterial material [71]. Raghupathi et al., synthesized ZnO NPs by solvothermal method and studied size-dependent bacteriostatic bacterial growth inhibition and the mechanism of antibacterial activity of zinc oxide nanoparticle against drug sensitive and resistant *Staphylococcus aureus*. Use of organic solvents or additional processing like grinding or calcinations are not

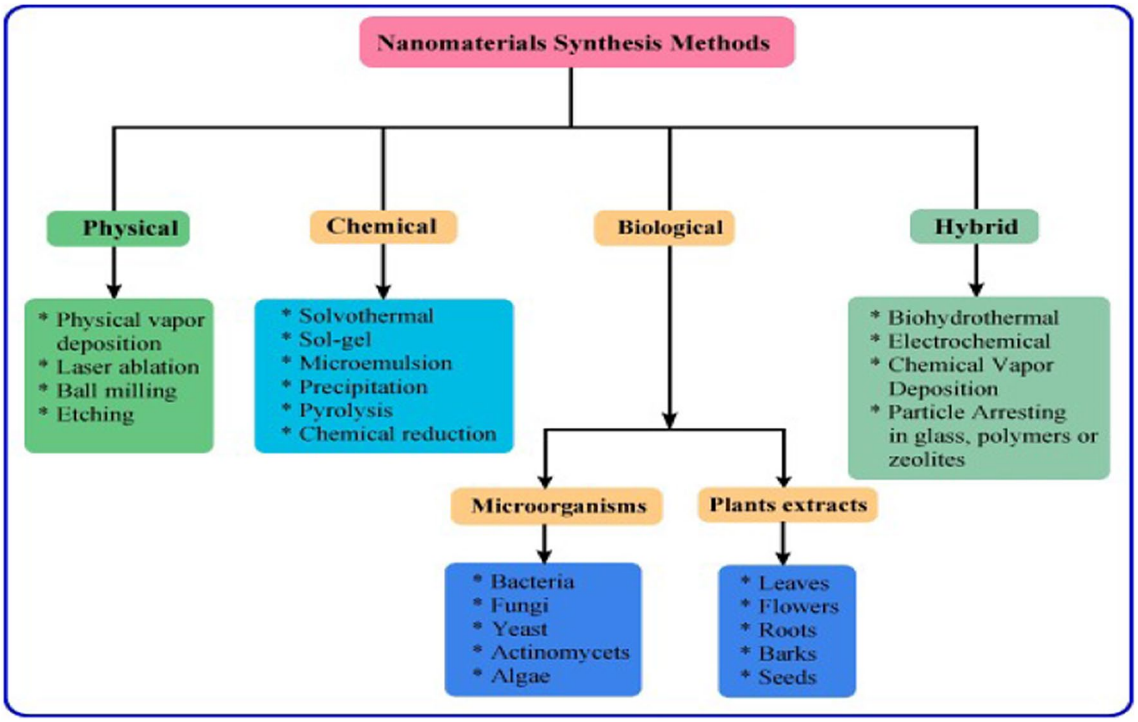


Fig. 3 Various synthesis methods used for ZnO NPs synthesis [113]

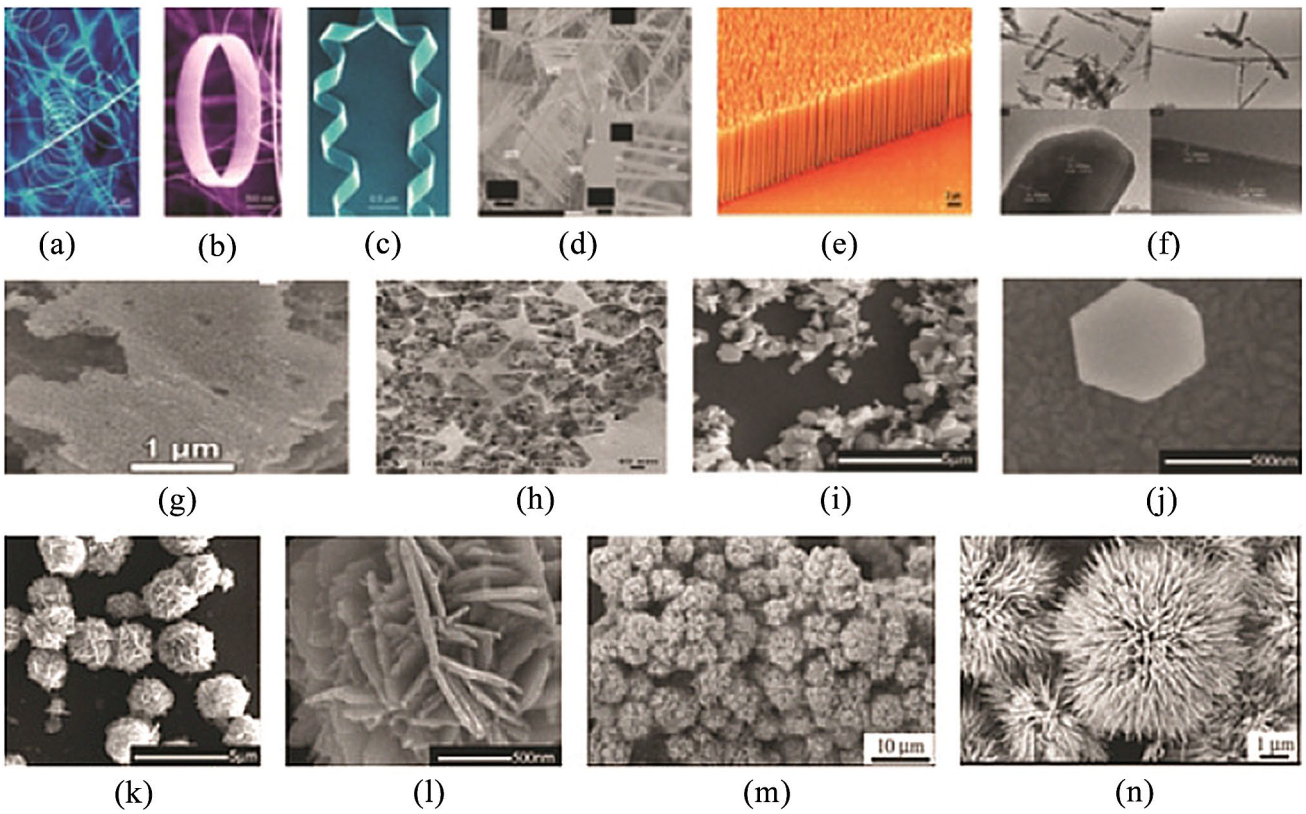


Fig. 4 Different structures of ZnO; (**a**) nanosprings, (**b**) nanorings, (**c**) nanohelix, (**d**) nanocombs, (**e**) nanowires, and (**f**) nanorods, (**g**) nanosheets, (**h**) nanopellets, and (**i**) nanoplates, (**k**) & (**l**) nanoflowers. (**m**) Nanomesosphere. (**n**) Nanourchins [78]

restricted in these synthesis methods which make them easy and convenient methods of synthesis [91].

Stankovic et al. reported the synthesis of ZnO NPs hydrothermally by additions of different stabilizing agents that led to different nanostructures. Obtained nanoparticles exhibit rod shaped morphology with hexagonal prismatic and pyramid like structure, along with spherical and ellipsoid morphology. These different particles showed different degree of antibacterial activity against targeted bacteria [107].

Elkady et al., proposed synthesis of rod and tube shaped ZnO nanoparticles via sol–gel and hydrothermal route of synthesis. Morphology was controlled by optimizing parameters like reaction temperature, time, and use of stabilizing agents. The obtained ZnO nanotubes displayed strong antimicrobial activity against Gram negative bacteria as compared with Gram positive bacteria [25] (Table 2).

The use of polyols in the synthesis process allows nanoparticles with high crystallinity by avoiding agglomeration and controlled morphology as they possess unique properties like high boiling point, high dielectric constant, and solubility of metal salt precursors. Pranjali et al. synthesized ZnO NPs by using different approaches like regular synthesis in polyols in diethylene glycol and tri-ethylene glycol, in the presence of sodium acetate, and by increasing reaction time. They observed size-dependent antibacterial and antibiofilm activity against *Staphylococcus aureus* and *E. coli*, in which they concluded that as particle size decreases their antimicrobial activity increases [63]. The mechano-chemical synthesis method uses zinc salts such as $ZnCl_2$, Zn $(NO_3)_2$, $ZnSO_4$ and carbonate salts such as Na_2CO_3, $(NH_4)_2CO_3$ to form zinc carbonate ($ZnCO_3$) by exchange reaction [8, 114].

In the vacuum phase method, the metal zinc is processed under high vacuum pressure and temperature in a vacuum chamber. The vaporized form of zinc is converted into particles form when it is mixed with cold gas. The precursor is evaporated, followed by sputtering and laser process [27]. Ali et al. described a continuous process for the preparation of functionalized ZnO NPs using vapor phase method, whereas the gas phase method of synthesis typically uses the spray pyrolytic method. Inert gas condensation method is further subdivided into physical vapor deposition and chemical vapor deposition [4].

Factors influencing antifungal action of ZnO NPs

Different factors affect the intensity of antifungal activity of ZnO NPs which can be elaborated as follows.

