

Impact of zinc oxide nanoparticles on seed germination and its growth

DISSERTATION REPORT

Submitted in partial fulfilment of the requirement for the award of the degree of

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In

Biotechnology

Submitted by:

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CERTIFICATE

This is to certify that this thesis entitled "**Impact of zinc oxide nanoparticle on seed germination and its growth**" submitted by Ms. Malvika Mehta (Roll. No. 301601016) in partial of the fulfilment requirement for the award of Master in Science Degree in Biotechnology from Department of Biotechnology, Thapar Institute of Engineering and Technology, Patiala (Punjab), India. It is an exclusive original record of candidate's own research work carried under my supervision. This Thesis in part or full has not been submitted in any other institute for award of such kind of degree.



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DECLARATION

I, the undersigned, hereby declare that the research work presented in M.Sc. project entitled **“Impact of zinc oxide nanoparticle on seed germination and its growth”** has been carried out by me under the supervision and guidance of Dr. Dinesh Goyal, Department of biotechnology, Thapar Institute of Engineering and Technology, Patiala. Further, I declare that no part of this dissertation has been submitted for a degree or any other qualification of any other university or examining body in India/elsewhere.



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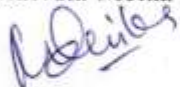
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LIST OF ABBREVIATIONS

AFM	Atomic force microscope
BG11	Blue green medium
HAuCl ₄	Chloroauric acid
CdS	Cadmium sulphide
CO ₂	Carbon dioxide
CeO ₂	Cesium oxide
CVD	Chemical vapour deposition
DLS	Dynamic light scattering
eV	Electron volt
Et al	Et alii
FTIR	Fourier transformed infrared spectroscopy
Hrs	Hours
Fe ₂ O ₃	Iron oxide
Kg	Kilogram
Khz	Kilo hertz
Mg	Miligram
ml	Mililiter
M	Molar
Min.	Minutes
Nm	Nanometer
NPs	Nanoparticles
ppm	Parts per million
pH	Potential hydrogen
SEM	Scanning electron microscope
TEM	Transmission electron microscope
UV	Ultra violet
H ₂ O	Water
XRD	X-ray powder diffraction
Zn(CH ₃ COOH) ₂	Zinc acetate
ZnO	Zinc oxide
ZnSO ₄	Zinc sulphate
Zn(NO ₃) ₂	Zinc nitrate

LIST OF SYMBOLS

%	Percentage
°C	Degree calcium
Ag	Silver
Au	Gold
Cu	Copper
Zn ²⁺	Zinc ion
α	Alpha
β	Beta
λ	Lambda
Θ	Theta

ABSTRACT

In the present investigation, impact of pre-synthesized ZnO nanoparticles as micronutrient was evaluated on germination of seed and seedlings growth of different crops such as mung (*Vigna radiate*), wheat (*Triticum aestivum*) and rice (*Oryza sativa*) under *in vitro* conditions. The study was carried out in triplicates with different concentrations of nano-ZnO particles in suspension form under dark conditions at 25°- 35°C. In wheat, 500 ppm of ZnO NPs promoted radical growth, in mung 1000 ppm promoted radical growth, where as in rice maximum radical growth was observed at 100 ppm and maximum plumule length was observed at 50 ppm. 100% seed germination was observed in rice and mung at all tested concentrations of ZnO NPs along with control, whereas in wheat 100% seed germination was observed at 100-500 ppm of ZnO NPs. Zinc oxide (ZnO) nanoparticles had both positive as well as negative effect on different crops such as mung (*Vigna radiate*), wheat (*Triticum aestivum*) and rice (*Oryza sativa*). Nanoparticles can be synthesized by various physical and chemical methods, which are generally toxic, releases hazardous chemicals and require high temperature. In the present investigation, green approach was used for the synthesis of Zinc oxide (ZnO) nanoparticles in which Zinc oxide (ZnO) nanoparticles were synthesized from *Haematococcus* using zinc acetate as metal precursor. After the synthesis of nanoparticles, characterization of nanoparticles for composition, structure, shape and size is done *via*. FTIR, SEM, XRD, EDS etc.

CHAPTER 1

INTRODUCTION

Nanotechnology involves synthesis of nanoscale particles ranging from 1-100 nm in size referred to as nanoparticles or nanoclusters often used to fill the gap between bulk materials and atomic or molecular structures (Sharma *et al.* 2015). Nanoparticles have been exploited by many researchers due to their abilities which allow them to withstand the harsh conditions (Fu *et al.* 2005). The physical, chemical or other properties of material varies with shape and size of the material. The reduced size and large surface area of nanoparticle is very much responsible for optical, catalytic as well as magnetic properties. Due to these properties, nanoparticles have wide range of applications in agriculture as nanofertilizers, in drugs and medication, in the manufacturing of sunscreens, in developing biosensors, optical devices and in energy harvesting (Srilatha, 2011, Singh *et al.*, 2013).

Metal oxide nanoparticles are of great interest including zinc oxide (ZnO), copper oxide (CuO), iron oxide (Fe₂O₃), titanium oxide (TiO₂) etc. Zinc oxide (ZnO) nanoparticles due to its large band width, high and exciton binding energy with large surface area to volume ratio have potential antimicrobial, antioxidant, optical properties etc. There are various methods to synthesize Zinc oxide (ZnO) nanoparticles such as chemical, physical, biological methods (Fig. 3). Nanoparticle synthesis in the field of chemistry and biology has been a major purpose due to their controlled shape, size, chemical nature, crystalline structure (Antonyraj *et al.* 2013, Frances *et al.* 2009, Staniland, 2007). Due to large rate of toxicity and other environmental issues concept of green synthesis came into existence. The presence of natural extracts and other biological entities release some extracellular as well as intracellular enzymes, which help in reducing metal ions to synthesize nanoparticles. In agriculture, ZnO NPs have the ability to improve crop yield, chlorophyll content, growth of plumule and radical by regulating the synthesis of Indole Acetic Acid (IAA) (Prasad *et al.*, 2012, Khan *et al.*, 2003). Zinc as micronutrient source act as cofactor for carbohydrate metabolism, protein metabolism, conversion of sugars to starch and auxin (growth regulator) metabolism (Singh *et al.*, 2013)

Many studies have been done in order to understand the influence of nanoparticles on plant growth and seed germination. Most of the studies and experiments, which have been performed on nanoparticles, have focused on accumulation, uptake, biotransformation and translocation of nanoparticles in plants. Type of plant, size, shape and chemical composition

of nanoparticle greatly influence their uptake by plant roots (Bagheri *et al.*, 2012, Ghormade *et al.*, 2011, Khiew *et al.*, 2011). Different plant species behave differently towards different nanoparticle treatments and this variation occurs because of different stages of plant growth, surface structure, shape, size, concentration, solubility of nanoparticles, method and duration of exposure etc (Aslani *et al.*, 2014). Interaction between nanoparticles and plants leads to change in gene expression and other genetic constituents of plants as well as change in different biological pathways thereby affecting growth of plants, percentage seed germination and development of seeds (Aslani *et al.*, 2014). There are many pathways by which nanoparticles can integrate into plants. The uptake of NPs follows active transport mechanism, which involves signalling, regulation of plasma membrane and various other cellular approaches (Rajput *et al.*, 2017). In soil, large nanoparticles directly disintegrate and form smaller nanoparticles, which further get absorbed by roots and enter plants tissues. The smaller nanoparticles further divide to form ions. These ions further penetrate cell wall of roots and enter plants vascular system (Arruda *et al.*, 2015). Translocation and distribution of nanoparticles to leaves is done *via* xylem (Rajput *et al.*, 2017). There have been some reports which showed the toxic effects of ZnO NPs in plants. Toxicity can occur due to stress that is caused by nanoparticle's shape, size, its surface to volume ratio or it can be chemical toxicity due to chemical composition of nanoparticles (Aslani *et al.*, 2014). Nanotoxicity studies have been done in order to determine the inhibitory effect of nanoparticles at different concentrations and conditions on plant species such as soybean (*Glycine max*), maize (*Zea mays*), ryegrass (*Secale cereale*) and barley (*Hordeum vulgare*). It was observed that several aspects of the plant growth, including seed germination, shoot length along with biomass and gene expression, were affected (Dimpka *et al.*, 2012). For example, the biomass of rye grass was reduced, root tip was shrunken and root epidermal as well as cortical cells became highly vacuolated and collapsed (Sabir *et al.*, 2014). Nanofertilizers are mostly ecofriendly, nontoxic, free of impurities, required in fewer amounts and improve plant growth, yield and seed development. In the present investigation, impact of pre-synthesized ZnO NPs as micronutrient was evaluated on seed germination and growth of seedlings under *in vitro* conditions.

