

Zinc Oxide Nanoparticles: Different synthesis approaches and applications

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Abstract

Nanotechnology deals with the synthesis and usage of materials with nanoscale dimension (1-100 nm). Nanoscale dimensions of the particles provide large surface to volume ratio and thus very specific properties. Synthesis of zinc oxide nanoparticles (ZnO NPs) has gained prime importance in recent arena due to its high excitation binding energy and large bandwidth and it has potential applications like anti-diabetic, antibacterial, anti-inflammatory, antifungal, wound healing, antioxidant and optical properties. Zinc (Zn) is a common mineral element in nature which plays an immense role in many biological processes. It is defined as an essential trace element or micronutrient which is very much crucial for the normal growth and the development of all higher plants as well as animals. Zinc directly involves in enzyme function associated with the photosynthesis and energy process in plants. It also plays an important role in maintenance of membrane integrity, formation and production of growth hormone, insulin, thyroid etc. Due to the involvement of large rate of toxic chemicals and requirement of extreme environment, chemical and physical methods of nanoparticle synthesis often became inappropriate. Whereas, green methods are used in a wide range of biological samples including plants, fungus, bacteria, and algae, which act as both reducing and capping agent. Biologically synthesized zinc nanoparticles have been reported for versatile applications in the field of medicine and pharmacy, for bio-imaging and bio-sensor production, in gene therapy and drug delivery system. Zinc nanoparticles also play vital role in agricultural sector including plant growth and development, enhancement of crop yield and post-harvest processing. In spite of being great potential of ZnO NPs for abiotic and biotic stress management, research works in this field is considerably less. This review described the summary of the recent works in the synthesis mechanism, characterization techniques, and applications of biosynthesized ZnO NPs in medicine and agriculture with special reference to application on plant growth, development and abiotic stress management.

Keywords: Zinc oxide nanoparticles, Nanoparticles, Green synthesis, Plant growth, Abiotic stress

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Introduction

In modern science, one of the rapidly developing concepts in last decade is nanotechnology (Kalpana et al. 2018). The nanomaterials embrace very distinct physiochemical properties, which have the potential to develop new age technology, systems, devices, structural forms, and engineering platforms which leads to the wide-ranging variety of disciplines (Mirzaei et al. 2017; Arruda et al. 2015). Nanomaterials are the specialized nanoscale particles of size below 100 nm with improved

conductivity, nonlinear optical performance, actively catalytic and have chemical stability due to their large surface to volume ratio (Agarwal et al. 2017). Nanoparticles can be synthesized through conventional methods (physical synthesis and chemical synthesis) in efficient way but they often require stabilizing agents which may lead to toxicity in the nature and environment. To deal with the problems, incorporation of biological or green way of nanoparticles (NPs) synthesis arises which serves as an alternative environment friendly, non-toxic, economically beneficial approach. Extracts of

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biological elements especially plants act as both reducing and capping agent during reduction of metallic precursor to nano-form as a result of which involvement of external capping agent is not required in this process (Salam et al. 2014). Nanoparticles including metallic and metal oxide shows beneficial effect on health (Bhattacharya et al. 2008) having a wide range of applications such as for medical imaging (Nune et al. 2009), drug delivery (Nasimi et al. 2013), cancer therapy (Siddique and chow 2021), textile (Yetisen et al. 2016), renewable energy (Hussein 2015), environment (Martínez et al. 2021), electronics (Matsui 2005), food, agriculture (Paramo et al. 2020) etc.

Metallic nanoparticles (such as Ag, Au, Pt, Cd etc.) and metal oxide nanoparticles (such as ZnO, TiO₂, ZrO₂, CeO₂, etc.) are the general classification of nanoparticles. Among different metal oxide nanoparticles, ZnO attracts great attention due to their special physiochemical properties, along with unique shape and size (Theerthagiri et al. 2019). According to ancient literature, ZnO had been used since at least two millennia B.C. in ancient Egyptian civilization and later in Rome as ointments for the treatment against skin diseases and wounds (Frederickson et al. 2005). Currently ZnO is being used across different industries such as brass production (Habashi 2001; Biswas 1987), rubber industry (Eastaugh et al. 2008), ceramic industry (Moezzi et al. 2012), concrete manufacturing (Brown 1957), electronic devices, food and others (Borysiewicz 2019).

Several researches described ZnO as a structurally functional, strategic, promising and resourceful material with broad range of applications (Neumark and Kuskovsky 2007). ZnO NPs typically contain neutral hydroxyl groups attached to their surface, which play important role in surface attachment and structural change behaviour (Qu and Morais 1999; 2001). The isoelectric point of ZnO NPs ranges in between 9-10, which indicate ZnO NPs contain a strong positive surface charge under physiological condition. ZnO NPs have great advantages in catalytic reaction due to their large surface to volume ratio (Huang et al. 2006). ZnO is a one-dimensional nanoparticle which exhibits interesting electronic and optical properties and due to their low dimensionality, they show quantum confinement effect (Baruah and Dutta 2009). One of the interesting facts is that the cancer cells frequently contain high concentrations of negatively charged phospholipids on their outer membrane with high membrane potentiality; therefore, interaction with positively charged ZnO NPs is probably achieved by

intermingling with electrostatic interactions, thus promoting cellular nutrition uptake, phagocytosis and ultimate cytotoxicity (Abercrombie and Ambrose 1962; Bockris and Habib 1982; Papo et al. 2003). ZnO NPs exhibits piezoelectric property, which is very much advantageous for the fabrication of devices, such as electromagnetic coupled sensors and actuators (Minne et al. 1995).