Morphology

The size and shape of antifungal agent play a significant role in the determination of antifungal intensity. The morphology of ZnO NPs can be controlled by selecting appropriate synthesis methods. Padmavathy et al. proposed that the antifungal efficacy of ZnO NPs get increases with decreasing particle size and concentration. This is due to the small size of the particles making it easier for them to penetrate into the cell [82]. The shape of the ZnO NPs also plays a important role in the intensity of antifungal activity during interactions. Antifungal agent surfaces with sharp edges or spikes, pillars, protrusions, etc. can easily penetrate and destroy fungal cells due to local stress [111]. Hui et al. synthesized cerium –doped ZnO NPs with flower shaped morphology through microwave-mediated hydrothermal route. Because of the flower-like morphology, ZnO nanoforms showed more potent anti-foaming activity compared to pure znO crystals, against pathogenic fungal species such as *Candida alkalensis* and *A. flavus*. It has been also suggested that the antifungal activity of ZnO NPs is due to surface defects and orientations [41]. Pariona et al. studied in-vitro antifungal activity

Table 2 Advantage and disadvantages of different synthesis procedures

Sr. no.	Synthesis method	Advantage	Disadvantage	References
1	Mechano-chemical process	Simple, can be used on large scale, low production cost, small particle size, high crystallinity	Large number of impurities	[8]
2	Co-precipitation method	Simple, cheap, reaction can be controlled by controlling reaction parameters	Agglomerated particles	[53]
3	Sol–gel method	Simple, cheap, reliable, repeatable	Agglomerated particles, large scale production is difficult	[53]
4	Solvothermal and hydrothermal method	High degree of crystallinity, high purity	High pressure and high temperature is required	[75]
5	Microemulsion method	Easy, reversible, thermodynamically stable	Require surfactants for stability, limited solubilizing capacity for substance having high melting point	[98]
6	Biological method	Cost effective, Ecofriendly	Lack of uniform size distribution	[1]

of ZnO NPs with three different shapes as nanoparticles, lamellar platelets (pls) and hexagonal rods (Rds), against three different fungal species namely *C. gloesporioides*, *F. solani*, and *F. oxysporum*. Antifungal activity was in order of ZnO pls > ZnO NPs > ZnO Rds [83].

High surface area to volume ratio

One of the most significant determinants of antimicrobial activity is the surface area-to-volume ratio. The surface area is the area of a particle's surface per unit volume. This ratio is expressed as the number of times the particle's surface area is divided by the number of times its volume. As previously discussed, nanoparticles have a smaller size and consequently a higher surface area–volume ratio when compared to their bulk counterparts [55]. Smaller zinc oxide nanoparticles (ZnO NPs) have higher mobility and significantly higher uptake across the bio-molecular interface. Therefore, the ratio is largely determined by the particle size and shape. For instance, spherical ZnO NPs have a minimum surface area–to-volume ratio (SAR). Antimicrobial activity is largely based on the physical–chemical properties of nanoparticles (NPs) [52]. NPs have a dimension < 100 nm, which is much less than that of conventional antibiotics. As previously mentioned, alterations to fungal cell membranes are complicated by genetic mutation, as the fungal cells are highly conserved due to their highly conservational nature. Consequently, greater inhibition is achieved with smaller particle sizes and higher SAR [12, 56].

Pranjali et al. synthesized ZnO NPs by using various aspects like by using two solvents like diethylene glycol (DEG) and triethylene glycol (TEG) and in presence and in absence of sodium acetate refluxed at 2 h and 3 h. In this work, they observed that use of these different parameters altered morphology of NPs from oval shape to rod shape. The rod-shaped NPs demonstrated better antimicrobial activity than the spherical ones, which was attributed to their small size and high surface area-to-volume ratio, which enabled them to interact with bacterial cells more effectively than the spherical NPs [64].

Kalika et al. reported green synthesis of three different metal and metal oxide NPs like silver, ZnO and FeO with the use of *trichoderma harzianum* hyphal or mycelia extract. Synthesized NPs possessing different sizes (Ag NPs with 5–50 nm), (ZnO NPs with 20–60 nm), and (FeO NPs with 10–40 nm) which were confirmed by UV visible spectroscopy, SEM and TEM analysis. These three nanoscale dimensions showed maximal antifungal actions against test *Fusarium* culture. After 3 days incubation with Ag NPs, they showed more inhibition (over 50%) of radio-labelled mycelial growth (*Fusarium monilifarme*) due to the difference in size of the NPs [47].

Anjali et al. reported the ZnO NPs by using aqueous extract of seaweeds *ulva lactuca* (Ul) and *stoechospermum marginatum* (SM) for evaluation of antibacterial, antifungal and anticancer activity. The NPs were of spherical and round in shape with the sizes1 ranging from 12–17 nm for Ul-ZnO NPs and 6–11 nm for SM-ZnO NPs. The SM-ZnO NPs exhibited a maximum zone of inhibition against *A. flavus* (9 mm) and *Fusarium oxysporum* (3–5 mm) as compared with Ul-ZnO NPs. This difference is attributed to less size and higher surface area to volume ratio, which increases surface properties and subsequently antimicrobial action due to particle size reduction [7]. Velmurugan et al. compared antibacterial and antifungal activities of biosynthesized ZnO NPs with bulk and commercial ZnO NPs. In this study, they observed highest antimicrobial activity for biosynthesized ZnO NPs against pathogens as compared with commercial ZnO NPs and bulk material. The observed results exhibited the importance of use of natural sources for ZnO NPs in various biomedical applications. The features of nanostructures like high surface area to volume ratio and enhanced particle surface reactivity, increased antibacterial activity of biosynthesized ZnO NPs as compared with bulk material [26].

UV Illumination effect

ZnO NPS show high photocatalytic efficiency than other inorganic photocatalytic material and it is biocompatible in nature. ZnO NPs have greater ability to absorb UV light, which increases their photoconductive properties. ZnO NPs in aqueous solution when exposed to UV radiations exhibit release of reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2) and superoxide ions (O_2^-). These ROS species can get easily penetrated into the cells and kill microorganisms. Therefore, antimicrobial activity of ZnO NPs can be increased by exposing ZnO NPs to UV radiations [118].

Photo-excitation of the ZnO NPs surface can activate the desorption of the oxygen molecules and release a series of different types of reactive oxygen species (ROS). The photo-induced oxidation process is responsible for the inactivation of various microbes. The anti-microbial activity of photo-activated zinc oxide nanoparticles (ZnO NPs) has been reported by Kairyte et al., who observed that photo-activated ZnO NPs destroy more populations of microorganisms as compared to normal ZnO NPs. These photo-activated ZnO NPs showed anti-fungal activity against the fungi *Botrytis cinearea* by affecting cellular function which damages the structure of the fungal hypha. This was confirmed by SEM images which showed a distorted conical head as well as damaged hyphal walls of the *Botrytis cineaurea* after treatment with the ZnO NPs. The effect of illumination on anti-fungal action is a major factor [99, 61].

Effect of thermal annealing of nanoparticles

Functionalization of NPs leads to improved antimicrobial activities. Annealing of ZnO NPs results in increased inhibition. Results of EDX measurements indicate that, oxygen annealing enhances the release of oxygen atoms on the ZnO surface, thereby increasing antimicrobial activity due to the generation of more ROS species which results in high oxidative stress. Ann et al. examined zinc and oxygen atoms on the surface of ZnO NPs using ESI method, which increased remarkable increase of O:Zn ratio in annealed samples Increasing the surface area of zinc nanoparticles increases the release of Zn^{2+} ions and stimulates the production of ROS [19]. Mamat et al. annealed ZnO nano-dods by air and oxygen annealing to increase the rate of nanohole formation to increase surface area, resulting in high surface absorption and oxygen diffusion after UV light exposure, resulting in increased surface reactive oxygen species (ROS) [68]. A study by Sharma et al. examined the effects of the temperature of the annealing site on morphology, specific area of the surface, and the total volume of the annealed ZnO NPs. They found that the ZnO NPs that were annealed at 300 and 400 °C demonstrated the highest level of anti- fungal activity against the *C. albicans*, as compared to the normal ZnO NPs. This indicates that the temperature of an annealing site plays an important role in antimicrobial activity [96].

Surface defects

Surface defects and surface charges play a significant role as the ZnO NPs surfaces contain numerous edges and corners that provide many reactive sites. Padmavathy and Vijayaraghavan et al. reported that antibacterial action of ZnO NPs is because of membrane disruption by edges and corners resulted from abrasive ZnO surface [81]. Pramod Kumar et al. synthesized CuO NPs by doping in different concentrations of Zn to check antifungal activity against *Alternaria alternate* and *Alternata alternate*. The effect of localized defects was tested and interpreted for the changes in illuminiscence spectral bands and oxidation states of CuO-Zn. It was noted that Zn doped CuO samples calcinated at 450 °C showed surface defects on CuO such as oxygen vacancies that generate more ROS. These ROS interact with fungal cell membrane and ruptured cell wall. From these observations it was concluded that Zn^{2+} and Cu^{2+} ions influence on fungi cell membrane [56].

Biocompatibility

Now a days implantation of foreign bodies has become very common. Biocompatibility of antifungal agent is an important criterion for the implantation of foreign bodies. Mahalaxmi et al. suggested that the biocompatibility of green synthesized ZnO NPs was higher than that of chemically synthesized ZnO NPs. They also observed that the effect of ZnO NPs on the intensity of cell toxicity are concentration and shape dependent [63]. Zanni et al. prepared grapheme nanoplatelets decorated by ZnO nanorods to inhibit pathogens *S. aureus* and *P. aeruginosa*. They used this material as anti-bio-deteriorative approach. Better biocompatibility was evaluated in animal models *C. elegans* which were not shown by pure ZnO NPs [116].

pH

One of the most important factors affecting antifungal activities of ZnO NPs is the pH. The pH value of the culture condition can influence physico-chemical and biological characteristics of NPs [16]. It was reported that extreme reaction conditions like high acidic and alkaline pH lowers the growth of fungal cells. Many researchers highlighted the effect of the ZnO NPs solubility on the inhibition of some microorganisms which is more complex as compared with particle size [24]. pH plays an important to increase stability, biocompatibility, functionality, sensitivity, and selectivity for different species. Mostly metal containing NPs are soluble in aqueous solution to some extent. Generally antifungal activity is attributed to source of zinc ions [28]. Solubility of zinc ions depends greatly upon pH of the surrounding medium. Most importantly environmental factors influencing experiment was hardness of water, composition of testing media and pH. Interaction between nanoparticles and organic components present in the medium like protein, amino acids and other natural organic substances has been reported to show altered dispersion of ZnO NPs. Solubility of ZnO NPs get enhanced mostly at acidic pH [60].

Other factors

Functionalized ZnO NPs can be used for various applications. Functionalization improves the stability, sensitivity and functionality of the surfaces. The functionalization can be done directly or by grafting [63]. Concentration of ZnO NPs is another important factor that affects antimicrobial activity. Navale et al. reported concentration dependent antimicrobial activity of ZnO NPs against *S. aureus*, *S. typhimurium*, *Aspergillus flavus* and *A. fumigates*. In antifungal activity it was observed that at 20 µg/ml concentration of ZnO NPs, there was inhibitory action after 7 days incubation of both fungi at different concentration. Fungal biomass was remarkably decreased with increased concentration of ZnO NPs up to 100 µg/ml. These results interpret that ZnO NPs show concentration dependent antifungal activities [76].