CHAPTER 2

REVIEW OF LITERATURE

Nanotechnology is a discipline of science and technology that involves synthesis of various nanomaterials that range in size from 1-100 nm. It involves manipulation of matter at [atomic](#), [molecular](#) and [supramolecular](#) scale. By managing nanoscale shape and size, designing, characterization, production and application of structures can be done. Nanotechnology in biomedical research has revealed that nanoscale materials can be very useful in clinical methodologies including imaging, therapeutics, diagnostic, drug delivery and tissue engineering, labelling of immunoassays, hyperthermia treatment of bacteria, antibacterial as well as antifungal drugs (Srilatha, 2011). They are widely used in consumer products, pharmaceuticals, cosmetics, semiconductors, microelectronics and agriculture (Singh *et al.*, 2013).

Nanoparticles have large variety of geometric shapes and conformations like tubes, wires, plates, sheets, nanorods, whiskers, nanocables, clusters, nanofibers, spheres etc (Fawcett *et al.*, 2016). Many of the studies shown unique electronic, thermal, mechanical, chemical, optical, physical and biological properties of these nanoscale particles due to its small size, shape and size distribution (Fawcett *et al.*, 2016). Nanoparticles interact efficiently with biological system due to their nanoscale size, shape and large surface area (Brayner *et al.*, 2010). The physiochemical properties of these nanomaterials are mainly influenced by their larger surface area to volume ratio.

2.1 Types of nanoparticles

Nanoparticles types are divided in two main groups: organic and inorganic NPs (Fig.1). The organic nanoparticles include micelles, dendrimers, liposomes, hybrid and compact polymeric NPs. The inorganic nanoparticles include fullerenes, quantum dots, silica and gold nanoparticles (Orive *et al.*, 2005).

1. **Quantum dots** : They are semiconductor nanosized crystals which are basically made up of elements of group II–VI or III–V that emits light of all rainbow colours depending on their size. These nanostructures enclose conduction band electrons, valence band holes, or excitons in all three spatial directions.

2. **Dendrimers** : Dendrimers are star shaped, highly branched nano sized structure having large-scale use in medical field particularly in the field of nano- medicine because of numerous molecular "hooks" present on their surfaces to attach cell- identification tags, enzymes, fluorescent dyes and other molecules (Bhatia, 2016).

3. Nanorods: They are mainly 1-100 nm in length, often made-up of semiconducting materials used in nanomedicine as contrast and imaging agents. These nanorods are made- up of small cylinders of silicon, gold or inorganic phosphate, among other materials.

4. Nanoshells: They are also known as core-shells. These are spherical cores of a particular compound surrounded by an outer coating or shell of another, which is few nanometers thick (Bhatia, 2016).

5. **Carbon tubes**: Carbon tubes are basically carbon-based, lattice- like, porous molecules. They are the part of the fullerene structural class.

6. Micelles: They are nanostructures made of amphiphilic molecules, like polymers or lipids. When exposed to aqueous environments, they hide their hydrophobic groups inside the structure and expose the hydrophilic groups (Orive *et al.*, 2005)

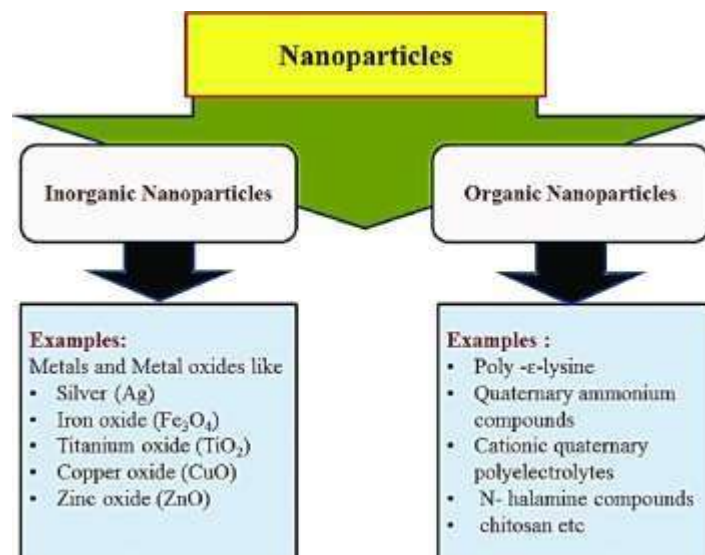


Fig 1: Types of nanoparticles (Source: Oscar *et al.*, 2016)

The inorganic nanoparticles are basically metals and metal oxides like Zinc oxide (ZnO), copper oxides, silver, iron oxide, titanium oxide. Organic nanoparticles comprises poly-ε-lysine, cationic quaternary polyelectrolytes, quaternary ammonium compounds, N-halamine

compounds, and chitosan. At high temperature, organic nanoparticles are less stable as compared to inorganic ones which make inorganic nanoparticles more preferable as antimicrobial polymers (Oscar *et al.*, 2016).

Nanoparticles are broadly categorised on the basis of dimension, phase composition and manufacturing method (Hett *et al.* 2004)

2.1.1. Dimension

a. One dimension nanoparticles

Thin film, coatings, multilayers comes under one dimension. Thin films sizes ranging between 1–100 nm and monolayer are used in the solar cells, in different applications such as chemical and biological sensors, optical device, magneto-optic and fiber-optic systems, information storage systems.

b. Two dimension nanoparticles

Carbon nanotubes, fibres, wires etc

c. Three dimension nanoparticles

Dendrimers, Fullerenes, hollow spheres, Quantum Dots

2.1.2. Composition (Luther *et al.* 2004):

a. Single-solid phase- amorphous and crystalline particles

b. Multi-phase solids- coated particles, matrix composites etc

c. Multi-phase system- aerogels, colloids etc

2.1.3. Manufacturing methods (Luther *et al.* 2004):

a) Gas phase reaction- CVD, flame synthesis, condensation etc.

b) Liquid phase reaction- sol-gel, hydrothermal processing, precipitation etc

c) Mechanical phase reaction- plastic deformation, ball milling etc.

2.2. Metal oxide nanoparticles

Metal oxide nanoparticles have principle role in many sections of biology, materials science, chemistry and physics. Different variety of oxide compounds can be formed by metal oxide nanoparticles. Metal oxides exhibit metallic, insulator or semiconductor character due to enormous number of structural geometries with some electronic structures (Garcia *et al.*, 2007). Metal oxides are used for fabrication of microelectronic circuits, sensors, piezoelectric devices, fuel cells, coatings for the passivation of surfaces against corrosion, and as catalysts (Garcia *et al.*, 2007). Due to their metallic, insulator and semiconductor characters these have applications in magnetic storage media, gas sensors catalysis, electronics etc (Ramgir *et al.* 2016, Jani *et al.* 2013, Shalana *et al.* 2013, Ahmadi *et al.* 2011). The characteristics of metal oxide are more complex than pure metal because metal oxide bonding differs from nearly ionic to highly covalent and even metallic in nature (Fawcett *et al.*, 2016). Metal oxide nanoparticle due to large surface area, limited size and a high density of corner or edge surface sites exhibit unique physical and chemical properties. Due to size dependent properties such as fluorescence, magnetism and photocatalytic degradation, these metal oxide nanoparticles have wide range of use in agrochemicals as well as soil remediation (Aslani *et al.*, 2014). Some structural characteristics like the lattice symmetry and other cell parameters of nanoparticles are mainly influenced by particle size of metal oxide nanoparticles (Garcia *et al.*, 2007). The most studied metal oxide nanoparticles are mainly ZnO, TiO₂, Fe₃O₄ and CeO₂. Bulk oxides have proper crystallographic, robust and stable structures (Garcia *et al.*, 2007).