Zinc serves as essential micronutrients in plant system (Marschner 1993). Zn act as cofactor of more than 300 proteins, among of which the majority are zinc finger proteins, DNA and RNA polymerases (Coleman 1998; Lopez Millan et al. 2005). Also, it is the only metal which is present in all the six class of enzyme viz. oxidoreductase, transferase, hydrolases, lyases, isomerases and ligases (Gupta et al. 2016). Zn being a part of the structural and catalytic unit regulates the activity, function, stabilization and folding of the various proteins and enzymes. It also involves in hormone regulation (in tryptophan synthesis Zn is very essential component which is a precursor of IAA) (Castillo-González et al. 2018), mitogen-activated protein kinase regulation which directly involves in signal transduction pathway (Lin et al. 2005; Hansch and Mendel 2009), control PS II complex in photo-inhibition process (Monnet et al. 2001; Bailey et al. 2002; Lu et al. 2011), and are found to involve in the Rubisco activity (Peck and McDonald 2010). Zinc has impressive role in maintenance and regulation of gene expression corresponding to environmental stress (such as high temperatures, high light intensity) tolerance in plants (Cakmak 2000).

Globally 49% of the soils are deficient of zinc making it the most noticeable micronutrient deficiency in the World (Graham 2008). Following the trend, on an average, 36.5% of the Indian soils are potentially deficient of zinc being highest than the rest of the micronutrients (Shukla et al. 2018). For restoration of this deficiency, several commercial products are used which are mainly synthetic chemical fertilizers which leads to soil mineral imbalance, abolishing soil texture and fertility and showing long-term negative effect in our ecosystem (Elemike et al. 2019). To overcome this problem, supplementation of biofertilizers might play important role in the improvement of crop production and soil fertility (Bhardwaj et al. 2014), but large-scale industrial production of biofertilizers and their appropriate usage are not easy. To deal with it, nanotechnology generated nano-fertilizer arises which serves as the latest technology in precision agriculture. Specifically, use of zinc oxide nanoparticles can able to restore the zinc deficiency

in soil and also can able to enhance plant growth and development (Prasad et al. 2012; Laware et al. 2014; Singh et al. 2016; Elizabeth et al. 2017). Also zinc oxide nanoparticles could be used in management of several abiotic stresses viz. drought stress (Taran et al. 2017; Hassan et al. 2020; Sun et al. 2020) and salt stress (Sanaciostovar et al. 2012; Soliman et al. 2015; Alharby et al. 2016). This present review describes the synthesis of zinc oxide nanoparticles, its characterization, its application in plant growth and development and abiotic stress management with detailed description of uptake, translocation and accumulation of zinc in the plant system.

Synthesis methods of zinc oxide nanoparticles

There are mainly three strategies for synthesis of nanoparticles viz. (a) Physical synthesis, (b) Chemical synthesis and (c) Biological synthesis (Dhand et al. 2015) (Figure 2). Among these Physical and Chemical way of NPs synthesis is most conventional one but it possesses certain level of toxic substances (Li et al. 2011). On the other hand, biological or green way of synthesis is quite advanced with special properties such as cost effectiveness, eco-friendly, easily available and less-toxic in nature (Ingale et al. 2013).

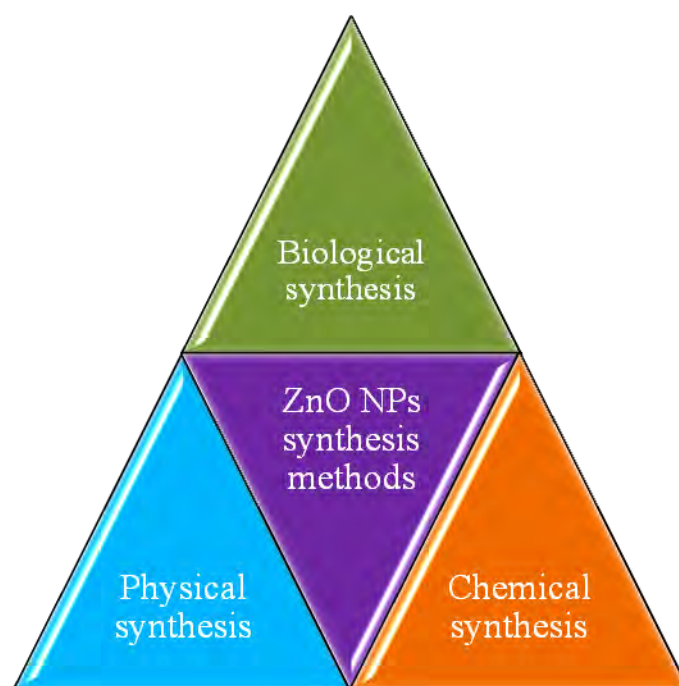


Fig. 1 Different strategies of zinc oxide nanoparticles synthesis

Physical method of zinc oxide nanoparticles synthesis

In this process, the physical forces are used as an external agent for the formation of stable and distinctly shaped nanoparticles, e.g., colloidal dispersion, physical fragmentation, vapour condensation, etc. (Krupa 2016). In case of ZnO NPs synthesis, plasma method, laser ablation method and thermal evaporation are the most commonly used physical strategies (Brintha and Ajitha 2015). Laser ablation method offers some unique benefits because it allows properly sized, shaped and well-purified ZnO nanoparticles (Ma et al. 2013), where the ablation time and wavelength are important factor for ZnO NPs synthesis (Manzoor et al. 2015). In

thermal evaporation method powder material are vaporized at very high temperature and the vapour phase condensed under high pressure to obtain nanoparticles (Happy et al. 2017). ZnO nanoparticles synthesized through physical method shows a strong potential photo-catalytic activity which is very much beneficial for photo-degradation (Parita Basnet et al. 2018). Physical methods of nanoparticles synthesis have some limitations because of the large expensive equipments with high pressure and temperature, also this process often leads to irregular particle formation (Chandrasekaran et al. 2016).