Use of ZnO NPs and ZnO nanocomposites in antifungal nano-therapy

Nanotechnology offers a viable alternative to traditional treatments for the treatment of systemic mycotic infections. Current treatments are limited in terms of bioavailability, selectivity, efficacy, and adverse reactions. Nano-based nanoparticles offer a more precise and controlled delivery system for drug delivery, which can improve the efficacy of mycotic treatment without compromising the patient's quality of life. The most attractive nanoparticle among all available options is ZnO NPs [2]. ZnO NPs possess great antimicrobial ability to enhance the inhibition of microbial growth and metabolism. The antibacterial and antifungal activity of ZnO NPs has been reported widely against wide range of bacteria [3, 10]. Obviously, there is different mechanism of antifungal action from that of antibacterial action due to basic differences in the cell wall compositions in bacteria and fungi. Therefore, it is the area of research with more attention by the scientist. Varieties of assays can be used for detection of antifungal activity like agar or broth dilution, disc diffusion and micro-titer plate methods. ZnO NPs are majorly used in sunscreens lotions and cosmetics as it is photo-stable and low photoallergic. It is widely reported that ZnO NPs can be easily penetrated from the outer skin layer to the dermal layer. The compatibility of ZnO NPs with human skin makes it as a most preferred additive in textile fields as well as in treating fungal skin infections. Also, ZnO NPs in nano-emulsion form are less oily, with improved texture and more deep penetration ability in skin and hairs. According to various studies, spherical shaped ZnO NPs were non-toxic to human cells and can be used in sunscreen, antifungal ointments and cosmetics. Grijalba et al., investigated the effect of ZnO NPs synthesized using different parameters such as the initial concentration of precursor on the antifungal activity of ZnO NPs [76]. Joshaghani et al. examined suppression of *Candida albicans* at very low dosages ranging from 1.013–296.0 g/mL. Morphology of ZnO NPs influences the potential of antifungal activities [15]. Melendz et al. observed that flower shaped ZnO NPs inhibited the growth of *Aspergillus flavus* and formation of aflatoxin [37]. Sonia et al. studied that colloidal ZnO NPs can be effectively used in topical cold cream formulations. In this study, ZnO NPs were synthesized using *Adatoda vasica* leaf extract, with a particle size of about 10–12 nm having a hexagonal morphology. The antifungal activity of these ZnO NPs was calculated by measuring the inhibition zone from 9.0 to 19.00 mm [101]. Tiwari et al. tested biosynthesized ZnO NPs (100 nm) against *T. mentagrophytes* and *M. canis* alone and also in combination with ketoconazole. The results suggest that these ZnO NPs can exhibit enhanced antifungal activity when combined with ketoconazole compared with ZnO NPs alone [106].

In modern nanotechnologies, which improve materials quality, combining nanostructures with appropriate biotechnologically active agents is the most common practice. The surface functionalization of nanomaterials, which is very efficient in changing their properties on surfaces, may increase the antifungal activity of ZnO NPs [119]. ZnO NP functionalization may improve toxicity, blood compatibility, biodegradability and minimization in turn increase antibacterial activity. The antifungal activity of ZnO NPs can be increased by the surface functionalization of nanoparticles which is a very effective way of altering the surface properties. Functionalization of ZnO NPs can improve minimization in toxicity, blood-compatibility, and bio-degradability, and in turn enhance the antibacterial, antifungal, and anti-biofilm activity [86]. The significance of functionalization of ZnO NPs can be well explained in following example (Table 3).

Ikhechi et al. explained enhanced antifungal effect of ZnO-TiO_2 NPs against *Aspergillus flavus* as compared with only ZnO or TiO_2. Minimum inhibitory concentration (MIC), minimum fungicidal concentration and diameters of zones of inhibition of these nanomaterials are given in Table 3.

Results clearly showed that the functionalization of ZnO NPs with TiO_2 overcomes the limitations of pure ZnO NPs with remarkable increase in antifungal activity. This is becauseZnO-TiO2 nano-composite releases high numbers of reactive oxygen species as compared with only ZnO NPs. The possible mechanism for this antifungal activity can be related with release of higher amounts of ROS that

Table 3 Antifungal activities of ZnO NPs, TiO_2 NPs and ZnO- TiO_2 NPs [74]

Sr. no.	Type of nanoparticles	Target fungi	Minimum inhibitory concentration (MIC)	Minimum fungicidal concentration	Morphology of nanoparticles	Zone of inhibition (mm)
1	ZnO NPs	*Aspergillus flavus*	156 µg ml^{-1}	312 µg ml^{-1}	Hexagonal pyramidal	37.5
2	TiO_2		78 µg ml^{-1}	156 µg ml^{-1}	Spherical	75
3	ZnO-TiO_2		39 µg ml^{-1}	78 µg ml^{-1}	Mixture of pyramidal and spherical	150

can rupture the cell membrane, damage of enzymes, finally resulting into cell death [74].

Alshahrani et al. prepared ZnO-AMB-PEG NPs and tested it against *Candida albicans* and *Candida neoformans* for *in-vivo* and *in-vitro* studies. This conjugated material showed reduced amphotericin induced nephrotoxicity. This functionalization improved both solubility and therapeutic index of AMB, supported by remarkable lowering in MIC and MFC in ZnO-AMB-PEG NPs. They reported that ZnO-AMB-PEGylated nanoparticles with less nephrotoxicity can be safe for clinical use [5]. Ghosh et al. synthesized Cu-Ag-ZnO nanocomposites by mechanical alloying of Cu, ZnO and Ag by conjugating with antifungal drug fluconazole. It was observed that fluconazole conjugated Cu-Ag-ZnO nanocomposite showed 20 times enhanced antifungal activity as compared with pure fluconazole. This conjugation minimized side effects and lowered both dose and dose frequency of fluconazole because large surface area of Cu-Ag-ZnO nanocomposite showed higher affinity for fluconazole that loads larger amount of fluconazole on the surface of Cu-Ag-ZnO nanocomposites. The conjugation of Cu and ZnO NPs enhanced the generation of reactive oxygen species and doping with Ag increased antifungal activity due to synergism with fluconazole [31]. Dhananjaya et al. investigated anti-fungal activity and cytotoxicity of ZnO-chitosan nanocomposites against *C. albicans* and Hep2 (human epithelial type-2). Chitosan being natural polysaccharide biopolymeris widely used in drug delivery, bone healing, tissue engineering, food packaging, and antimicrobial agents due to less toxicity, biocompatibility, biodegradability, sufficient strength and water permeability. This organic–inorganic hybrid material showed synergistic effects as a potential antimicrobial agent and thus had ability to use as an alternative for existing treatments used to treat infections caused by *C. albicans* [21]. Hassan et al. proposed that antifungal activity can be enhanced by conjugating metallic nanoparticles with natural oils. The ZnO-Ag nanocomposite with essential oils suppressed the growth of both bacterial and fungal diseases. For this purpose, they conjugated ZnO-Ag nanocomposite with olive and cinnamon oil that inhibited growth of *Aspergillus flavus* and *Escherichia coli* and prevented related toxin production [31].

Filamentous fungal infections cause immune suppression and carcinogenic effects in the human body through secondary metabolites (mycotoxins) in pathogenic fungi. Various studies have shown that the production of aflatoxin, ochratoxin A and fumonisin B can be inhibited in pathogenic fungi by the addition of ZnO NPs [43, 108]. Lipovsky et al., suggested the marked antifungal activity of ZnO NPs against *C. albicans* is concentration-dependent and size dependent [95]. In addition, it was also suggested that ZnO NPs in aqueous solutions can form reactive oxygen species like hydroxyl radicals, singlet oxygen, and superoxide anion radicals. Also, the addition of histidine which acts as a scavenger of hydroxyl radical and singlet oxygen vanishes the effect of ZnO NPs on the viability of *C. albicancs* [52, 20]. Pragathiswaran et al. investigated nanocomposites of TiO_2-ZnO modified by decorating gold NPs, with the use of hydrothermal method. It was tested for antimicrobial activities against *E. coli*, *S. aureus* and *C. albicans*. Also, their anti-inflammatory activity and cell viability assay were performed. In this nanocomposite decoration of TiO_2-ZnO by gold enhanced physico-chemical and biological activities. Combination of TiO_2-ZnO being inorganic heterocatalyst provides variety of applications in pharmaceuticals and biotechnology as an antimicrobial and anticancer agent [88]. Nanocomposites possess lot of applications in various fields as they exhibit unique properties than their original form. Phiwdang et al. reported higher antifungal activity of Cu–ZnO nanocomposite (7:3) against *Aspergillus trichoderma* as compared with pure CuO/ZnO NPs. In this study, it was suggested that the presence of ZnO NPs in CuO matrix promoted antifungal properties due to charge transfer between CuO and ZnO NPs that mediate fungal inhibition more effectively [85]. Different ZnO NPs and ZnO nanocomposites are given in Table 4.

Process of functionalization of ZnO NPs

ZnO NPs can be modified to enhance specific properties like size, shape, structure, morphology, appearance, color, consistency, and strength of material. This modification may improve compatibility of ZnO NPs with organic, inorganic and polymers that incorporate valuable properties to materials like less time, improved optical, electrical properties and minimizes drawbacks of materials. Several techniques are used for the functionalization of ZnO NPs are given in Fig. 5.

Several reports are available showing silica and trimethoxysilane (TMS) are flexible for the functionalization of ZnO NPs. For the production of pure ZnO NPs, precursors Zn carbonate hydroxide (ZCH) were allowed to be calcinated. Ammonium solution (NH_4OH), ammonium bicarbonate (NH_4HCO_3), and zinc sulfate heptahydrate ($ZnSO_4.7H_2O$) substrates were used in the production of ZCH with precipitation technique. ZnO NP modification can also overcome the agglomeration process [32, 92].