2.3 Zinc oxide (ZnO) nanoparticles

Zinc oxide (ZnO) is inorganic white powdered compound that is nearly insoluble in water (Sabir *et al.*, 2014). It has a wide band gap of 3.37 eV having short-wavelength optoelectronic applications (Wellings *et al.*, 2007), large exciton binding energy of 60 meV at room temperature and large bond strength (Garcia *et al.*, 2007, Sabir *et al.*, 2014). Crystalline ZnO, at ambient conditions, displays a wurtzite (B4) crystal structure that exhibits sp³ covalent bonding (Sabir *et al.*, 2014). Zinc oxide (ZnO) is more preferred as they are safe and very easy to synthesise. Zinc oxide NPs are mentioned as generally recognized as safe (GRAS) by US FDA (Agarwal *et al.*, 2017). They are non-toxic, have neutral n-type electrical conductivity. Zinc oxide (ZnO) nanoparticles exhibit high thermal conductivity, binding energy, high refractive index as well as anti bacterial and UV protection (Vaseem *et*

al., 2010). Zinc oxide (ZnO) is reported to be required for fertilization, biomass production, chlorophyll production, pollen function and germination (Prasad *et al.*, 2014, Milani, McLaughlin 2012, Pandey *et al.*, 2010, Genc *et al.*, 2006, Khan *et al.*, 2003). Many pigments, proteins, sugar contents and nitrate reductase activities in crops have been increased by Zinc oxide (ZnO) nanoparticles (Siddiqui *et al.*, 2015). Zinc oxide (ZnO) nanoparticle is most flexible metal oxide nanomaterial, due to its different properties, functionalities and applications. They possess unique physical, chemical as well as optical properties. They also possess antimicrobial activities against some bacteria and fungi. They can be synthesized from various biological agents like plant extract, bacteria, yeast, fungi, algae, actinomycetes etc. Zinc oxide (ZnO) has best selectivity, heat resistance, best biocompatibility and durability than other organic and inorganic materials (Kumar *et al.*, 2008, Padmavathy *et al.*, 2008, He *et al.*, 2011). These biological entities secrete large number of enzymes which helps in hydrolyzing metal by enzymatically reducing metal ions to form nanoparticles (Rai *et al.*, 2009). There are various physical or chemical methods for the synthesis of Zinc oxide (ZnO) including green synthesis. The green synthesis of ZnO NPs is safe and environment friendly as compared to chemical synthesis because there is no formation of toxic by-products. Zinc is important for the synthesis of hormones and is important for carbohydrate metabolism (Vilosh *et al.*, 1994). Zinc protects cell organelles from the reactive oxygen species thus forms a defence system in the cells (Singh *et al.*, 2013). Zn deficiency results in reduced internodal growth, leading to a rosette habit of plants. The leaves may be small and distorted with puckered margin. ZnO is not soluble in water but is used as fertilizer with zinc sulphate (Singh *et al.*, 2013). In some studies it has been shown that chromosomal as well as cellular aspects of plants are affected by Zinc oxide (ZnO) nanoparticles (Aslani *et al.*, 2014). Zinc oxide (ZnO) nanoparticle toxicity can be due to two reasons: Chemical toxicity mainly due to chemical composition and other is stress conditions caused by shape, size of nanoparticle (Wang *et al.*, 2004).

In agriculture, ZnO NPs play a very important role, where colloidal solution of ZnO NPs is used as nanofertilizers. Despite the harmful factors of chemical fertilizers, nanofertilizer act as plant nutrient which not only provides nutrients for the plant but also improves soil quality to an organic state without harming the crop as well as the soil (Sabir *et al.*, 2014). Nanofertilizer can be used in very small amounts which makes it more beneficial than other fertilizers. Plant growth and yield can be increased by using Zinc oxide (ZnO) nanoparticles. These particles effectively increase the growth of plumule and radicle (Prasad *et al.*, 2012).

ZnO NPs have very good absorption rate, therefore can be quickly and completely absorbed by the plants.

2.4 Distinguishing properties of ZnO nanoparticles

1. Optical Properties of ZnO NPs

The optical properties of ZnO nanostructures have been used for the execution of photonic devices. Photoluminescence spectra of ZnO nanostructure is used for this execution. ZnO NPs shows great effect on the photoresponse due to the presence of O₂. The photo response of ZnO nanowire is basically affected by desorption-adsorption process (Sabir *et al.*, 2014).

2. Semiconducting properties of ZnO NPs

ZnO NPs have large band gap and high exciton binding energy because of which they show immense semiconducting properties. Some of the semiconducting properties of ZnO NPs are antiinflammatory, optic, wound healing, UV filtering properties and high catalytic activity (Patel *et al.*, 2014, Stan *et al.*, 2015). ZnO NPs exhibit UV blocking property hence are used in sunscreens (Aggarwal *et al.*, 2017)

3. Antimicrobial Properties of ZnO NPs

Biologically synthesized Zinc oxide (ZnO) nanoparticles show more antimicrobial activity than chemically synthesized nanoparticles (Sabir *et al.*, 2014). In order to quantitatively evaluate the antimicrobial effects of *Staphylococcus aureus*, *Escherichia coli*, or fungi culture media are used (Brayner *et al.*, 2010). Maximum inhibition of bacterial as well as fungal growth occurs due to enhanced bioactivity which depends on higher surface area of nanoparticles (Sabir *et al.*, 2014). The main mechanism involved in antibacterial activity depends on reactive oxygen species that are generated by these metal oxide particles (Sirelkhatim *et al.*, 2015). ZnO NPs follow antibacterial mechanism because of which they act as antimicrobial agent. This mechanism involves interaction of ZnO nanoparticles directly with microbial cell surfaces affecting permeability of cell membrane. These nanoparticles when enter into a bacterial cell stimulate oxidative stress, which in turn inhibits cell growth and eventually causes cell death (Molina *et al.*, 2006).

2.5 Applications and uses of zinc oxide (ZnO) Nanoparticles

ZnO NPs have unique versatile applications in bio-sensors, transparent electronics, nanogenerators, ultraviolet (UV) light absorption and piezoelectric devices, chemical sensors, and spin electronics (Nomuro *et al.*, 2003, Nakada *et al.*, 2004). Zinc oxide (ZnO) is nontoxic thus can be used as photo catalytic degradation materials of environmental pollutants. ZnO nanostructures exhibit high catalytic efficiency, as well as strong adsorption ability, and are more frequently used in the manufacturing of sunscreens (Singh *et al.*, 2013). Apart from antifungal and antibacterial properties, Zinc oxide (ZnO) nanoparticles have important role in central nervous system during different development stages of diseases by mediating neuronal excitability or even release of neurotransmitters. It has been shown in many studies many functions of different cells and tissues, neural tissues and biocompatibility gets affected by ZnO NPs (Kato *et al.*, 2011, Rasmussen *et al.*, 2010, Song *et al.*, 2008). These nanoparticles are very effective in cancer treatment as well (Hanley *et al.*, 2008).

In agriculture, nanoparticles and nanocapsules due to large surface area help in efficient distribution of pesticides and fertilizers with high site specificity. These nanofertilizers reduce collateral damage and are rapidly absorbed by plants (Sabir *et al.*, 2014). Nanoencapsulated slow release fertilizers can save fertilizer consumption and minimize environmental pollution. The yield and growth of food crops can be improved by the use of ZnO NPs. For example, peanut seeds when treated with different concentrations of Zinc oxide (ZnO) nanoparticles (25 nm), at 1000ppm ZnO NPs improvement in seed germination, seedling vigor, and plant growth was observed and these nanoparticles were also effective in increasing stem and root growth in peanuts (Prasad *et al.*, 2012). Nanofertilizers required amount in farms can be decided by nanosensors. These nanosensors detect soil moisture, nutrients requirement etc (Sabir *et al.*, 2014). Studies have shown that the level of zinc in soil ranges between 10-300ppm (Jala *et al.*, 2006). As a fertilizer, colloidal solution of zinc oxide (ZnO) nanoparticles is used in order to increase the level of zinc in soil. Nanofertilizer acts as fertilizer to plants and also revives soil quality without the harmful factors of chemical fertilizer (Batsmanova *et al.*, 2013, Selivanov *et al.*, 2001). One of the advantages of nanofertilizers is that they can be used in very small amounts.