Chemical methods of zinc oxide nanoparticles synthesis

In chemical method of nanoparticles synthesis, one or more chemical reactions are carried out through which the final product of nanoparticles synthesized. Chemical synthesis methods involve two major phases i.e., gas phase and liquid phase. The gas phase is divided into pyrolysis and gas condensation method and liquid phase is sub-divided into precipitation method, hydrothermal method, colloidal method, sol-gel method and oil emulsion method. (Naveed UIHaq et al. 2017). During ZnO NPs certain stabilizers (citrate or polyvinyl pyrrolidone) are used to control the morphology and avoid agglomeration of ZnO NPs (Naveed et al. 2017). However, the chemical method of synthesis has some sort of drawbacks because it requires high energy, toxic chemicals, highly expensive equipments and several researches showed that there are certain traces of toxic reagents in the chemically synthesized zinc nanoparticles which could be hazardous for application in several sectors (Anshuman et al. 2014).

Biological method of zinc oxide nanoparticles synthesis

Biological synthesis or green synthesis approaches

provide an environment friendly, low-toxic, cost effective and efficient protocol to synthesize and fabricate the NPs. These methods employed several biological elements viz. bacteria (Klaus et al. 1999; Rohet al. 2001; Nair et al. 2002; Yong et al. 2002; Husseiny et al. 2007), fungi (Mukherjee et al. 2001a; Mukherjee et al. 2001b; Ahmad et al. 2005), yeast (Kowshik et al. 2003), actinomycetes (Ahmad et al., 2003a; Ahmad et al. 2003b; Sastry et al. 2003), virus (Shenton et al. 1999; Lee et al. 2002; Merzlyak et al. 2006) and plant extracts for the production of ZnO nanoparticles. Despite of the advantages of using microorganisms as reducing agent in synthesis of ZnO NPs, it also may contain some number of toxic substances which directly or indirectly affect the quality of ZnO NPs (Guldiken et al. 2018). Whereas, plants contain several types of phytochemicals or secondary metabolites such as methyl xanthenes, phenolic acids, tannins, alkaloids, flavonoids etc. have been reported as very good reducing agents of metallic precursors (Altemimi et al. 2017). The use of plant sample has some benefits over the others such as it is safe, cost-effective, eco-friendly, non-hazardous, easy to handle and synthesis (Abdul et al. 2014; Folorunso et al. 2019; Akintelu and Folorunso 2019a; Akintelu and Folorunso 2019b) (Figure 3).

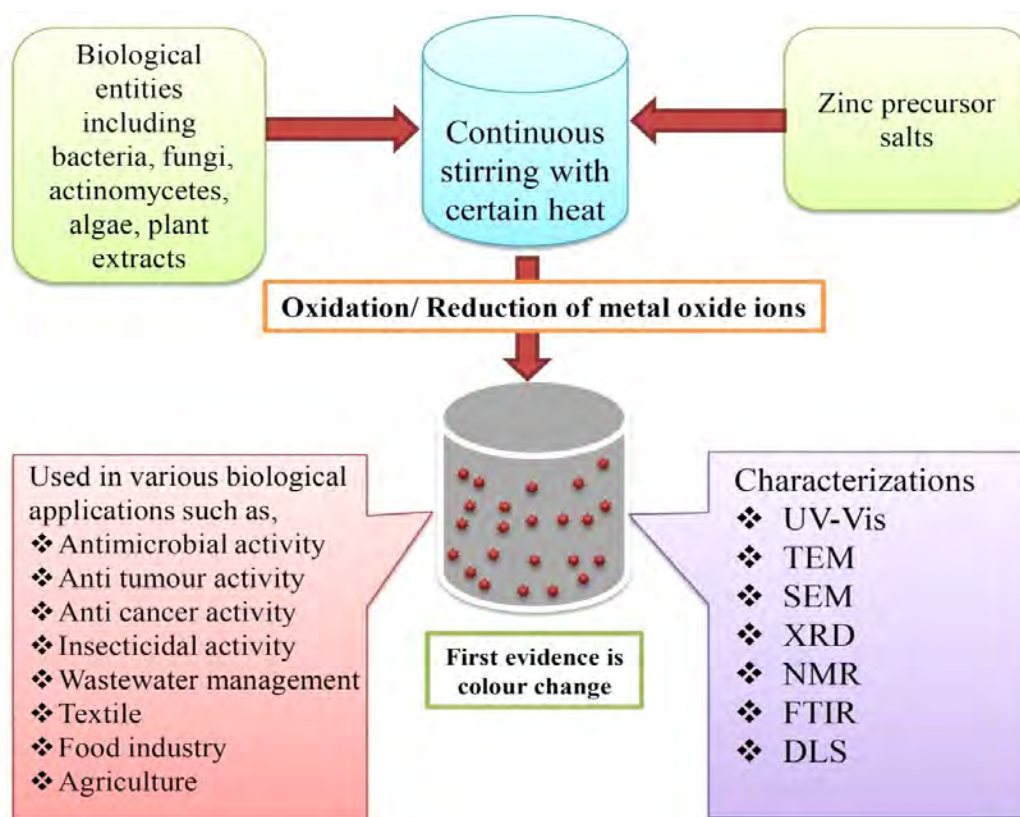


Fig. 2 Flowchart represents the green synthesis of zinc oxide nanoparticles, their potential use and characterization methods

Bacteria mediated synthesis of zinc oxide nanoparticles

ZnO NPs synthesis using bacterial species gain more attention in past recent years. ZnO nanoparticles were synthesised by *Bacillus licheniformis* through an environment friendly approach showed photo-

catalytic and dye degradation activity (Raliya and Tarafdar 2013). Tripathi et al. (2014) describes synthesis of ZnO nanoparticles by using *Rhodococcus pyridinivorans* bacteria. Some study shows the usage of *Aeromonas hydrophila* as reducing agent for synthesis of ZnO NPs (Mehta et al. 2009) (Table 1).