Inorganic compounds like silica showed lowered photocatalytic activity when present on ZnO surface. In contrast, when organic molecule is present on ZnO surface they increase compatibility of ZnO NPs with organic matrix. To get required results, modified ZnO surface was exposed as a transparent material that provided protection against UV radiations and further their antifungal and antialgal action were evaluated effectively. Dhanalakshmi et al. synthesized

Table 4 Different ZnO Nanocomposites for antifungal activities

Sr. no.	Materials used	Synthesis strategy	Concept	Tested Organisms	Dose	Remark
1	Recycled Polyethylene Terephthalate r-PET functionalized with different concentration of ZnO nanofibers [109]	Solvothermal method (For ZnO NPs synthesis) and electrospinning method (for fibers)	To reduce environmental problems due to plastic waste, recycled r-PET that possess low density, corrosion and chemical resistance, maximum availability is used, while synthesizing nanofibers offers large surface area, surface flexibility, mechanical properties with controlled pore size. ZnO NPs were added in nanofibers to add antimicrobial properties	*E. coli, Bacillus subtilis, Penicillium* sp. *Fusarium graminearum*	Maximum inhibition effect was observed against *E. coli* (38.5 mm), *B. subtilis* (34.25 mm) at 6% w/w ZnO NPs concentration. Also strong antifungal effectagainst *Penicillium* sp. *Fusarium graminearum* were observed by ZnO-6-r-PET nanofibers	This material is useful in textiles for medical or surgical purposes like apron etc
2	Green synthesized ZnO NPs by using *Terminalia bellerica* Tb-(ZnO NPs) [23]	Green synthesis	Phyto-fabricated ZnO NPs (Tb-ZnO NPs) are synthesized biologically which is effective as compared with ZnO NPs synthesized by chemical method	*Alternaria brassicae*	Tb-ZnO NPs were effective against *Alternaria brassicae* at 200 ppm concentration	Disadvantages of use of microorganisms as a bio-control agents can be overcome by use of such green synthesized ZnO NPs. This material showed more antifungal activity as compared with available fungicide and chemically synthesized ZnO NPs
3	Xanthan gum/ZnO/TiO_2 [30]	Taguchi method	Antimicrobial, antioxidant, cost effective, anticancer, non-toxic, and biocompatible nature of ZnO, TiO_2 NPs and xanthan with non-toxic, cheap, high biocompatibility, thermal stability, resistance to acid–base, water solubility and antimicrobial properties offered in one nanocomposite	*C. albicans*	Optimum inhibition (95%) were observed at composition 0.01: 0.009: 0.009 M (Xanthan gum:ZnO:TiO_2)	Antifungal activity of xanthan gum/ZnO/TiO_2 NPs against *C. albicans* were optimum (95%). Due to its antifungal activity, it can be used in medicine and dentistry that can inhibit pathogenic microbes. However, clinical studies are required to be used in treatment of vaginal candidititis

Table 4 (continued)

Sr. no.	Materials used	Synthesis strategy	Concept	Tested Organisms	Dose	Remark
4	Pullulans- Piscean collagen-ZnO nanocomposite [30]	Solution casting method	To avoid adverse effects of plastic, biopolymers like collagen used being nontoxic, eco-friendly, renewable, cost effective. Pullulans act as a matrix of such films. It is preferred due to adhesive, biodegradable and with high tensile strength. ZnO NPs added as an antimicrobial activity. FDA approved composite with fabricated film	*B. subtilis, S. aureus, P. aeruginosa, C. albicans, A. niger*	Pullulans- Piscean collagen-ZnO nanocomposite with 1% of ZnO NPs showed 14 mm inhibition zone against *A. niger* while others were resistant	These nanomaterials can be used as edible food packaging
5	Fe_3O_4/ZnO/AgBr nanocomposite [38]	Facile microwave assisted method	ZnO NPs have high chemical, physical stability, cost effective, non-toxic, biocompatible, eco-friendly and don't affect soil fertility. Limitation of alone ZnO NPs being less effective as antifungal agent and recovery of ZnO from inactivation systems is difficult, time consuming, expensive that leads to secondary pollution. This can overcome by using ZnO based nanocomposite with magnetic NPs that can help in removing suspended NPs with applications of external magnetic field	*Fusarium grarinearum, Fusarium oxysporum*	Out of all prepared samples Fe_3O_4/ZnO-AgBr nanocomposite (1:10) showed higher antifungal activities. However, due to its poor magnetic separation ability Fe_3O_4/ZnO/AgBr nanocomposite with 1:8 ratio selected as best composite that showed 99.4% inactivation of fungal spores after 120 min	This ultifunctional Fe_3O_4/ZnO/AgBr nanocomposite (1:8) that can be used as effective fungicide in agriculture and waste water treatments, also can be recycled from place of action with the use of magnetic field

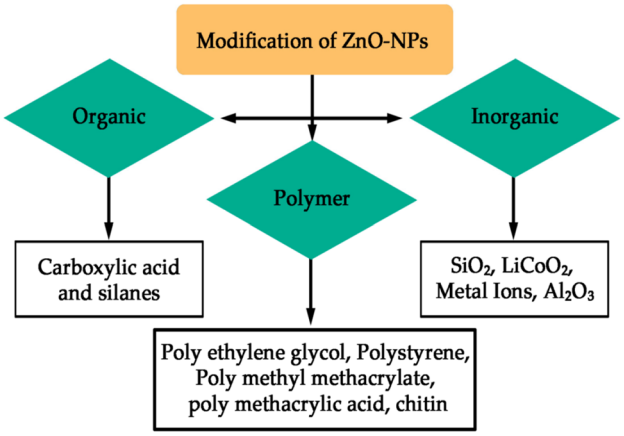


Fig. 5 Diagram of different modification methods of ZnO NPs [120]

ZnO-TiO_2 nanocomposite materials (ZnO-TiO_2) NCM by using simple chemical method, and further added into various amine functionalized silicate sol gel matrices (FSG). There decomposition ability towards 4-nitrophenol by obtaining photocatalytic performance was obtained. Combined antimicrobial action of TiO_2 and ZnO NPs released higher amount of metal ions, and ROS that acts as a strong oxidant. Release was maximum due to photocatalytic activity of TiO_2 and increased release of H_2O_2 by ZnO NPs [22]. Vazquez et al. synthesized polymeric fibers from recycled polyethylene terephthalate (r-PET) of post-consumer water bottles and modified it with ZnO NPs of different concentration (0%, 1.5%, 3% and 6%). These nanocomposites were further tested for antibacterial and antifungal activity. In this work, ZnO NPs were synthesized by using solvothermal method with diameter of 38.15 nm and fibers were synthesized by electrospinning technique with diameter of 200–5000 nm. Functionalization of ZnO NPs was performed by simply adding ZnO NPs in the solution of r-PET into TFA solvent by constant magnetic stirring for 1–2 h [109].

Antifungal mechanism of ZnO NPs

Antifungal mechanisms of ZnO NPs have been described in many literatures (Fig. 6). But due to insufficient data availability about inhibitory modes, synthetic approaches and experimental conditions there is less clarity of antifungal mechanism of ZnO NPs. Still, the exact mechanism of fungal cell responses towards nanoparticles is unresolved. On the basis of literature available on antifungal mechanism of ZnO NPs two ways can be considered: first one is photon induced release of ROS due to its potential photo-catalytic activity and toxic effect due to Zn^{2+} ions [13].

According to Mahamuni-Badiger et al., antimicrobial activity of ZnO NPs is mainly due to the presence of oxygen radicals or hydrogen peroxide on ZnO NPs surface. The release of ROS (reactive oxygen species) creates oxidative stress that causes cell death. According to some reports, hyphal cells tried to escape the interaction with ROS by promoting the oxidative stress reactions such as superoxide

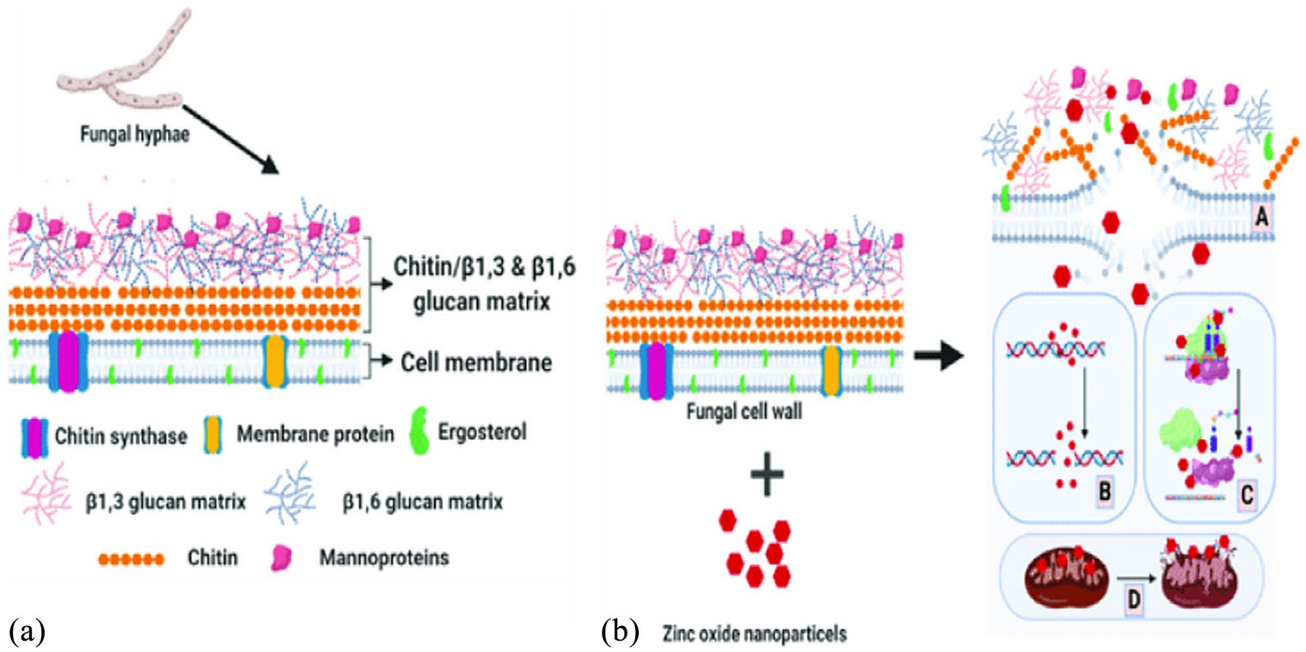


Fig. 6 Different mechanisms of antifungal activity of ZnO NPs. (a) Structure of fungal cell wall; (b) Mechanism of action; (A) Disruption of fungal cell wall; (B) DNA damage; (C) Inhibition of protein synthesis; (D) Mitochondria damage [12]

dismutase and peroxidase activities [67]. But, higher concentration of ZnO NPs can cause the rupturing of hyphal cells and oxidation of protein. The release of ROS due to ZnO NPs can cause other physiological changes along with cellular damages. Lipovsky et al. reported the concentration-dependent antifungal activity against *Candida albicans* which is pathogenic yeast. They confirmed that the cytotoxic effect of ZnO was due to reactive oxygen species by addition of scavenger of hydroxyl radical and singlet oxygen, histidine [61].