2.6 Effect of ZnO NPs on plants

Nanoparticles have unique physicochemical properties which allow them to be used as nanofertilizers. Depending on the chemical composition, size, surface area, and dose, the efficacy of nanoparticles can be determined (Siddiqui *et al.*, 2015). Nanoparticles interact with plants causing some physiological changes as well as morphological changes. These nanoparticles therefore show both stimulatory and inhibitory effect on plant growth, germination of seeds, biomass of roots and shoots etc (Siddiqui *et al.*, 2015). Some of the studies explain the effect of nanoparticles of different metal oxides on different plants crops based on different nanoparticle concentration, size.

2.6.1 Effect of nanoparticles on different crops

Zhao *et al.*, (2013) showed the action of CeO₂ or ZnO NPs on cucumber plants. Cucumber was treated at different concentration (0, 400, and 800 mg/kg) of ZnO nanoparticles (size 8±1 nm) and observed negative effect on gaseous exchange in leaves and chlorophyll content at 800 mg/kg concentration. Phytotoxicity in tissues was observed above 200 mg/kg concentration. At 400 and 800 mg/kg, increase in biomass of roots by 10.5% and 63% was reported respectively.

A study was carried out by Prasad *et al.*, (2012), in which peanuts (*Arachis hypogaea*) was exposed to concentrations of 400, 1000 and 2000 ppm ZnO NPs with of 25 nm and there was increase in germination, stem and root growth and chlorophyll was observed as compared to plants exposed to ZnSO₄. At 1000 ppm, there was maximum seedling vigor index and at 2000 ppm it was decreased.

Similarly, Burman *et al.*, (2013) worked on chickpea and found increased biomass on foliar application of 1.5ppm Zinc oxide (ZnO) nanoparticle suspension with size 0.8 to 1.2mm as compared to ZnSO₄ with size 0.8 to 1.2mm but negative impact on root biomass was observed when 10 ppm nanoparticle suspension was applied (Burman *et al.*, 2013). But there was overall increase in the biomass in Zinc oxide (ZnO) treated seeds of chickpea.

Nanoparticle concentration, size and different plant species mainly affect the germination of plants. Rosa *et al.*, (2013) studied the effect of different concentrations of Zinc oxide (ZnO)

nanoparticles on tomato, cucumber and alfalfa and found that germination of seed was increased only in cucumber with the application of ZnO NPs.

Raliya and Tarafdar (2013) reported that Zinc oxide (ZnO) nanoparticles size ranging from 1.2 to 6.8 nm when applied on cluster bean (*Cyamopsis tetragonoloba*) via foliar spray method improved plant biomass, chlorophyll content, shoot and root growth, protein synthesis, rhizospheric, acid phosphatase, alkaline phosphatase, microbial population and phytase activity in cluster bean rhizosphere was observed.

Mahajan *et al.*, (2011) showed that on application of different concentrations of nano-ZnO particle suspension on mung (*Vigna radiata*) and gram (*Cicerarietinum*) seedlings, It was observed that with increase in nanoparticle concentration there was retardation in growth of seedlings. Best result was shown at controlled condition.

Helaly *et al.*, (2014) reported that when nano ZnO added to MS media, it was observed that there was reduced proline synthesis, regeneration of plantlets, shooting activity of superoxide dismutase, peroxidase, catalase activity and somatic embryogenesis thereby improves tolerance to biotic stress.

Kouhi *et al.*, (2015) studied the comparative effect of Zinc oxide (ZnO) nanoparticles and bulk ZnO NPs and Zn²⁺ on *Brassica napus*. Size range between 155±10 and 590± 27 nm of Zinc oxide (ZnO) nanoparticles and bulk particles was used at different concentration levels. It was found that there was adverse effect of Zinc oxide (ZnO) nanoparticles or bulk particles on *B. napus* which was due to the toxic effects of Zn²⁺ ions dissolution. Retardation of root length was seen.

Some studies showed that clear root germination effects, due to the presence of ZnO were observed for the species of Buckwheat (*Fagopyrum esculentum*). Furthermore, the presence of the ZnO nanoparticles also promoted the permeation of onion (*Allium cepa*) roots and affected the root's elongation, genetic materials, and metabolisms. The ZnO suspension meaningfully inhibited root growth of corn, with the termination of root development (Aslani *et al.*, 2014).

2.7 Synthesis of nanoparticles

The synthesis of nanoparticles can be done *via* two main approaches: Bottom up approach and Top down approach (Fig. 2). Top down approach mainly leads size reduction of material via physical and chemical methods. In this method large-scale particles are initially synthesized and then reduced to nanoscale structures. The second method involves assembly of atoms, molecules and smaller particles or monomers in order to form nanoparticles. In bottom-up approach, [chemical](#) properties of single molecule are being used to self-assemble single- molecule component into useful conformation and shape.

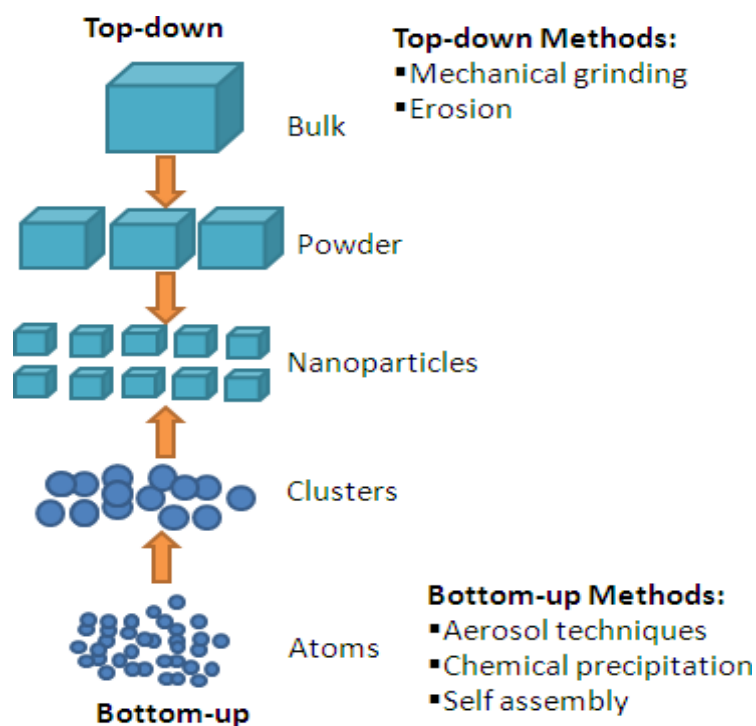


Fig 2: Schematic flowchart of Top-down and Bottom-up approaches

2.7.1 TOP DOWN APPROACH

a) **Mechanical milling:** This method involves different mixtures of powders that are placed in high energy mill and subjected to mechanical grinding. In this method there is reduction in particle size and formation of new particle. Different varieties of mills are used for this purpose like tumber mills, attrition mills, shaker mills etc (Koch *et al.*, 1996).

b) **Etching:** This is a chemical method that involves formation of regular arrangement of nano-sized structures formed on planar substrate. So silicon nano-wires are fabricated through non-uniform etching on silicon substrate on aqueous acid solution (Peng *et al.*, 2002).

c) **Electro-explosion:** In this process, very thin metallic wires are used to provide high current in short time period through reactive or inert gas to attain high temperature in order to obtain infinite metallic resistivity. Electromagnetic field disappears and expansion of superheated metal plasma occurs. Around the wire, a supersonic velocity is created that creates a shock wave in ionised gas (Luther *et al.*, 2004).

d) **Sputtering:** This process involves ejection of particles from solid target material. These particles are bombarded energetically on very cold substrate at low pressure (Luther *et al.*, 2004).

e) **Laser ablation:** Basically it is referred as removal of material through pulse laser. It is a process which involves irradiation of material from a solid surface using a laser beam. Evaporation or sublimation of material occurs when heated by absorbed laser energy at low laser flux. Whereas the material is typically converted to [plasma](#) at high laser flux (Ullmann *et al.*, 2002)

f) **Attrition:** It is a process which involves grinding of particulates typically by size reduction mechanism. Then the particulates are being separated according to their size with the help of air-classifiers and thus oxidized nanoparticles are recovered.

g) **Pyrolysis :** It is a thermochemical process of classification and separation of molecules. In this, precursors are being passed through an orifice at high temperature and pressure and are burned down in the absence of oxygen. The ashes obtained are air-classified to recovered nanoparticles.