Table 1 Bacterial specimens involved in the synthesis of zinc oxide nanoparticles

Sl. No.	Bacteria Name	Family	Size and Morphology	Application	References
1	<i>Aeromonas hydrophila</i>	Aeromonadaceae	57-72 nm, Spherical	Antibacterial and Antifungal activity	Jayaseelana et al., 2012
2	<i>Bacillus megaterium</i>	Bacillaceae	45-95 nm	Antimicrobial activity	Saravanan et al., 2018
3	<i>Halomonas elongata</i> IBRC-M 10214	Halomonadaceae	18.11 nm	Antimicrobial activity	Taran et al., 2018
4	<i>Sphingobacterium thalpophilum</i>	Sphingobacteriaceae	40 nm	Antimicrobial activity	Rajabairavi et al., 2017
5	<i>Staphylococcus aureus</i>	Staphylococcaceae	10-50 nm	Antimicrobial activity	Rauf et al., 2017

Fungus and yeast mediated synthesis of zinc oxide nanoparticles

Synthesis of ZnO nanoparticles through fungus facilitates large-scale production, highly precise size and shaped NPs (Azizi et al. 2014). Sometimes fungal strains are chosen over bacterial strains because fungal cells contain metal bioaccumulation property and high tolerance (Pati et al. 2014). Some yeast species were also found to be involved in the process of ZnO NPs synthesis (Moghaddam et al. 2017) (Table 2).

Algae mediated synthesis of ZnO nanoparticles

Algae are chlorophyll containing organisms ranges from unicellular to multicellular in nature. Several microalgae draw special attraction because of its capability to degrade toxic metals and also have the capacity to convert them into smaller amount toxic forms (Bird et al. 2015). *Sargassum muticum* and *S. myriocystum* are reported for their role used in ZnO nanoparticles synthesis (Rajiv et al. 2013) (Table 3).

Plant mediated synthesis of ZnO nanoparticles

Plant mediated synthesis of nanoparticles is also termed as ‘Green Synthesis’ (Shankar et al. 2004). Plants contain a large number of metabolites though their potential impact on synthesis of NPs not fully known and utilized. Using *Vitex negundo* plant extract as reducing agent, Ambika and Sundarajan (2015) reduces zinc nitrate into nano-form which shows antimicrobial activity against *Escherichia coli* and *Staphylococcus aureus*. Kalpana et al. (2017) describes synthesis of ZnO nanoparticles by using *Lagenaria siceraria* pulp extract, its antimicrobial, anti-dandruff and anti-arthritis efficiency. Demissie et al. (2020) reported synthesis of ZnO nanoparticle through *Lippiaadoensis* (Koseret) leaf extract and evaluate its antibacterial activity. Fakhari et al. (2019) used *Laurusnobilis* L. plant extract with zinc acetate and zinc nitrate as Zn precursor. *Cassia fistula* and *Melia azadarach* leaf extracts are used for successful synthesis of ZnO NPs that shows strong antibacterial activity (Naseer et al. 2020) (Table 4).

Table 2 Fungus and yeast mediated synthesis of zinc oxide nanoparticles

Sl. No.	Fungus Name	Family	Size and Morphology	Application	References
1	<i>Aspergillus niger</i>	Trichocomaceae	53-69 nm	Dye degradation and antibacterial activity	Kalpana et al., 2018
2	<i>Candida albicans</i>	Saccharomycetaceae	25 nm	Synthesis of steroidal pyridines	Mashrai et al., 2017
3	<i>Fusarium keratoplasticum</i> A1-3	Nectriaceae	10-42 nm	Antibacterial, cytotoxic activity and loaded on textile	Mohamed et al., 2019
4	<i>Aspergillus niger</i> G3-1	Trichocomaceae	8-38 nm	Antibacterial cytotoxic activity and loaded on textile	Mohamed et al., 2020
5	<i>Aspergillus terreus</i>	Trichocomaceae	10-45 nm	Antibacterial cytotoxic activity, loaded on textile, UV protection	Fouda et al., 2018
6	<i>Pichia kudriazevii</i>	Saccharomycetaceae	10-61 nm	Antibacterial & Antioxidant activities	Moghaddam et al., 2017
7	<i>Aspergillus terreus</i>	Trichocomaceae	8 nm, Spherical	Catalysts, bio sensing, drug delivery, solar cell, cell-labelling, optoelectronics and imaging	Raliya and Tarafdar, 2014
8	<i>Pichia kudriavzevii</i>	Saccharomycetaceae	10-61 nm	Antimicrobial and antioxidant activity	Moghaddam et al., 2017

Table 3 Algae reported for synthesis of zinc oxide nanoparticles

Sl. No.	Algae Name	Family	Size and Morphology	Applications	Reference
1	<i>Chlorella</i> extract	Chlorellaceae	20±2.2 nm	Photocatalytic activity	Khalafi et al., 2019
2	<i>Sargassum muticum</i>	Sargassaceae	30-57 nm	Supplemental drug in cancer treatments	Sanacimehr et al., 2018
3	<i>Chlamydomonas reinhardtii</i>	Chlamydomonadaceae	55-80 nm	Photocatalytic activity	Rao and Gautam, 2016
4	<i>Sargassum muticum</i>	Sargassaceae	30-57 nm	One-pot method for synthesis	Azizi et al., 2014