It has been suggested in many reports that ZnO NP activity is caused by toxicity of released ZnO ions. Most of the time, ZnO NP solubility depends on various factors such as water quality, media components and pH. Some of the studies have shown that in the absence of light, there is oxidative stress in the ZnO NP due to oxygen vacancies. This can be explained by the fact that ZnO solubility causes ZnO ions to be released which in turn cause fungitoxic effects. In these cases, water solubility and intact particle, metal ion or metal complexes have become important criteria for the development of anti-fungal effects [2]. According to some studies, the mechanism of the fungicidal action is the disruption of cell structure, such as cell wall or cell organelles, which prevents inhibition of cell metabolism. In the filamentous fungi the dominant principle of the anti-fungal activity may be oxidative stress due to the presence of reactive oxygen species (ROS). Treatment with ZnO NP may also reduce glutathione levels by inhibiting the GSH synthesis enzymes, thereby reducing the antioxidant capacity of the cells [59]. Interaction of ZnO NPs with fungal cell wall alters the cytoskeleton of the cell by surface shrinkage, cell aggregation, pore formation and destruction. Microscopic analysis depicts inner membrane destruction, organelle dysfunction, and reduced cytoplasmic content. Alvarez et al. reported cell leakage of *candida albicans* increased intracellular and extracellular amount of glucose and trehalose that can act as protectant during stress conditions and can act as carbon source [84].

It was noted that after inhibition of fungal growth carbohydrate and nucleic acid level decreased. In addition, interaction with ZnO NPs didn't alter protein and lipid level showing that cell wall may not be affected and particles may internalize acting intracellularly rather than extracellularly [45]. Smaller sized ZnO NPs can cause fluid phase endocytosis escaping cell wall damage. This includes mitochondrial fragmentation, ribosomal deactivation and chitin destruction. ZnO NPs can also have effect on fungal hyphae and spores, that showed hyphae deformation, distortion and shrinkage, changed growth patterns, agglomeration and thinning of hyphal fibers. ZnO NPs formed bulges on *B. cinerea* hyphae surface, affecting growth [36]. Wani et al. reported ZnO treatment on *Penicillium expansum* and external mycelia colonies exhibited destroyed conidia and hampered germination ability. Interaction with ZnO NPs can also affect gene expression. ZnO NPs lowered HWP1 gene expression in *C. albicans* [112]. ZnO exposure down regulated expression of ALS 1 and ALS 3 in fluconazole resistant *C. albicans* strain more as compared with fluconazole treatment [73].

Changes in the redox state and release of ROS in hyphal cell maintained the transcript levels of *ShSOD2* and *Shgst1* that encodes superoxide dismutase and glutathione S-transferese. Even exposure to ZnO NPs can alter the gene expression levels which are related to oxidative stress, zinc ion binding function, and oxidative phosphorylation function. Detection of intermolecular interaction will be helpful to understand the antifungal mechanism of nanoparticles [70].

ZnO NPs are promising antimicrobial agent due to their excellent antimicrobial property. However, the exact mechanism of ZnO is not fully resolved. There is need to focus on theoretical experiments and toxicity assessment experiments, like assays available for oxidative stress, and quantification of metal ion release and ROS release [83].

Nanotoxicity of ZnO NPS

Despite the various applications of ZnO NPs as an antibacterial agent, their toxicity is one of the important factors that can restrict their wide use. Mechanism of ZnO nanotoxicity is attributed mainly to the generation of reactive oxygen species that can produce oxidative stress in the tissues. Generally, in mitochondria, ATP gets synthesized through reduction of oxygen into water by electron transfer reaction. However, minute amount of oxygen is not reduced completely which produce superoxide anion radicals and O_2 containing radicals, termed as a reactive oxygen species (ROS). ROS includes superoxide anion radicals, hydroxyl radicals, singlet O_2 and hydrogen peroxide (H_2O_2). It is widely reported that nano-ZnO enhances cytotoxicity which is attributed to release of ROS that resulted into oxidative injury, production of inflammatory mediators, causing cell death in phagocytic RAW-2647 cells and human bronchial epithelial BEAS-2B cells. Toxicity of ZnO NPs get altered with various mechanisms. Solubility of ZnO NPs and amount of unreacted Zn^{+2} inside the cell has major role in nanotoxicity of ZnO NPs. Yang et al. evaluated changes in the cytotoxicity of ZnO NPs by observing all proliferation in the uniformity of cell membrane and morphological alterations of nucleus and cell death after treating with ZnO NPs [115]. For toxicological assay of ZnO NPs information of routes of ZnO intake is important. Intake routes considered are gastrointestinal tract, and oral. As ZnO is present in large number of cosmetic products, dermal exposure is obvious. It was reported that ZnO NPs were unable to observe in healthy or intact human skin. However, ZnO absorbed in hair follicles are removed by sebum flow. But skin damage

can harm this protective layer and causes toxicological influence of NPs.

Genotoxic effect occurs at the basal layer of epidermis that limits ZnO applications for injured skin. The ingestion of ZnO NPs and contact with mucosa can be observed equally. Exposure through air is common in workers of chemical or cosmetic industries. These NPs easily reach to peripheral tissues like bronchiolar and alveolar regions. If they remain as it is, can affect alveolar cells and cause toxic and hazardous genetic effects. Inhalation of ZnO NPs of 10–100 nm size can get deposited in alveolar and tracheobronchial region. If they escape from phagocytosis mechanism their deposition may result into inflammation and fibrosis [94]. ZnO NPs dissolution release Zn^{+2} ions which may induce necrosis. Maximum amount of Zn^{+2} was observed in BAL cells and white blood cells in rats after inhalation of 38 nm ZnO nanoparticles [48]. Sharma et al. studied that ZnO NPs one of the commonly used materials in dermatological preparation and cosmetics, cause DNA damage in human epidermal cell line A431 [97]. Prasanth et al. reported that ZnO NPs showed time and dose dependent activity in nasopharyngeal cancer cells and cell respiration rate decreases with time [89]. Heng et al. studied cellular association, cytotoxic and inflammatory potential of spherical and sheet shaped ZnO nanoparticles on mouse and human cell lines (RAW-264.7 and BEAS- 2B) cells, in which it was observed that there is no significant effect of shape on cytotoxicity potential of ZnO nanoparticles [18]. Confluent C2C12 cells are more resistant to ZnO nanoparticles. Cytotoxicity is observed at time interval of 24, 48 and 72 h [24]. Use of ZnO in toothpaste is common which causes their accumulation in digestive tract. RKO human colon carcinoma cells showed particle cell interaction dependent cytotoxicity [72]. ZnO NPs can cause cytotoxicity in Ana-1 murine macrophages [100].

For the validation of risk assessment of ZnO NPs it is crucial to understand molecular mechanics of genotoxicity. Autophagy is the degradation process that is dependent upon lysosomal degradation which get triggered under stress situation. Auffan et al. studied that chemically stable NPs don't have remarkable cellular toxicity while NPs which can get oxidized reduced or dissolved are toxic for cells of organisms. ROS generation was observed as a major trigger to induce autophagy. According to previous reports p53 is responsible for enhancement of cytotoxicity by ZnO NPs. The interaction of ZnO NPs at lower concentration can promote the regulation of p53 that express antioxidant gene that further promotes the expression of antioxidant genes like ALDH4A, GPX1, SOD2, SEN1 and SESN2. In contrast higher concentration of ZnO NPs cause causes release of ROS with triggering p53 mediated apoptosis. Cancer cell without p53 like DLD-1ani SW480 cells showed more sensitivity for ZnO mediated toxicity [34].

Conclusion and future perspectives

Because of their complexity and antibiotic resistance, fungal infections are essential to treat. For fungal inhibition, nanoparticles are an effective way to treat severe infections, especially in drug-resistant fungi. Of all nanoparticles, ZnO NPs are considered to be promising candidates for various biomedical applications. ZnO NPs have broad spectrum antimicrobial activity potential by increasing the release of reactive oxygen species (ROS), release of zinc ions (Zn^{+2}), deposition of hydrogen ions (H_2O_2), and easy intracellular penetration. This review concentrates on the primary role of zinc oxide nanoparticles in antifungal action.

The discussion focuses on multiple drug resistance in fungi, ideal properties of ZnO NPs as an antifungal agent; synthesis approaches, factors influencing antifungal activities of ZnO NPs, applications of ZnO NPs and their nanocomposites in antifungal nano-therapy along with significance of functionalization, and antifungal mechanism of ZnO NPs. The different factors influence intensity of antifungal activity. The release of ROS species, Zn^{+2} ions, H_2O_2 ions is responsible fungal cell damage. ZnO NPs can act as an effective agent against multiple drug resistant microorganisms and as an alternative for conventional antibiotics. There is a need of further non-clinical and clinical studies in terms of biocompatibility, safety and tolerance to design potential commercial applications.

Acknowledgements The authors express their gratitude towards Rayat Shikshan Sanstha's S. M. Joshi College Hadapsar, Pune for providing lab facilities and infrastructure.

Author contributions PM conceived and designed the index and theme of the review along with manuscript preparation. CN helped literature search, VG and NP helped in editing, PM performed and finalized the revision.

Funding No funding.

Declarations

Ethical approval No approval was required for the study.

Informed consent and conflict of interest All authors consented to publish and declare no conflict of interest.

References

1. Agarwal H, Venkat Kumar S, Rajeshkumar S. A review on green synthesis of zinc oxide nanoparticles—an eco-friendly approach.