2.7.2 BOTTOM-UP APPROACH

a) **Supercritical fluid synthesis:** In this method, supercritical CO₂ and H₂O are used in the preparation of large number of metal oxide nanoparticles (Byrappa *et al.*, 2008).

b) **Spinnig**: In this process, fine fibres are produced by spinning the dilute polymer solutions in a high voltage electric field from a liquid which are mostly in nano scale (Luther *et al.*, 2004).

c) **Sol gel method**: In this process, colloidal nanoparticles are formed from liquid phase (Sekine *et al.*, 2009).

d) **Laser pyrolysis**: This method in vapour phase when sufficient degree of supersaturation of condensable products is reached formation of nanoparticles is initiated. Rapid synthesis of nanoparticles takes place by coagulation during nucleation stage (Amato *et al.*, 2013).

e) **Vapour deposition**: The precursor material is put into the working chamber with a stable arc. The chamber is filled by reactive gas that becomes ionized then molecular clusters are formed and cooled to produce nanoparticles.

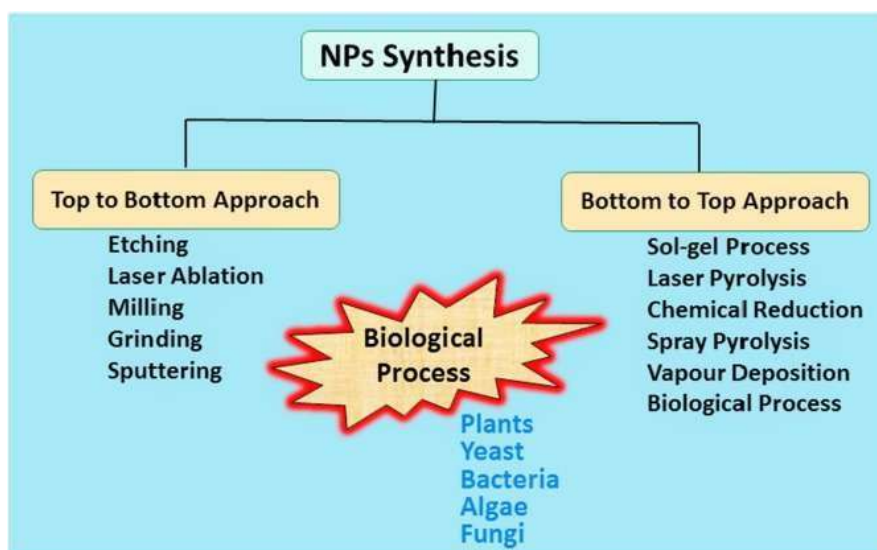


Fig 3: Different approaches of nanoparticle synthesis

In case of biological as well as chemical synthesis of nanoparticles, the synthesis of nanoparticle requires capping agents, some stabilizers, surfactants etc. These capping agents like biomolecules, polysaccharides stabilize and functionalize nanoparticles by maintaining shape, size and by inhibiting aggregation (Sharma *et al.* 2015). But in many studies it has been shown that, these capping agents are used to get the desired shape and size of the nanoparticles but they can be hazardous, difficult to remove and are difficult to degrade (Gittins *et al.*, 2000, Sharma *et al.*, 2015, Liu *et al.*, 2005). Many precursors are used during

synthesis process like $\text{Zn}(\text{CH}_3\text{COOH})_2$ (zinc acetate), HAuCl_4 (chloroauric acid), ZnSO_4 (zinc sulphate), $\text{Zn}(\text{NO}_3)_2$ (zinc nitrate) etc. The thermal stability of these precursors depends on their interaction with the solvent (Vaseem *et al.*, 2010). These aqueous metal ion precursors are reduced from metal salts to NPs due to which there is a change in colour of the reaction mixture.

But many of the physical and chemical method involves several drawbacks such as low material conversion rates, are technically complex, have high energy requirement and mostly are expensive. Lot of studies shown that synthesizing nanoparticles via conventional method involve the use of chemicals, toxic materials, some hazardous materials like such as reducing agents, capping agents, some organic solvents and non-biodegradable stabilizing agents (Sharma *et al.*, 2015). Presence of these toxic components limits the clinical as well as biomedical applications of nanoparticles (Shah *et al.*, 2015). In order to synthesis environment friendly, clean, biologically compatible and nontoxic nanoparticle, green approach is required.

2.7.3 Green approach

Nowadays, researchers are focused on using biological method for the synthesis of nanoparticles. Biological approaches are generally nontoxic, free of impurities, cost effective and eco friendly. Many different unicellular and multi-cellular forms such as actinomycetes, yeast, bacteria, fungi, algae, plant extract and enzymes are being used for nanoparticle synthesis. Large scale production of nanoparticles occurs *via* green approach. One of the most interesting features of these biological forms is their capacity to act as template in the synthesis, organisation and assembly of nanoscale material to synthesize well defined micro and macro scale structures (Shah *et al.*, 2015). These natural extracts and plant extract release some intracellular or extracellular enzymes that act as capping agents, reducing agents, as well as stabilization agents (Agarwal *et al.*, 2017).

For green synthesis, in order to produce stable and well-characterized nanoparticles some points need to be considered (Iravani *et al.*, 2011):

1. Selection of the best organism: For nanoparticle production, different biochemical pathways, organism's enzymatic activity is important.

2. Optical conditions required for cell growth and enzyme activity: Optical growth conditions like, light, pH, temperature, inoculum size, mixing speed should be optimized.

3. Optical reaction conditions: The bioreduction conditions for the synthesis for nanoparticles in the reaction mixture are required to be optimized for good yield and quality (Ahmad *et al.*, 2003). The electron donor and its concentration, pH, time, light, temperature, buffer strength, the catalyst concentration, buffer strength, mixing speed and substrate concentration requires optimization. This mainly affects rate of reaction, size as well as morphology of nanoparticle formed.

2.7.3.1. Synthesis from algae

Algae are photosynthetic organisms that include wide variety of unicellular and multicellular forms. They are autotrophic and aquatic organism that lack some of the tissues like xylem and phloem found in higher plants as well as some secondary cells. The chlorophyll content present in algae is used to capture light energy as fuel for the formation of sugars (Elumalai *et al.*, 2013). Algae can be used for the synthesis of nanoparticles (Table 1). For this, dry biomass of algae is used. Nanoparticle synthesis *via* algae can be done either by extracellular method or by intracellular method depending on the type of algae used.

Intracellular synthesis: In this synthesis, enzymes which are present inside the cell are responsible for the synthesis of nanoparticles. These enzymes remain inside the cell until they are extracted out of the cell (Zhang *et al.*, 2011).

Extracellular synthesis: In this, nanoparticles are formed by the enzymes that are present out of the cell. The enzymes secreted by the cell wall are mainly responsible for this. In the presence of enzyme, reduction of metal ions and trapping of metal ions on the cell surface occurs during nanoparticle synthesis (Zhang *et al.*, 2011).

Steps involved in the algae-mediated biosynthesis of metal nanoparticles are the following (Sharma *et al.*, 2015)

(i) Algal extracts are prepared in water or in an organic solvent by heating or boiling it for certain duration.

(ii) Molar solutions of ionic metallic compounds are prepared.

(iii) Further, algal solutions and molar solutions of ionic metallic compounds are incubated for certain duration under controlled conditions. They are either incubated with continuous stirring conditions or without stirring.

Algae have been extensively used in the synthesis of gold and silver nanoparticles but its application in the synthesis of ZnO nanoparticle is less and is very less reported. Dry biomass of algae is mainly used for the synthesis of nanoparticles. During nanoparticle synthesis, algal cell disruption is very important (Fig. 4).