Table 4 Plant reported for synthesis of zinc oxide nanoparticles

Sl. No.	Plant Name	Family	Size and Morphology	Applications	Reference
1	<i>Acalypha fruticosa</i>	Euphorbiaceae	50 nm; Spherical, hexagonal	Antimicrobial activity	Vijayakumar et al. 2020
2	<i>Allium cepa</i> (bulb), <i>Allium sativum</i> (bulb), <i>Petroselinum crispum</i> (leaves)	<i>Amaryllidaceae</i> <i>Apiaceae</i>	70 nm, Hexagonal wurtzite	Photodegradation of methylene blue	Stan et al. 2015
3	<i>Aloe socotrina</i>	Asphodelaceae	15-50 nm	Used in Drug delivery	Fahimmunisha et al. 2020
4	<i>Anisochilus carnosus</i>	<i>Lamiaceae</i>	20-40 nm, Hexagonal wurtzite	Antimicrobial activity	Anbuvaran et al. 2015a
5	<i>Artocarpus gomezianus</i>	Moraceae	30-40 nm	Cytotoxicity, antibacterial, and antifungal activities	Anitha et al. 2018
6	<i>Artocarpus gomezianus</i>	Moraceae	30-50 nm	Active against urinary tract infection pathogen	Santhosh kumar et al. 2017
7	<i>Calliandra haematocephala</i>	Fabaceae	19.45 nm	Photocatalytic dye degradation	Vinayagam et al. 2020
8	<i>Calotropis gigantea</i>	Apocynaceae	31 nm; Hexagonal and pyramidal	Nitrite sensing, photocatalytic, and antibacterial activities	Kumar et al. 2020
9	<i>Celosia argentea</i>	<i>Amaranthaceae</i>	25 nm, Spherical	Antibacterial activity, drug delivery	Vaishnav et al. 2017
10	<i>Ceropegia candelabrum</i>	<i>Apocynaceae</i>	12-35 nm, Hexagonal	Antibacterial activity	Murali et al. 2017
11	<i>Couroupita guianensis</i>	<i>Lecythidaceae</i>	57 nm, Hexagonal	Antimicrobial activity	Sathish kumaret al. 2017
12	<i>Euphorbia Jatropa</i>	<i>Euphorbiaceae</i>	15 nm, Hexagonal	As semiconductor	Geetha et al. 2016
13	<i>Jacaranda mimosifolia</i> (flower)	<i>Bignoniaceae</i>	2-4 nm, Hexagonal	Antimicrobial activity	Sharma et al. 2016
14	<i>Limonia acidissima</i>	<i>Rutaceae</i>	12–53 nm, Spherical	Shows antibacterial activity	Patil and Taranath 2016
15	<i>Olea europaea</i> (leaf extract)	Oleaceae	40.5-124 nm	Antibacterial activity	Ogunyemi et al. 2019

16	<i>Parthenium hysterophorus</i>	Asteraceae	27-84 nm, Spherical and hexagonal	Antifungal activity	Rajiv et al. 2013
17	<i>Phyllanthus niruri</i>	Phyllanthaceae	25.61 nm, Quasi spherical	Catalytic activity	Anbuvaran et al. 2015
18	<i>Prosopis juliflora</i>	Fabaceae	31.80-32.39 nm, Irregular	Degradation of methylene blue dye	Sheik Mydeen et al. 2020
19	<i>Rhamnus virgata</i>	Rhamnaceae	20 nm	Cytotoxic, antimicrobial, and antioxidant activities	Iqbal et al. 2019
20	<i>Sedum alfredii</i>	Crassulaceae	53.7 nm; Hexagonal and pseudo-spherical	As nano-electronics	Qu et al. 2011
21	<i>Solanum nigrum</i>	Solanaceae	29 nm, Quasi spherical	Antimicrobial activity	Ramesh et al. 2015
22	<i>Tecoma castanifolia</i>	Bignoniaceae	70-75 nm	Antioxidant, bactericidal, and anticancer activities	Sharmila et al. 2019
23	<i>Urtica dioica</i>	Urticaceae	20-22 nm, Spherical	Antidiabetic activity	Bayrami et al. 2020

Characterization of zinc oxide nanoparticles

UV-Visible Spectrophotometry

UV-Vis spectroscopy is most common instrument used to characterize a newly formed nanoparticles by scanning the synthesized nanoparticles with electromagnetic wave around 200-700 nm (Jamdagni et al. 2016). Distinct peak showed by the formed particles is specific to each nanoparticle. Fakhari et al. (2019) observed characteristic peak around 350 nm for ZnO NPs. This kind of results satisfy the standard pattern of ZnO nanoparticles because all metal oxides show wide band gaps and shorter wavelengths, also if the materials are in nanosize, the wavelengths get more shorter (Naseer et al. 2020). Santoshkumar et al. (2017) reported absorption spectra of newly synthesised ZnO NPs within the wavelength range of 300-500 nm. Karthik et al. (2017) reported absorption peaks ranges between 200-400 nm and also describes amount of plant extract and other external entities affecting the band gap and particle size.

Scanning Electron Microscopy (SEM)

In SEM, high powered electron beam is used for obtaining the data regarding the structure and

morphology in micro and nano scale materials (Alejandro et al. 2019). Depending on the high magnification, large field depth and electron density of the surface, SEM images are very relevant for topological assessment of ZnO nanoparticles (Mona et al. 2018). Raut et al. (2015) reported synthesis of hexagonal ZnO nanoparticles using *OcimumTenuiflorum* leaves extract with a size range of 11-25 nm.

Transmission Electron Microscopy (TEM)

TEM characterization is based on the interaction between the highly sophisticated high density electron beam and nano material (Saha et al. 2018). The interaction between the sample and electron beam produces an image by which the size and morphology of nanoparticles can be determined (Ambika et al. 2015; Aljabali et al. 2018). Demissie et al. (2020) reported spherical shaped agglomerated ZnO NPs as observed through TEM. Suresh et al. (2018) reported synthesis ZnO particles using *Costus pictus* D. Don showed variable morphology (hexagonal and spherical in shape). Thi et al. (2020) synthesized ZnO NPs at various temperatures between 400°C and 700°C and showed that with increasing temperature, size of the formed nanoparticles gradually increased.

Fourier Transform Infrared Spectroscopy (FTIR)

FTIR was used to examine the surface adsorption of functional groups on biosynthesised ZnO nanoparticles which help for identification of functional groups which are directly or indirectly responsible for the reduction process (Esrat et al. 2018). Earlier research shows that FTIR describes the effectiveness of biological entities in the process of synthesis of ZnO nanoparticles; thus, FTIR characterization unravels the specific role of various biomolecules in formation of ZnO nanoparticles (Feng et al. 2018). Through FTIR evaluation, Fakhari et al. (2019) established the involvement of different phytochemicals like alcohols, phenols, amines, carboxylic acids in ZnO NPs formation and surface stabilization. Happy et al. (2019) reported that phenolic groups, amines, ether carboxylic acid and hydroxyl group act as perfect capping agent for formation of ZnO NPs and are found obtain strong band at 3377.36 for O-H of phenolic group, 1587.42 for C=C stretching of alkyl ethers, 1419.61 for N=O bend of secondary amines, band at 1004.91 for C-O stretch of ether, and 476.42 cm^{-1} band for carboxylic acid and ZnO stretching.