Resour Technol. 2017;3:406–13. https://doi.org/10.1016/j.reffit.2017.03.002.
2. Akhtar N, Khan S, Rehman SU, Rehman ZU, Mashwani ZUR, Rha ES, et al. Zinc oxide nanoparticles enhance the tolerance and remediation potential of *Bacillus* spp. against heavy metal stress. Adsorpt Sci Technol. 2021;2021:1–16.
3. Alavi M, Nokhodchi A. Synthesis and modification of bio-derived antibacterial Ag and ZnO nanoparticles by plants, fungi, and bacteria. Drug Discov Today. 2021;26:1953–62. https://doi.org/10.1016/j.drudis.2021.03.030.
4. Ali M, Donakowski MD, Mayer C, Winterer M. Chemical vapor functionalization: a continuous production process for functionalized ZnO nanoparticles. J Nanoparticle Res. 2012;14:0–10.
5. Alshahrani SM, Khafagy E, Riadi Y, Al Saqr A, Alfadhel MM, Hegazy WAH. Amphotericin B-PEG conjugates of ZnO nanoparticles: enhancement antifungal activity with minimal toxicity. Pharmaceutics. 2022;14:1646.
6. Alvarez-Peral FJ, Zaragoza O, Pedreño Y, Argüelles JC. Protective role of trehalose during severe oxidative stress caused by hydrogen peroxide and the adaptive oxidative stress response in *Candida albicans*. Microbiology. 2002;148:2599–606.
7. Anjali KP, Sangeetha BM, Raghunathan R, Devi G, Dutta S. Seaweed mediated fabrication of zinc oxide nanoparticles and their antibacterial, antifungal and anticancer applications. ChemistrySelect. 2021;6:647–56.
8. Ao W, Li J, Yang H, Zeng X, Ma X. Mechanochemical synthesis of zinc oxide nanocrystalline. Powder Technol. 2006;168:148–51.
9. Arastehfar A, Gabaldón T, Garcia-Rubio R, Jenks JD, Hoenigl M, Salzer HJF, et al. Drug-resistant fungi: an emerging challenge threatening our limited antifungal armamentarium. Antibiotics. 2020;9:1–29.
10. Arciniegas-Grijalba PA, Patiño-Portela MC, Mosquera-Sánchez LP, Guerrero-Vargas JA, Rodríguez-Páez JE. ZnO nanoparticles (ZnO-NPs) and their antifungal activity against coffee fungus Erythricium salmonicolor. Appl Nanosci. 2017;7:225–41.
11. Aslani P, Mohammadi SR, Roudbary M. Novel formulated zinc oxide nanoparticles reduce Hwp1 gene expression involved in biofilm formation in candida albicans with minimum cytotoxicity effect on human cells. Jundishapur J Microbiol. 2018;11:1–6.
12. Babayevska N, Przysiecka Ł, Iatsunskyi I, Nowaczyk G, Jarek M, Janiszewska E, et al. ZnO size and shape effect on antibacterial activity and cytotoxicity profile. Sci Rep. 2022;12:1–13. https://doi.org/10.1038/s41598-022-12134-3.
13. Bailore NN, Balladka SK, Doddapaneni SJDS, Mudiyaru MS. Fabrication of environmentally compatible biopolymer films of pullulan/piscean collagen/ZnO nanocomposite and their antifungal activity. J Polym Environ. 2021;29:1192–201. https://doi.org/10.1007/s10924-020-01953-y.
14. Berman J, Krysan DJ. Drug resistance and tolerance in fungi. Nat Rev Microbiol. 2020;18:319–31.
15. Bhattacharyya S, Gedanken A. A template-free, sonochemical route to porous ZnO nano-disks. Microporous Mesoporous Mater. 2008;110:553–9.
16. Bondarenko O, Juganson K, Ivask A, Kasemets K, Mortimer M, Kahru A. Toxicity of Ag, CuO and ZnO nanoparticles to selected environmentally relevant test organisms and mammalian cells in vitro: a critical review. Arch Toxicol. 2013;87:1181–200.
17. Chen WJ, Liu WL, Hsieh SH, Tsai TK. Preparation of nanosized ZnO using a brass. Appl Surf Sci. 2007;253:6749–53.
18. Chin B, Xinxin H, Eng Z, Tan C, Khamis N, Loo JS. Evaluation of the cytotoxic and inflammatory potential of differentially shaped zinc oxide nanoparticles. Arch Toxicol. 2011;85:1517–28.
19. Chuo L, Mahmud S, Khadijah S, Bakhori M, Sirelkhatim A. Effect of surface modification and UVA photoactivation on antibacterial bioactivity of zinc oxide powder. Appl Surf Sci. 2014;292:405–12. https://doi.org/10.1016/j.apsusc.2013.11.152.
20. da Silva BL, Abuçafy MP, Manaia EB, Junior JAO, Chiari-Andréo BG, Pietro RCLR, et al. Relationship between structure and antimicrobial activity of zinc oxide nanoparticles: An overview. Int J Nanomedicine. 2019;14:9395–410.
21. Dananjaya SHS, Kumar RS, Yang M, Nikapitiya C, Lee J, De ZM. Department of marine life sciences, school of marine biomedical sciences, Jeju National SC. Int J Biol Macromol. 2017. https://doi.org/10.1016/j.ijbiomac.2017.11.046.
22. Dhanalakshmi R, Pandikumar A, Sujatha K, Gunasekaran P. Photocatalytic and antimicrobial activities of functionalized silicate sol-gel embedded ZnO-TiO2 nanocomposite materials. Mater Express. 2013;3:291–300.
23. Dhiman S, Varma A, Prasad R, Goel A. Mechanistic insight of the antifungal potential of green synthesized zinc oxide nanoparticles against *Alternaria brassicae*. J Nanomater. 2022;2022:1–13.
24. Dimkpa CO, Calder A, Britt DW, McLean JE, Anderson AJ. Responses of a soil bacterium, Pseudomonas chlororaphis O6 to commercial metal oxide nanoparticles compared with responses to metal ions. Environ Pollut. 2011;159:1749–56. https://doi.org/10.1016/j.envpol.2011.04.020.
25. Elkady MF, Hassan HS, Hafez EE, Fouad A. Construction of zinc oxide into different morphological structures to be utilized as antimicrobial agent against multidrug resistant bacteria. Bioinorg Chem Appl. 2015;2015:1–20.
26. Elumalai K, Velmurugan S. Green synthesis, characterization and antimicrobial activities of zinc oxide nanoparticles from the leaf extract of *Azadirachta indica* (L.). Appl Surf Sci. 2015;345:329–36. https://doi.org/10.1016/j.apsusc.2015.03.176.
27. Espitia PJP, Soares NFF, Coimbra JSR, de Andrade NJ, Cruz RS, Medeiros EAA. Zinc oxide nanoparticles: synthesis, antimicrobial activity and food packaging applications. Food Bioprocess Technol. 2012;5:1447–64.
28. Fabrega J, Luoma SN, Tyler CR, Galloway TS, Lead JR. Silver nanoparticles: behaviour and effects in the aquatic environment. Environ Int. 2011;37:517–31. https://doi.org/10.1016/j.envint.2010.10.012.
29. Frade T, Melo Jorge ME, Gomes A. One-dimensional ZnO nanostructured films: effect of oxide nanoparticles. Mater Lett. 2012;82:13–5. https://doi.org/10.1016/j.matlet.2012.05.028.
30. Ghorbani F, Gorji P, Mobarakeh MS, Mozaffari HR, Masaeli R, Safaei M. Optimized synthesis of xanthan gum/ZnO/TiO_2 nanocomposite with high antifungal activity against pathogenic *Candida albicans*. J Nanomater. 2022. https://doi.org/10.1155/2022/7255181.
31. Ghosh M, Mandal S, Roy A, Chakrabarty S, Chakrabarti G. Enhanced antifungal activity of fl uconazole conjugated with Cu-Ag-ZnO nanocomposite. Mater Sci Eng C. 2020;106: 110160. https://doi.org/10.1016/j.msec.2019.110160.
32. Gu T, Yao C, Zhang K, Li C, Ding L, Huang Y, et al. Toxic effects of zinc oxide nanoparticles combined with vitamin C and casein phosphopeptides on gastric epithelium cells and the intestinal absorption of mice. RSC Adv. 2018;8:26078–88.
33. Hall-stoodley L, Costerton JW, Stoodley P. Bacterial biofilms: from the natural environment to infectious diseases. Nat Rev Microbiol. 2004;2(2):95–108. https://doi.org/10.1038/nrmicro821.
34. Hameed ASH, Karthikeyan C, Ahamed AP, Thajuddin N, Alharbi NS, Alharbi SA, et al. In vitro antibacterial activity of ZnO and Nd doped ZnO nanoparticles against ESBL producing *Escherichia coli* and *Klebsiella pneumoniae*. Sci Rep. 2016;6:1–11. https://doi.org/10.1038/srep24312.