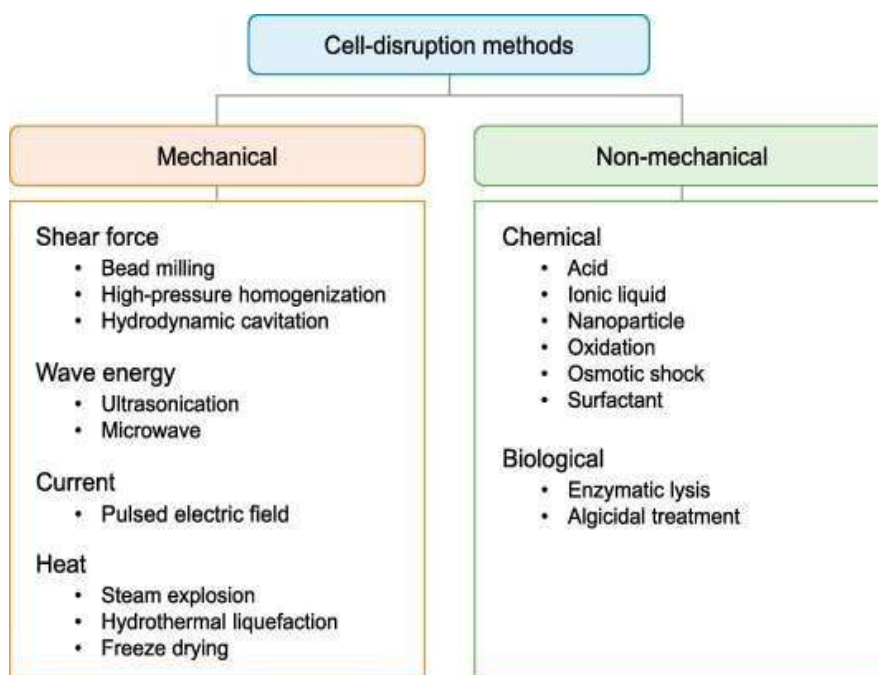


Fig 4: Methods of algal cell disruption

Table 1: Synthesis of nanoparticles from different species of algae

Algae	Specie	Nanoparticles	Shape	NP size (nm)	Reference
Cyanobacteria					
<i>Cyanophyta</i>	<i>Lyngbya majuscula</i>	Au	Spherical	>20	Chakraborty <i>et al.</i> , (2009)
	<i>Nostoc ellipsosporum</i>	Au	Nanorods	137–209 (length) 33–69 (diameter)	Parial <i>et al.</i> , (2012b)
	<i>Microcoleus sp.</i>	Ag	Spherical	44–79	Sudha <i>et al.</i> , (2013)
	<i>Anabaena sp. L 31</i>	ZnO	Spherical	80	Singh <i>et al.</i> , (2014)
Microalgae					
<i>Chlorophyta</i>	<i>Chlamydomonas reinhardtii</i>	Ag	Rounded and rectangular	5–15 <i>in vitro</i> 5–35 <i>in vivo</i>	Barwal <i>et al.</i> , (2011)
	<i>Chlorella pyrenoidosa</i>	Au	Spherical	25–35	Oza <i>et al.</i> , (2012)
	<i>Auxenochlorella pyrenoidosa</i>	Ag	Spherical	5–10	Elumalai <i>et al.</i> , (2013)
Macroalgae					
<i>Chlorophyta</i>	<i>Codium capitatum</i>	Ag	Spherical	3–44	Kannan <i>et al.</i> , (2013)
	<i>Caulerpa racemosa</i>	Ag	Spherical, triangular	5–25	Kathiraven <i>et al.</i> , (2015)
<i>Phaeophyta</i>	<i>Sargassum wightii</i>	Au	Thin planner structure	8–12	Singaravelu <i>et al.</i> , (2007)
	<i>Bifurcaria bifurcate</i>	Cu	Spherical	20.6	Abboud <i>et al.</i> , (2014)
	<i>Sargassum vulgare</i>	Ag	Spherical	~10	Govindaraju <i>et al.</i> , (2015)
<i>Rhodophyta</i>	<i>Gelidiella acerosa</i>	Ag	Spherical	22	Marimuthu <i>et al.</i> , (2011)
	<i>Gracilaria dura</i>	Ag	Spherical	6	Shukla <i>et al.</i> , (2012)

2.7.3.2. Green synthesis from bacteria

Bacteria are a prokaryotic organism having few micrometers length. They are present in most of the habitats including water, soil, hot springs, some radioactive waste as well as deep inside the earth too. They have number of shapes and live in parasitic as well as symbiotic association with some plants as well as animals. These bacteria are helpful in green synthesis of nanoparticles (Table 2). But has many disadvantages such as (Aggarwal *et al.*, 2017):

- Screening of microbes is time consuming process which requires careful monitoring of broth.
- The cost of media associated with bacterial growth is very high.
- Shape and size of nanoparticle formed also gets compromised.

Table 2: Synthesis of nanoparticles from different bacteria

Bacteria	Type	Shape and mode of synthesis	Size of NPs (nm)	Reference
<i>Pseudomonas stutzeri</i>	Ag	Intracellular	~200	Klaus <i>et al.</i> , 1999
<i>Thermomonospora</i> sp.	Au	Extracellular	8	Ahmad <i>et al.</i> , 2003
<i>Morganella</i> sp.	Ag	Extracellular	20-30	Parikh <i>et al.</i> , 2008
<i>Aeromonas hydrophila</i>	ZnO	spherical and oval	57-72(AFM), 42-64(XRD)	Jayaseelan <i>et al.</i> , 2012
<i>Pseudomonas aeruginosa</i>	ZnO	Spherical	35-80(TEM),81(DLS), 27(XRD)	Singh <i>et al.</i> , 2014
<i>Escherichia coli DH5α</i>	Au	Intracellular	25–33	Liangwei <i>et al.</i> , 2007

2.7.3.3 Green synthesis from fungi

Many studies have revealed that different species of fungi can be used in synthesis of different nanoparticles (Table 3). Extracellular synthesis of nanoparticles is very effective due to large scale production, metal bioaccumulation property, better tolerance, economic variability and downstream processing (Aggarwal *et al.*, 2017, Thakkar *et al.*, 2010).

Table 3: Synthesis of nanoparticles from different fungi

Fungi	Type	Shape and mode of synthesis	Size of NPs (nm)	Reference
<i>Verticillium</i>	Ag	Intracellular	25±12nm	Mukherjee <i>et al.</i> , 2001
<i>Aspergillus fumigates</i>	Ag	Extracellular	5-25nm	Bhainsa <i>et al.</i> , 2006
<i>Aspergillus strain</i>	ZnO	Spherical aggregates	50–120- SEM	Pawani <i>et al.</i> , 2012

<i>Aspergillus fumigates</i> TFR-8	ZnO	Oblate spherical and hexagonal forms aggregate	1.2–6.8 - DLS, 100 (agglomerate)	Raliya <i>et al.</i> , 2012
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2.7.3.4. Synthesis from yeast

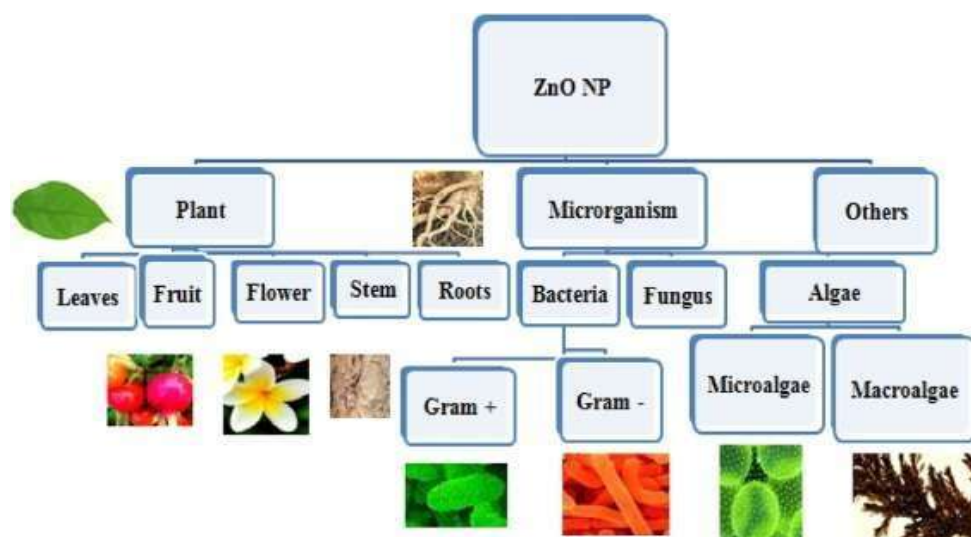
There are few studies done on the synthesis of nanoparticles through yeast (Table 4). The yeast strains possess more benefits over bacteria (Moghaddam *et al.*, 2015). Yeast production is easy controlled in laboratory conditions, the rapid grow of yeast strains and the use of simple nutrients possess several benefits in the mass production of metal nanoparticles (Skalickova *et al.*, 2017). Yeast produces nanomaterials like quantum dots, organic and inorganic nanoparticles by using their reducing enzymes intracellularly as well as extracellularly (Skalickova *et al.*, 2017).