Energy Dispersive X-ray Spectroscopy (EDX)

EDX is very suitable method for analysis of composition of ZnO nanoparticles. For confirming the components, EDX analysis is playing a very prominent role because each element has unique structural properties which lead to distinct peaks on the X-ray spectrum (Taziwa et al. 2017). The purity of synthesised ZnO nanoparticles can be detected through this EDX method (Bala et al. 2015). Nagarajan et al. (2013) prepared ZnO nanoparticles by using seaweeds showed an elemental composition of zinc (52%) and oxygen (48%).

Whereas, Abdul Salam et al. (2014) reported the presence of 79.33% zinc and 20.67% oxygen in formed ZnO nanoparticles synthesized using *Ocimum basilicum L. var. purpurascens* Benth plant extracts.

X-Ray Diffraction (XRD)

XRD technique is used to determine the crystalline nature of a material (Agarwal et al. 2019). In this process, ZnO nanoparticles are exposed to high energy electron beam which absorbed and diffracted from the nanoparticles surface thereby providing useful data about the structure and morphology (Aldabahi et al. 2020). Debye-Scherrer's equation is used for determination of crystalline structure of ZnO nanoparticles (Rabiei et al. 2020) which is: $D = \frac{k \lambda}{\beta \cos \theta}$, where K=Scherer constant (0.9), λ = X-ray wavelength, β = width at half maximum of the diffraction peak, θ = measured Bragg angle and D= structure and size of ZnO nanoparticles. Generally biosynthesised ZnO NPs showed diffraction peaks at 31.61°, 34.26°, 47.37°, 56.40°, 62.68° and 67.72 with corresponds to 100, 002, 101, 102, 110, 103, and 112 reflection planes indicating the purity and crystalline nature of ZnO nanoparticles (Pelicano et al. 2017).

Application of Zinc nanoparticles

ZnO nanoparticles gain prime attention due to their unique properties and versatile application from electric sensors to cancer treatment (Sabir et al. 2014) (Figure 1 & 4). ZnO nanoparticles are generally non-toxic materials, and it can be used in photo-catalytic degradation, substantial materials of environments pollutants (Ryu et al. 2003). Several applications of ZnO nanoparticles are described as follows:

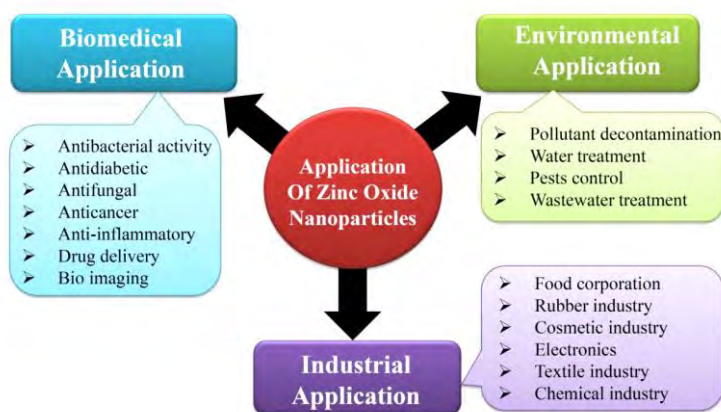


Fig. 3 Major applications of zinc oxide nanoparticles



Fig. 4 Various applications of zinc oxide nanoparticles

Anticancer activity

Several scientist including Ruenraroengsak et al. (2019), Mahdizadeh et al. (2019), and Hu et al. (2019) reported effectiveness of ZnO nanoparticles against MCF-7 cell lines. Subramaniam et al. (2019) reported effectiveness and use of ZnO nanoparticles against colon cancer. Akintelu and Folorunso, (2020) described that ZnO nanoparticles are able to induce the selective toxicity against only cancerous cells without damaging normal cells.

Antioxidant activity

Antioxidant effect of ZnO nanoparticles is highly reported by several researchers throughout the world. Umar et al. (2019) reported the antioxidant properties of ZnO nanoparticles. Another study showed antioxidant effects of ZnO nanoparticles in monosodium glutamate (MSG)-treated rats (El-Shenawy et al. 2019). Reason behind the antioxidant property of ZnO NPs is electro density to the oxygen molecule (Stan et al. 2016).

Antimicrobial activity

ZnO nanoparticles show strong antimicrobial activity against a wide range of microbes (Akbar et al. 2019). Antimicrobial properties of ZnO

nanoparticles showed promising results against several bacterial strains such as *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas fluorescens*, *Staphylococcus aureus*, and *Salmonella typhimurium* and several fungal species viz. *Aspergillus flavus* and *Aspergillus fumigatus* (Keerthana and Kumar 2020).

Antidiabetic activity

ZnO nanoparticles show remarkable antidiabetic activity especially in glucose tolerance, improvement and reduction of sugar level, increase insulin level, certain level of reduction in triglyceride levels (Raguraman et al. 2020). Siddiqui et al. (2020) reported very promising results on zinc nanoparticles efficiency against diabetes.