35. Hasnidawani JN, Azlina HN, Norita H, Bonnia NN. Synthesis of ZnO nanostructures using sol-gel method. Procedia Chem. 2016;19:211–6. https://doi.org/10.1016/j.proche.2016.03.095.
36. He L, Liu Y, Mustapha A, Lin M. Antifungal activity of zinc oxide nanoparticles against Botrytis cinerea and *Penicillium expansum*. Microbiol Res. 2011;166:207–15. https://doi.org/10.1016/j.micres.2010.03.003.
37. Hernández-Meléndez D, Salas-Téllez E, Zavala-Franco A, Téllez G, Méndez-Albores A, Vázquez-Durán A. Inhibitory effect of flower-shaped zinc oxide nanostructures on the growth and aflatoxin production of a highly toxigenic strain of *Aspergillus flavus* link. Materials (Basel). 2018;11(8):1265. https://doi.org/10.3390/ma11081265.
38. Hoseinzadeh A, Habibi-yangjeh A, Davari M. Antifungal activity of magnetically separable Fe_3O_4/ZnO/AgBr nanocomposites prepared by a facile microwave-assisted method. Prog Nat Sci Mater Int. 2016;26:334–40. https://doi.org/10.1016/j.pnsc.2016.06.006.
39. Hossain CM, Ryan LK, Gera M, Choudhuri S, Lyle N, Ali KA, et al. Antifungals and drug resistance. Encyclopedia. 2022;2:1722–37.
40. Huang Y, He J, Zhang Y, Dai Y, Gu Y, Wang S, Zhou C. Morphology, structures and properties of ZnO nanobelts fabricated by Zn-powder evaporation without catalyst at lower temperature. J Mater Sci. 2006;41:3057–62.
41. Hui A, Liu J, Ma J. Synthesis and morphology-dependent antimicrobial activity of cerium doped flower-shaped ZnO crystallites under visible light irradiation. Colloids Surf A Physicochem Eng Asp. 2016;506:519–25. https://doi.org/10.1016/j.colsurfa.2016.07.016.
42. Jesionowski T. Zinc oxide—from synthesis to application: a review. Materials. 2014;7(4):2833–81.
43. Jin T, Sun D, Su JY, Zhang H, Sue HJ. Antimicrobial efficacy of zinc oxide quantum dots against Listeria monocytogenes, Salmonella Enteritidis, and *Escherichia coli* O157:H7. J Food Sci. 2009;74:M46–52.
44. Jose M. Surface diffusion and coalescence of mobile metal nanoparticles. J Phys Chem B. 2005;109:9703–11.
45. Joshaghani HR, Eskandari M. Antifungal effect of Sodium Dodecil Sulfate and Nano particle ZnO on growth inhibition of standard strain of *Candida albicans*. J Gorgan Univ Med Sci. 2011;12:2011.
46. Kairyte K, Kadys A, Luksiene Z. Antibacterial and antifungal activity of photoactivated ZnO nanoparticles in suspension. J Photochem Photobiol B Biol. 2013;128:78–84. https://doi.org/10.1016/j.jphotobiol.2013.07.017.
47. Kalia A, Kaur J, Kaur A, Singh N. Antimycotic activity of biogenically synthesised metal and metal oxide nanoparticles against plant pathogenic fungus *Fusarium moniliforme* (*F. fujikuroi*). Indian J Exp Biol. 2020;58:263–70.
48. Kao Y, Chen Y, Cheng T, Chiung Y, Liu P. Zinc oxide nanoparticles interfere with zinc ion homeostasis to cause cytotoxicity. Toxicol Sci. 2012;125:462–72.
49. Katiyar A, Kumar N, Srivastava A. Optical properties of ZnO nanoparticles synthesized by co-precipitation method using LiOH. Mater Today Proc. 2018;5:9144–7. https://doi.org/10.1016/j.matpr.2017.10.034.
50. Khalid M, Ur-Rahman S, Hassani D, Hayat K, Zhou P, Hui N. Advances in fungal-assisted phytoremediation of heavy metals: a review. Pedosphere. 2021;31:475–95. https://doi.org/10.1016/S1002-0160(20)60091-1.
51. Kischkel B, Rossi SA, Santos SR, Nosanchuk JD, Travassos LR, Taborda CP. Therapies and vaccines based on nanoparticles for the treatment of systemic fungal infections. Front Cell Infect Microbiol. 2020;10:463.
52. Klink MJ, Laloo N, Taka A, Pakade V, Monapathi M, Modise J. Synthesis, characterization and antimicrobial activity of zinc oxide nanoparticles against selected waterborne bacterial and yeast pathogens. Molecules. 2022;27:3532.
53. Kołodziejczak-Radzimska A, Jesionowski T, Krysztafkiewicz A. Obtaining zinc oxide from aqueous solutions of KOH and Zn(CH3COO)2. Physicochem Probl Miner Process. 2010;44:93–102.
54. Kong XY, Ding Y, Yang R, Wang ZL. Single-crystal nanorings formed by epitaxial self-coiling of polar nanobelts. Science (80-). 2004;303:1348–51.
55. Król-Górniak A, Rafińska K, Monedeiro F, Pomastowski P, Buszewski B. Comparison study of cytotoxicity of bare and functionalized zinc oxide nanoparticles. Int J Mol Sci. 2021;22:9529.
56. Kumar P, Kumar G, Chandra M, Ghosh S, Roos D, Swart HC. Defects induced enhancement of antifungal activities of Zn doped CuO nanostructures. Appl Surf Sci. 2021;560: 150026. https://doi.org/10.1016/j.apsusc.2021.150026.
57. Lakshmeesha TR, Murali M, Ansari MA, Udayashankar AC, Alzohairy MA, Almatroudi A, et al. Biofabrication of zinc oxide nanoparticles from Melia azedarach and its potential in controlling soybean seed-borne phytopathogenic fungi. Saudi J Biol Sci. 2020;27:1923–30. https://doi.org/10.1016/j.sjbs.2020.06.013.
58. Lamoth F, Lockhart SR, Berkow EL, Calandra T. Changes in the epidemiological landscape of invasive candidiasis. J Antimicrob Chemother. 2018;73:i4-13.
59. León-Buitimea A, Garza-Cervantes JA, Gallegos-Alvarado DY, Osorio-Concepción M, Morones-Ramírez JR. Nanomaterial-based antifungal therapies to combat fungal diseases aspergillosis, coccidioidomycosis, mucormycosis, and candidiasis. Pathogens. 2021;10:1303.
60. Levard C, Hotze EM, Lowry GV, Brown GE. Environmental transformations of silver nanoparticles: impact on stability and toxicity. Environ Sci Technol. 2012;46:6900–14.
61. Lipovsky A, Tzitrinovich Z, Friedmann H, Applerot G, Gedanken A, Lubart R, et al. EPR study of visible light-induced ROS generation by nanoparticles of ZnO. J Phys Chem C. 2009;113:15997–6001.
62. Lipovsky A, Nitzan Y, Gedanken A, Lubart R. Antifungal activity of ZnO nanoparticles-the role of ROS mediated cell injury. Nanotechnology. 2011;22:105101.
63. Mahalakshmi S, Hema N, Vijaya PP. In vitro biocompatibility and antimicrobial activities of zinc oxide nanoparticles (ZnO NPs) prepared by chemical and green synthetic route—a comparative study. Bionanoscience. 2020;10:112–21.
64. Mahamuni PP, Patil PM, Dhanavade MJ, Badiger MV. Synthesis and characterization of zinc oxide nanoparticles by using polyol chemistry for their antimicrobial and antibiofilm activity. Biochem Biophys Rep. 2019;17:71–80. https://doi.org/10.1016/j.bbrep.2018.11.007.
65. Mahamuni PP, Patil PM, Dhanavade MJ, Badiger MV, Shadija PG, Lokhande AC, et al. Synthesis and characterization of zinc oxide nanoparticles by using polyol chemistry for their antimicrobial and antibiofilm activity. Biochem Biophys Reports. 2019;17:71–80. https://doi.org/10.1016/j.bbrep.2018.11.007.
66. Mahamuni-Badiger PP, Patil PM, Badiger MV, Patel PR, Thorat-Gadgil BS, Pandit A, et al. Biofilm formation to inhibition: role of zinc oxide-based nanoparticles. Mater Sci Eng C. 2020;108:1–74. https://doi.org/10.1016/j.msec.2019.110319.
67. Mahamuni-Badiger PP, Patil PM, Patel PR, Dhanavade MJ, Badiger MV, Marathe YN, et al. Electrospun poly(3-hydroxybutyrate-co-3-hydroxyvalerate)/polyethylene oxide (PEO) microfibers reinforced with ZnO nanocrystals for antibacterial and antibiofilm wound dressing applications. New J Chem. 2020;44(23):9754–66.