Table 4: Synthesis of nanoparticles from different yeast

Yeast	Type	Shape and mode of synthesis	Size (nm)	Reference
<i>Schizosaccharomyces pombe</i>	CdS	Intracellular	200 nm	Dameron <i>et al.</i> , (1989)
<i>Candida glabrata</i>	CdS	Extrcellular,Hexamer	20 - 29 nm	Dameron <i>et al.</i> , (1989)
Yeast strain MKY3	Ag	Extracellular Twinned or multitwinned, some hexagonal	2-5 nm	Kowshik <i>et al.</i> , (2002)

2.7.3.5. Synthesis using plant extract

There have been many studies done on the biosynthesis of nanoparticles using plant extracts. There are different parts of plants which basically are responsible for synthesis of nanoparticles like leaf, stem, root, seed and fruit (Fig. 5). These plant parts produce many phytochemicals such as polysachharides, polyphenols, vitamins, amino acids, terpenoids, alkaloids etc which help in bioreduction of metal ions of metal oxides to 0 valence metal nanoparticles (Aggarwal *et al.*, 2017). Use of plant extract is advantageous because it involves cheap process and is ecofriendly. They lead to large scale production, does not involve the use of intermediate base groups, lead to large scale production and gives highly pure, stable enriched product free of impurities (Heinlaan *et al.* 2008, Qu *et al.* 2011).



(Aggarwal *et al.*, 2017)

Fig 5: Zinc oxide (ZnO) nanoparticle synthesis via different biological sources

Table 5: Synthesis of nanoparticles from different plant source

Plant	Plant extract	Type	Shape	Size (nm)	Reference
<i>Azadirachta indica</i>	Leaf	Au, Ag	Spherical	50-100nm	Shankar <i>et al.</i> , (2003)
<i>Pelargonium graueolens</i>	Leaf	Au	Spherical	16-40	Shankar <i>et al.</i> , (2003)
<i>Emblica officinalis</i>	Fruit	Au, Ag	Spherical	Au-10 - 20 nm, Ag- 15 - 25 nm	Ankamwar <i>et al.</i> , (2005)
<i>Cinnamomum camphora</i>	Leaf	Ag	Spherical	55-80	Huang <i>et al.</i> (2007)
<i>Azadirachta indica (Neem)</i>	Leaf	Zno	Spherical	9.6-25.5 (TEM)	Bhuyan <i>et al.</i> , 2015
<i>Cocus nucifera (Coconut)</i>	Coconut water	ZnO	Spherical	20-80 (TEM), 21.2 (XRD)	Krupa <i>et al.</i> , 2016
<i>Aloe vera (Liliaceae)</i>	Leaf	ZnO	spherical, oval, hexagonal	8-20 (XRD)	Ali <i>et al.</i> , 2016

After the synthesis of nanoparticles, characterization of nanoparticles for composition, structure, shape and size is done for example FTIR (Fourier-transform infrared spectroscopy) is used to study [infrared spectrum](#) of [absorption](#) (Kumar *et al.* 2013). TEM analysis is done in order to observe direct visualization of morphological change in nanoparticle (Langmuir 2010). [X-ray diffraction](#) (XRD) is used to study the structure, composition, and physical

properties of materials. It also provides phase identification and characteristic of chemical composition. DLS (dynamic light scattering) used to determine the size distribution profile of nanoparticles in [suspension](#) (Berne, 2000). SEM (scanning electron microscope) and EDS were done in order to know the shape and purity of synthesized ZnO nanoparticles. EDS allows the elemental composition of the specimen to be measured.

2.4) Factors affecting synthesis of nanoparticles

1. pH

During biological synthesis of nanoparticles, pH of the reaction mixture plays very important role. There are many studies which show that changes in pH of reaction medium brings about some shape and size variabilities. During nanoparticle synthesis, at lower acidic pH conditions, particles with large size tend to be synthesized as compared to high pH values (Shah *et al.*, 2015). For example, rod shaped Au nanoparticles synthesized using *Avena sativa* (oat) tend to be formed at pH 2 with size range between 25-85 nm and smaller at pH 3 and 4 with size range between 5-20 nm.

2. Concentration of reactant

The concentration of biomolecule found in plant extract affects the synthesis of metallic nanoparticles. Huang *et al.* 2007 worked on sundried camphor (*Cinnamomum camphora*) leaf extract and found that triangular shape of nanoparticles changed to spherical when chloroauric acid was added to increasing concentration of extract (Shah *et al.*, 2015). The reactant concentration mainly affects the shape of nanoparticle being synthesized.

3. Reaction time

The reaction time varies with biological extract and the precursor used. It can vary between 30 min to n number of hours to produce a change in particle size. Ahmed *et al.* worked on pineapple (*Ananas comosus*) extract and found that the reaction time to synthesize spherical silver nanoparticle produced a rapid change in colour within 2 min (Ahmed *et al.*, 2012).

4. Reaction temperature

Size, shape and yield of nanoparticle synthesized depend on the temperature of reaction mixture. In some cases, when reaction temperature increases, particle conversion rate, particle formation rate and reaction rate increases while average particle size decreases (Shah *et al.*, 2015). For example, Kaviya *et al.*, in 2011 worked on *Citrus sinensis* (sweet orange) peel extract and found that at reaction temperature of 25°C, Ag NPs with average size of 35nm was produced. But when temperature was increased to 60°C, the average size of Ag nanoparticles reduced to 10nm.

CHAPTER 3

MATERIAL AND METHODS

31 Effect of different concentration of Zinc oxide (ZnO) nanoparticles on percentage seed germination of mung (*Vigna radiate*) and its growth (Dhoke et al., 2013)

- a) Mung seeds were surface sterilized by rinsing with 1% bavistin and 0.1% mercuric chloride and further rinsed with sterile water several times.
- b) The sterile seeds were transferred to petri plates having moist germination paper.
- c) The nanoparticles with concentration 0 ppm, 50 ppm, 100 ppm, 200 ppm, 500 ppm, 1000 ppm, were directly suspended in deionized water and were dispersed by using mechanical stirrer and ultrasonicator (100 W, 40 khz) for 30 min.
- d) The seeds were incubated in triplicates for 3 days at 30-35°C in dark conditions.
- e) After 3 days, percentage germination and radical length was recorded.

32 Effect of different concentration of Zinc oxide (ZnO) nanoparticles on percentage seed germination of wheat (*Triticum aestivum*) and its growth

- a) Wheat seeds used were surface sterilized by rinsing with 1% bavistin and 0.1% mercuric chloride and further rinsed with sterile water several times.
- b) The sterile seeds were transferred to petri plates having moist germination paper.
- c) The nanoparticles with concentration, 0 ppm, 50 ppm, 100 ppm, 200 ppm, 500 ppm, were directly suspended in deionized water and were dispersed by using mechanical stirrer and ultrasonicator (100 W, 40 khz) for 30 min.
- d) The seeds were incubated in triplicates for 7 days at 25°C in dark conditions.
- e) After 7 days, percentage germination and radical length was recorded.

33 Effect of different concentration of Zinc oxide (ZnO) nanoparticles on percentage seed germination of rice (*Oryza sativa*) and its growth

- a) Rice seeds used were surface sterilized by rinsing with 1% bavistin and 0.1% mercuric chloride and further rinsed with sterile water several times.
- b) The sterile seeds were transferred to petri plates having moist germination paper.

- c) The nanoparticles with concentration, 0 ppm, 50 ppm, 100 ppm, 200 ppm, 500 ppm, 1000ppm, were directly suspended in deionized water and were dispersed by using mechanical stirrer and ultrasonicator (100 W, 40 khz) for 30 min.
- d) The seeds were incubated in triplicates for 7 days at 25°C in dark conditions.
- e) After 7 days, percentage germination and radical length was recorded.