Role of ZnO NPs in plant growth and development

Agriculture is the back bone of any developing economy but in present day it's facing various global challenges such as irregular rainfall, increasing temperature, shortage of agricultural land, pollution, high food demand in comparison to the production etc. It was estimated that world population will be hit 9 billion by the year 2050, so certain efficient

technologies must have to introduce to develop agricultural sector (Chen and Yada 2011). Continuous use of antibiotics and fungicides in the agricultural sectors leads to the development of multi-drug resistant microbial strains (Sirelkhatim et al. 2015). The application of ZnO nanoparticles works very efficiently against fungi and other microbial infections in both farm animals and crop plants. Application of ZnO NPs on *Arachis hypogea* plant showed increased seed germination; fast stem growth, and enhanced vigour index of the seedlings (Prasad et al. 2012). Another study on *Solanum lycopersicum* showed efficacy ZnO NPs in plant growth improvement, seed germination rate enhancement as well as protein content increment (Singh et al. 2016). Sun et al. (2020) described physiological, transcriptome and metabolic analysis on tomato plant treated with ZnO nanoparticles and reported that ZnO nanoparticles reduced Fe deficiency, minimized oxidative stress and improved nutrient element contents in tomato plant. Some other experiments of ZnO NPs on carrot (*Daucus carota* L.) plants showed positive result on plant growth and yield (Elizabeth et al. 2017). Another experiment on ZnO NPs treated tomato plant showed improved antioxidant system and increment in the rate of proline accumulations that provide plants' stability and improved the photosynthetic efficiency (Faizan et al. 2017). Upadhyaya et al. (2017) reported that ZnO NPs protects rice plants from ROS damage by improving the antioxidant enzyme activity during the germination. ZnO NPs treatments on *Allium cepa* L. showed better growth, quick flower appearance than the control plant (Laware et al. 2014). Taheri et al. (2015) highlighted that ZnO nanoparticles treated corn (SC704) plants exhibited improved corn growth and yield in poor fertile soil. Phycocyanin coated ZnO nanoparticles treatment on *Gossypium hirsutum* L. plant showed an increase in growth and total biomass (Venkatachalam et al. 2017). Yusefi-Tanha et al. (2020) applied different concentrations of ZnO nanoparticles on soil-grown soybean (*Glycine max* cv. *Kowsar*) and reported improvement in antioxidant enzyme system and yield.

Effect of ZnO NPs in plant abiotic stress management

ZnO NPs play important role for minimizing the harmful effect of ROS in cell organelles of plant system and trigger various defence systems by activating cell signalling cascades and by activating or deactivating certain genes (Hancock et al. 2001). ZnO NPs are also very much effective against

various abiotic stresses by increasing the activities of several antioxidant enzymes and accumulating osmolytes along with amino acid residues and nutrients during stress condition (Taran et al. 2017; Venkatachalam et al. 2017; Wang et al. 2018)

Drought stress management

Drought stress in both natural and man-made conditions limits crop production and growth. To counter this drought-induced damage in plants, NPs have promising potential. Under stress condition, the stomatal movement is affected by the NPs (Faizan et al. 2020). Taran et al. (2017) reported that foliar application of ZnO NPs on wheat plants reduce the adverse effects of drought stress and improve the growth and yield. Dimkpa et al. (2019) published their intensive research on the mitigation of drought stress in *Sorghum bicolor* var. 251 plants through ZnO nanoparticles. Overall results confirmed that ZnO NP treated plants could improve grain yield (22-183%) and enhancement in nitrogen, phosphorous, potassium and zinc nutrient translocation in comparison with the non-treated plants. Another study showed that foliar application of ZnO nanoparticles on wheat plant can increase the yield and also able to mitigate water deficiency stress (Adrees et al. 2021). Dimkpa et al. (2017) reported positive impacts on application of ZnO, B₂O₃, CuO nanoparticles in minimizing drought stress in soybean plants. Drought stress mainly changes sub-cellular structural modification, and shows accumulation of malondialdehyde (MDA) and osmolytes in plant leaves. Application of ZnO nanoparticles induces synthesis of melatonin and activates several antioxidant enzymes to mitigate the drought stress as reported in maize plant (Sun et al. 2020). Semida et al. (2021) investigated foliar application of ZnO nanoparticles to promote drought stress tolerance in *Solanum melongena* L. and showed positive results by improving accumulation of micro and macro-nutrients, increasing relative water content (RWC) and alleviating the cell membrane damage. Tewari et al. (2019) reported that increased concentration of zinc affects the activity and production of zinc dependent enzymes such as carbonic anhydrase, which directly or indirectly regulates the CO₂ sensing pathway thereby influencing drought tolerance.

Salinity stress management

According to Soliman et al. (2015), ZnO nanoparticles are very much efficient to medicate the salt stress on *Moringa peregrina* plants. Sanaeiostovar et al. (2012) used ZnO NPs to increase antioxidant enzyme activity to stabilize the

stress condition. Alharby et al. (2016) reported that use of 15 mg L⁻¹ concentration of ZnO nanoparticles be able to mitigate the effect of NaCl toxicity on tomato. Hussein and Baker (2018) utilized zinc NPs to alleviate salinity stress on cotton plants. Foliar application of ZnO nanoparticles on wheat and mustard can able to diminish the harmful effect of the salinity stress (Torabian et al. 2016; Fathi et al. 2016).

Metal stress management

Various reports suggested that ZnO nanoparticles are very much effective against the metal stress (Aravind and Prasad 2005). Venkatachalam et al. (2017) reported that ZnO nanoparticles reduce the toxicity induced by Cd and Pd in *Leucaena leucocephala* seedlings. Garg and Kaur (2013) suggested that the presence of Zn decreased the Cd content on the plant body of *Cajanus cajan*, and enhanced growth, yield and survival of the plants.