68. Mamat MH, Khusaimi Z, Zahidi MM, Mahmood MR. Performance of an ultraviolet photoconductive sensor using well-aligned aluminium-doped zinc-oxide nanorod arrays annealed in an air and oxygen environment. Jpn J Appl Phys. 2011;50:06GF05. https://doi.org/10.1143/JJAP.50.06GF05.
69. Megri Y, Arastehfar A, Boekhout T, Daneshnia F, Hörtnagl C, Sartori B, et al. Candida tropicalis is the most prevalent yeast species causing candidemia in Algeria: the urgent need for antifungal stewardship and infection control measures. Antimicrob Resist Infect Control. 2020;9:1–10.
70. Miri A, Mahdinejad N, Ebrahimy O, Khatami M, Sarani M. Zinc oxide nanoparticles: biosynthesis, characterization, antifungal and cytotoxic activity. Mater Sci Eng C. 2019;104:109981.
71. Mishra A, Tripathy SK, Wahab R, Jeong SH, Hwang I, Yang YB, et al. Microbial synthesis of gold nanoparticles using the fungus *Penicillium brevicompactum* and their cytotoxic effects against mouse mayo blast cancer C 2C 12 cells. Appl Microbiol Biotechnol. 2011;92:617–30.
72. Moos PJ, Chung K, Woessner D, Honeggar M, Cutler NS, Veranth JM. ZnO particulate matter requires cell contact for toxicity in human colon cancer cells. Chem Res Toxicol. 2010;23:733–9.
73. Nair S, Sasidharan A, Divya Rani VV, Menon D, Nair S, Manzoor K, et al. Role of size scale of ZnO nanoparticles and microparticles on toxicity toward bacteria and osteoblast cancer cells. J Mater Sci Mater Med. 2009;20:235–41.
74. Najibi Ilkhechi N, Mozammel M, Yari Khosroushahi A. Antifungal effects of ZnO, TiO_2 and ZnO-TiO_2 nanostructures on *Aspergillus flavus*. Pestic Biochem Physiol. 2021;176:104.
75. Native OU, Chitosans M. Preparation and morphology studies of nano zinc oxide obtained using native and modified chitosans. Materials. 2013;6:4198–212.
76. Navale GR, Thripuranthaka M, Late DJ. Antimicrobial activity of ZnO nanoparticles against pathogenic bacteria and fungi. Sci Med Central. 2015;3:1–9.
77. Navale GR, Thripuranthaka M, Late DJ, Shinde SS. Antimicrobial activity of ZnO nanoparticles against pathogenic bacteria and fungi. JSM Nanotechnol Nanomed. 2015;3:1033.
78. Naveed Ul Haq A, Nadhman A, Ullah I, Mustafa G, Yasinzai M, Khan I. Synthesis approaches of Zinc oxide nanoparticles: a dilemma of ecotoxicity. J Nanomat. 2017;2017:1687–4110. https://doi.org/10.1155/2017/8510342.
79. Nikoobakht B, Wang X, Herzing A, Shi J. Scalable synthesis and device integration of self-registered one-dimensional zinc oxide nanostructures and related materials. Chem Soc Rev. 2013;42:342–65.
80. Novak-Frazer L, Anees-Hill SP, Hassan D, Masania R, Moore CB, Richardson MD, et al. Deciphering *Aspergillus fumigatus* cyp51A-mediated triazole resistance by pyrosequencing of respiratory specimens. J Antimicrob Chemother. 2020;75:3501–9.
81. Padmavathy N, Vijayaraghavan R. Enhanced bioactivity of ZnO nanoparticles—an antimicrobial study. Sci Technol Adv Mater. 2008;9:035004. https://doi.org/10.1088/1468-6996/9/3/035004.
82. Padmavathy N, Vijayaraghavan R. Enhanced bioactivity of ZnO nanoparticles - An antimicrobial study. Sci Technol Adv Mater. 2008;9.9(3):1–7.
83. Pariona N, Paraguay-Delgado F, Basurto-Cereceda S, Morales-Mendoza JE, Hermida-Montero LA, Mtz-Enriquez AI. Shape-dependent antifungal activity of ZnO particles against phytopathogenic fungi. Appl Nanosci. 2020;10:435–43. https://doi.org/10.1007/s13204-019-01127-w.
84. Patil PM, Mahamuni PP, Shadija PG, Bohara RA. Conversion of organic biomedical waste into value added product using green approach. Environ Sci Pollut Res. 2019;26:6696–705.
85. Phiwdang K, Phensaijai M, Pecharapa W. Study of antifungal activities of CuO/ZnO nanocomposites synthesized by co-precipitation method. Adv Mater Res. 2013;802:89–93.
86. Pillai AM, Sivasankarapillai VS, Rahdar A, Joseph J, Sadeghfar F, Anuf AR, et al. Green synthesis and characterization of zinc oxide nanoparticles with antibacterial and antifungal activity. J Mol Struct. 2020;1211: 128107. https://doi.org/10.1016/j.molstruc.2020.128107.
87. Polshettiwar V, Baruwati B, Varma RS. Self-Assembly of Metal Oxides into Synthesis and Application in Catalysis. ACS Nono. 2009;3:728–36.
88. Pragathiswaran C, Smitha C, Barabadi H, Al-Ansari MM, Al-Humaid LA, Saravanan M. TiO2@ZnO nanocomposites decorated with gold nanoparticles: Synthesis, characterization and their antifungal, antibacterial, anti-inflammatory and anticancer activities. Inorg Chem Commun. 2020;121: 108210. https://doi.org/10.1016/j.inoche.2020.108210.
89. Prasanth R, Gopinath D. Effect of ZnO nanoparticles on nasopharyngeal cancer cells viability and respiration. Appl Phys Lett. 2013;102:113702.
90. Purwaningsih SY, Pratapa S, Triwikantoro, Darminto. Synthesis of nano-sized ZnO particles by co-precipitation method with variation of heating time. AIP Conf Proc. 2016;1708:1–7.
91. Raghupathi KR, Koodali RT, Manna AC. Size-dependent bacterial growth inhibition and mechanism of antibacterial activity of zinc oxide nanoparticles. Langmuir. 2011;27:4020–8.
92. Raha S, Ahmaruzzaman M. ZnO nanostructured materials and their potential applications: progress, challenges and perspectives. Nanoscale Adv. 2022;4:1868–925.
93. Ramasamy M, Lee J. Recent nanotechnology approaches for prevention and treatment of biofilm-associated infections on medical devices. Biomed Res Int. 2016;2016:1–17.
94. Schraufnagel DE. The health effects of ultrafine particles. Exp Mol Med. 2020;52:311–7.
95. Schwartz VB, Thétiot F, Ritz S, Pütz S, Choritz L, Lappas A, et al. Antibacterial surface coatings from zinc oxide nanoparticles embedded in poly (N-isopropylacrylamide) hydrogel surface layers. Adv Funct mater. 2012;22(11):2376–86.
96. Sharma RK, Ghose R. Synthesis of zinc oxide nanoparticles by homogeneous precipitation method and its application in antifungal activity against *Candida albicans*. Ceram Int. 2014;41:1–9. https://doi.org/10.1016/j.ceramint.2014.09.016.
97. Sharma V, Shukla RK, Saxena N, Parmar D, Das M, Dhawan A. DNA damaging potential of zinc oxide nanoparticles in human epidermal cells. Toxicol Lett. 2009;185:211–8.
98. Singh PK, Iqubal MK, Shukla VK, Shuaib M. Microemulsions: current trends in novel drug delivery systems. J Pharm Chem Biol Sci. 2014;1:39–51.
99. Singh S, Singh SK, Chowdhury I, Singh R. Understanding the mechanism of bacterial biofilms resistance to antimicrobial agents. Open Microbiol J. 2017;11:53–62. https://doi.org/10.2174/1874285801711010053.
100. Song W, Zhang J, Guo J, Zhang J, Ding F, Li L, et al. Role of the dissolved zinc ion and reactive oxygen species in cytotoxicity of ZnO nanoparticles. Toxicol Lett. 2010;199:389–97. https://doi.org/10.1016/j.toxlet.2010.10.003.
101. Sonia S, Kumari HLJ, Ruckmani K, Sivakumar M. Antimicrobial and antioxidant potentials of biosynthesized colloidal zinc oxide nanoparticles for a fortified cold cream formulation: a potent nanocosmeceutical application. Mater Sci Eng C. 2017. https://doi.org/10.1016/j.msec.2017.05.059.
102. Sousa F, Ferreira D, Reis S, Costa P. Current insights on antifungal therapy: novel nanotechnology approaches for drug delivery systems and new drugs from natural sources. Pharmaceuticals. 2020;13:1–30.
103. Sun Q, Li J, Le T. Zinc oxide nanoparticle as a novel class of antifungal agents: current advances and future perspectives. J Agric Food Chem. 2018;66:11209–20.

104. Tanwar J, Das S, Fatima Z, Hameed S. Multidrug resistance: an emerging crisis. Interdiscip Perspect Infect Dis. 2014;2014:541340.
105. Tien LC, Pearton SJ, Norton DP, Ren F. Synthesis and microstructure of vertically aligned ZnO nanowires grown by high-pressure-assisted pulsed-laser deposition. J Mater Sci. 2008;43:6925–32.
106. Tiwari N, Pandit R, Gaikwad S, Gade A, Rai M. Biosynthesis of zinc oxide nanoparticles by petals extract of *Rosa indica* L., its formulation as nail paint and evaluation of antifungal activity against fungi causing onychomycosis. IET Nanobiotechnol. 2016;11(2):205–11.
107. Uskokovi D. Influence of size scale and morphology on antibacterial properties of ZnO powders hydrothemally synthesized using different surface stabilizing agents. Colloids Surf B Biointerfaces. 2013;102:21–8.
108. Van Loosdrecht MCM, Rittmann BE, Boltz JP, Daigger GT, Smets BF, Morgenroth E. From biofilm ecology to reactors: a focused review. Water Sci Technol. 2017;75:1753–60.
109. Vázquez K, Vanegas P, Cruzat C, Novoa N, Arrué R, Vanegas E. Antibacterial and antifungal properties of electrospun recycled pet polymeric fibers functionalized with zinc oxide nanoparticles. Polymers (Basel). 2021;13(21):3763.
110. Wahab R, Ansari SG, Kim Y, Seo H, Shin H. Room temperature synthesis of needle-shaped ZnO nanorods via sonochemical method. Appl Surf Sci. 2007;253:7622–6.
111. Wang L, Hu C, Shao L. The antimicrobial activity of nanoparticles: present situation and prospects for the future. Int J Nanomedicine. 2017;12:1227–49. https://doi.org/10.2147/IJN.S121956.
112. Wani AH, Shah MA. A unique and profound effect of MgO and ZnO nanoparticles on some plant pathogenic fungi. J Appl Pharm Sci. 2012;2:40–4.
113. Weldegebrieal GK. Synthesis method, antibacterial and photocatalytic activity of ZnO nanoparticles for azo dyes in wastewater treatment: a review. Inorg Chem Commun. 2020;120: 108140. https://doi.org/10.1016/j.inoche.2020.108140.
114. Wu JJ, Liu SC, Wu CT, Chen KH, Chen LC. Heterostructures of ZnO-Zn coaxial nanocables and ZnO nanotubes. Appl Phys Lett. 2002;81:1312–4.
115. Yang Y, Zhang C, Li K, Li Z. Fe2+ alleviated the toxicity of zno nanoparticles to pseudomonas tolaasii y-11 by changing nanoparticles behavior in solution. Microorganisms. 2021;9(11):2189.
116. Zanni E, Bruni E, Chandraiahgari CR, De BG, Santangelo MG, Leone M, et al. Evaluation of the antibacterial power and biocompatibility of zinc oxide nanorods decorated graphene nanoplatelets: new perspectives for antibiodeteriorative approaches. J Nanobiotechnol. 2017;2017:1–12.
117. Zhai B, Ola M, Rolling T, Tosini NL, Joshowitz S, Littmann ER, et al. High-resolution mycobiota analysis reveals dynamic intestinal translocation preceding invasive candidiasis. Nat Med. 2020;26:59–64. https://doi.org/10.1038/s41591-019-0709-7.
118. Zhang G, Xiao Y, Yan J, Xie N, Liu R, Zhang Y. Ultraviolet Light-degradation behavior and antibacterial activity of polypropylene/ZnO nanoparticles fibers. Polymers (Basel). 2019;11(11):1841. https://doi.org/10.3390/polym11111841.
119. Zhang J, Yang H, Zhang X, Morbidoni M, Burgess CH, Kilmurray R, et al. Effect of processing temperature on film properties of ZnO prepared by the aqueous method and related organic photovoltaics and LEDs. Inorg Chem Front. 2020;7:2809–17.
120. Zhou X, Hayat Z, Zhang D, Li M, Hu S, Wu Q, et al. Modification, and applications in food and agriculture. Processes. 2023;11(4):1193.

Authors and Affiliations

Pranjali Mahamuni-Badiger[1] · Vishrut Ghare[2] · Charushila Nikam[1] · Nilam Patil[3]

✉ Pranjali Mahamuni-Badiger
pranjalisamsung@gmail.com

[1] Rayat Shikshan Sanstha's, S. M. Joshi College, Hadapsar, Pune 411028, India

[2] Appasaheb Jedhe College, Swargate, Pune 411042, India

[3] Bharti Vidyapeeth's Dr. Patangrao Kadam Mahavidyalaya, Sangli 416416, India