Uptake, translocation and accumulation of Zinc nanoparticles in plant system

Plants are one of the most important components of food chain. Uptake, transport and accumulation of nanoparticles in the plant system directly affect the

food web (Wang et al. 2013). The mechanism of uptake, translocation and accumulation of nanoparticles in plant system mainly depend on the plant species and the size, configuration and concentration of nanoparticles. The transport of nanoparticles and its adhering property mainly depend on several physical properties such as, van der Waal forces, Brownian motion, gravity, surface tension etc. (Handy et al. 2008). Nanoparticles may dissolve in soil water and dissociate into ions which can move easily through the ion channels (Gupta et al. 2016). Vascular bundle (xylem) acts as most important transporter for distributing and translocation of NPs to the leaves and other terminal parts. Epidermis, cortex, endodermis, cambium, and vascular bundles are mostly interacted place of nanoparticles and accumulate high concentration of nanoparticles than the other plant parts (Faizan et al. 2020). On the other hand, the surface characteristics of nanoparticles are one of the key factors that regulate accumulation, transport and uptake (Nair et al. 2010). López-Moreno et al. (2010) describes accumulation of ZnO NPs in *Glycine max* seedlings. Another study of zinc nanoparticles uptake through the root system of *Lolium perenne* plant showed promising evidence that the particles

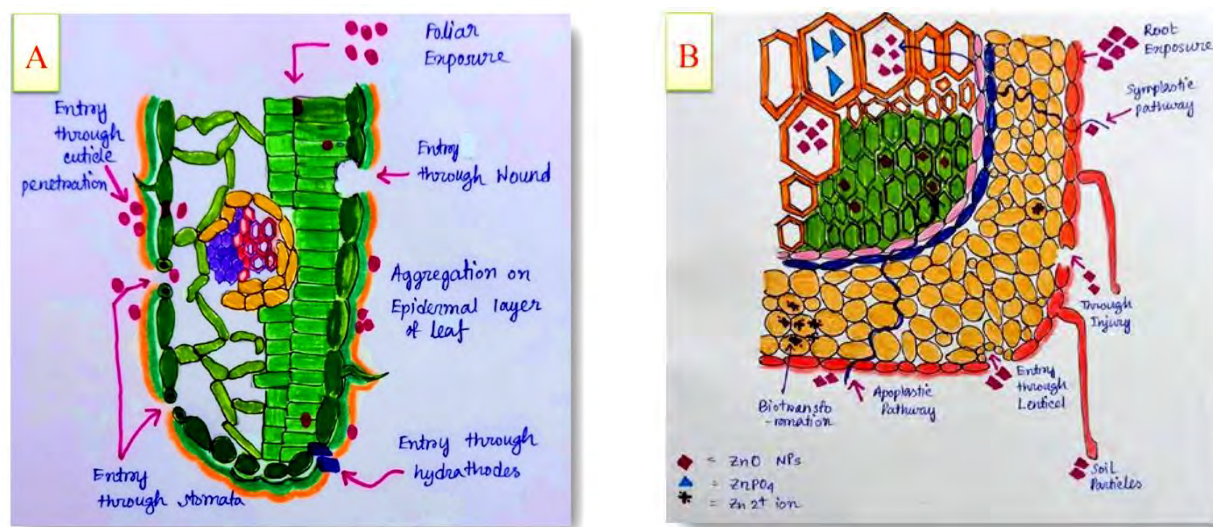


Fig. 5 An overview showing general uptake, translocation and biotransformation process of zinc oxide nanoparticles in plant system. Transverse-section of leaf (A) and root (B) showing entry of nanoparticles

scattered in the apoplast, cytoplasm, epidermal cells and vascular bundle cells (Lin and Xing 2008). Salt et al. (1999) previously described that carboxyl groups play important role in transport and storage of zinc in plant shoot. Lv et al. (2015) studied accumulation and uptake of ZnO NPs in maize (*Zea mays* L. cv. Zhengdan 958) plant and by

characterizing through XAS, μ -XRF, SEM, TEM, fluorescence labelling techniques, reported large amount of Zn²⁺ ions uptake and accumulation in the form of ZnPO₄. ZnO nanoparticles which adsorbed through the root surface directly affect cell division and elongation. ZnO NPs applied on the leaves may act differently, where they can only enter into the

plant system through stomata or cuticles and their cellular transport is occurring through the apoplastic and symplastic routes into the vascular bundles of the plant (Figure 5). Leaf morphology, anatomical characteristics, chemical components etc. are essential factors that affect the uptake, accumulation and translocation of nanoparticles in the leaf (Larue et al. 2014). Da Cruz et al. (2019) conducts an experiment to investigate absorption, transportation, and accumulation of ZnO NPs and ZnSO₄ on *Phaseolus vulgaris* (L.) plant and reported that smaller nanoparticles (<40 nm) are more likely to be taken up by root system. X-ray fluorescence spectroscopy confirms those root-to-shoot translocations are not very much promising but the radial movement of Zn nanoparticles simultaneously occurs through the xylem transport. Gene coding analysis shows involvement of ZIF1, MTP8, IRT3, HMA2, NRAMP3, NRAMP4, and MTP1 genes for Zn transport in *P. vulgaris* plant system. Al-Salama (2010) describes that when ZnO particles are accumulated in the rhizosphere in higher concentrations, may inhibit the seedling growth in barley plant. Atomic absorption spectroscopy (AAS) is one of the major technologies used for confirmation of nanoparticle uptake and translocation in the plant system (Ju et al. 2019). AAS data showed high accumulation of ZnO NPs in shoot than the root or leaves in rice plant as observed by Afzal et al. (2021). However, for detailed understanding of translocation and accumulation of ZnO nanoparticles in plant system, further investigations are required.

Conclusion and Future prospective

The possibility of synthesizing ZnO NPs using green methods holding a wide range of biological samples has been discussed. With the obtained comprehensive information from this review, it is considered that green synthesis of zinc oxide nanoparticles is much safer and environment friendly than the conventional physical and chemical way of synthesis. In this process, biological entities act as both reducing and capping agents for controlling the synthesis of required size and shape zinc nanoparticles. The generation of reactive oxygen species and easy penetration of ZnO NPs through cell wall has made it a potential therapeutic agent for treating cancer and microbial infections. ZnO NPs would act as nanofertilizer which not only promotes the yield and growth of the plants but also able to mitigate abiotic stresses. Overall, it acts as effective element for sustainable agriculture. Future prospective of biogenic synthesis of zinc oxide nanoparticles include extended laboratory-based

work for large-scale production and commercialization, evaluation of toxicity and environmental safety for use in different fields; genome analysis and gene expression studies for understanding the precise mechanism behind the plant growth, development and abiotic stress management.

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