34 Synthesis of Zinc oxide (ZnO) nanoparticles from *Hematococcus* (Azizi et al., 2014)

- a) *Hematococcus* was inoculated in BG 11 medium with pH 7.0-7.3 and was incubated for 30 days at 25±2°C with light condition of 1500-3000 lux.
- b) After 30 days, the algal culture was centrifuged at 4000 rpm for 10 min. in order to obtain the biomass.
- c) The harvested biomass was collected and dried for 48 hrs at 37°C.
- d) Dried biomass was crushed using mortar and pestle and weighed.
- e) 0.78 gm of dried algal biomass was taken and dissolved in 100 ml of distilled water.
- f) It was heated at 80°C on hot plate for 6 hrs with continuous stirring.
- g) It was further filtered using whatmann filter paper no.1 and concentrate was collected to synthesize ZnO nanoparticles.
- h) 60 ml of filtrate was taken in round bottom flask and diluted by adding 40 ml of distilled water.
- i) It was kept in oil bath with fixed temperature of 80°C.
- j) 10ml of 0.1M zinc acetate was added dropwise as precursor when temperature risen to 70°C.
- k) Further, it was heated at 100°C with continuous stirring at 500 rpm for 6 hrs.
- l) After the completion of the reaction, the reaction mixture was centrifuged at 8000 rpm for 10 min at 4°C.
- m) Nanoparticles were washed with distilled water 3-4 times.
- n) Then dried in oven at 80°C overnight.

These biologically synthesized nanoparticles were further characterized for composition, structure, shape and size is done for example FTIR (Fourier-transform infrared spectroscopy) was done to study [infrared spectrum](#) of [absorption](#) and to study different functional groups (Kumar et al. 2013). [X-ray diffraction](#) (XRD) was performed in order to study the structure, composition, and physical properties of materials. SEM-EDS were done in order to know the

shape and purity of synthesized ZnO nanoparticles. EDS allows the elemental composition of the specimen to be measured.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Effect of different concentration of zinc oxide nanoparticles on the growth and percentage seed germination of mung (*Vigna radiate*)

Mung seeds were surface sterilized with 1% bavistin and 0.1% HgCl₂ and transferred to petri plates having moist germination paper. The nanoparticles with different concentrations i.e. 0 ppm, 50 ppm, 100 ppm, 200 ppm, 500 ppm, 1000 ppm were applied (Table 11) and seeds were incubated for 3 days at 30-35°C under dark conditions. After 3 days of incubation, percentage seed germination, length of plumule and radicle were recorded (Fig.7). It was found that with increase in zinc oxide nanoparticle concentration, length of radicle also increased (Fig.8). Maximum radicle length was seen at 1000ppm concentration and 100 % seed germination was recorded (F ig.9).



Fig 6: Effect of ZnO NPs on mung

Table 6: Effect of different concentration of ZnO NPs on the growth of mung

ZnO NPs (ppm)	Radicle (cm)	Plumule (cm)
0 ppm	24.93 ± 1.25	0.54 ± 0.13
50 ppm	25.32 ± 0.74	0.63 ± 0.06
100 ppm	28 ± 1.05	1.24 ± 0.24
200 ppm	29.74 ± 2.13	1.63 ± 0.49
500 ppm	31.33 ± 1.45	1.25 ± 0.17
1000 ppm	31.43 ± 1.28	1.63 ± 0.38

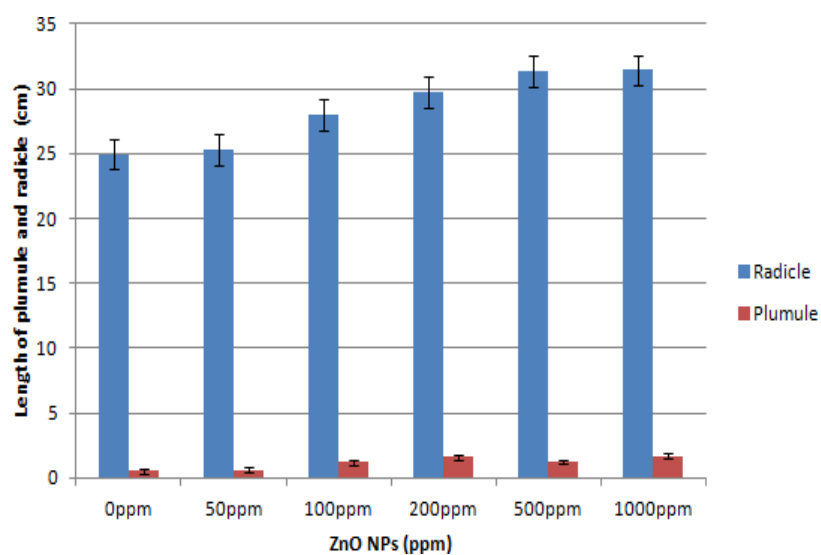


Fig 7: Effect of different concentration of ZnO NPs on the growth of mung

Table 7: Percentage seed germination in mung

ZnO NPs (ppm)	Percentage seed germination
0 ppm	100
50 ppm	100
100 ppm	100
200 ppm	100
500 ppm	100
1000 ppm	100

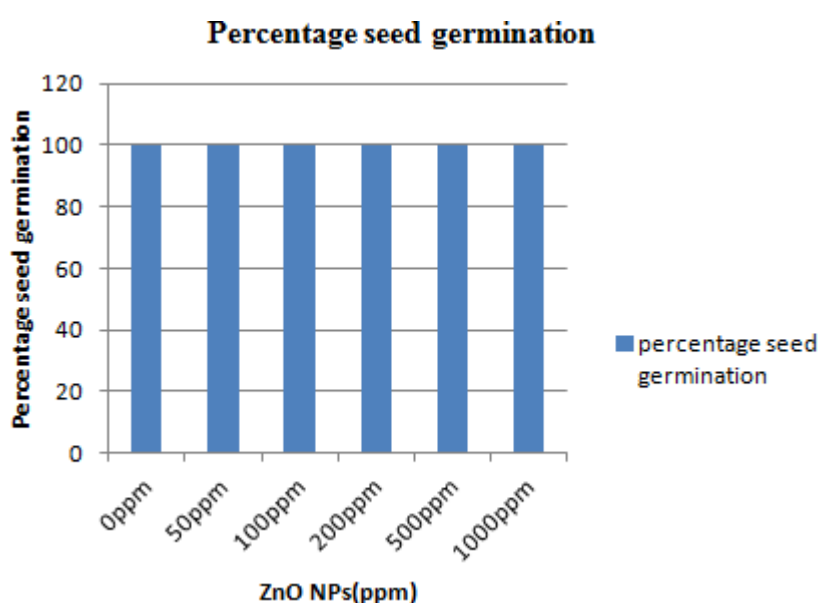


Fig 8: Percentage seed germination in mung

Increase in root-shoot length at 1000 ppm compared to that of control was observed when 400, 1000 and 2000 ppm ZnO NPs were applied *via* foliar spray (Prasad *et al.*, 2012). In this study ZnO nanoparticles (25nm) were chemically synthesized using oxalate decomposition technique and found that at 1000 ppm there was increase in root length and inhibitory effect at 2000 ppm. On comparing with our results, green synthesized NPs show increase in radicle length at 1000ppm compared to control and are nontoxic, biocompatible and are free of impurities (Agarwal *et al.*, 2017). Seeds coated with ZnO NPs show less wastage as compared to foliar spray method.

4.2 Effect of different concentration of zinc oxide nanoparticles on the growth and percentage seed germination of wheat (*Triticum aestivum*)

Wheat seeds used were surface sterilized with 1% bavistin and 0.1% HgCl₂ and transferred to petri plates having moist germination paper. The nanoparticles with different concentrations i.e. 0 ppm, 50 ppm, 100 ppm, 200 ppm, 500 ppm were applied and seeds were incubated for 7 days at 25°C under dark conditions (Table 12). After 7 days of incubation, percentage germination and length of plumule and radicle was recorded. It was found that with increase in zinc oxide nanoparticle concentration, length of radicle also increased (Fig.10). Maximum radicle length was seen at 500 ppm concentration and there was hardly any significant difference observed in plumule length (Fig.11). 96.6% percentage germination was recorded at 0 ppm and 50 ppm where as 100% percentage germination was recorded at 100 ppm, 200 ppm and 500 ppm (Fig.12, Table 13)

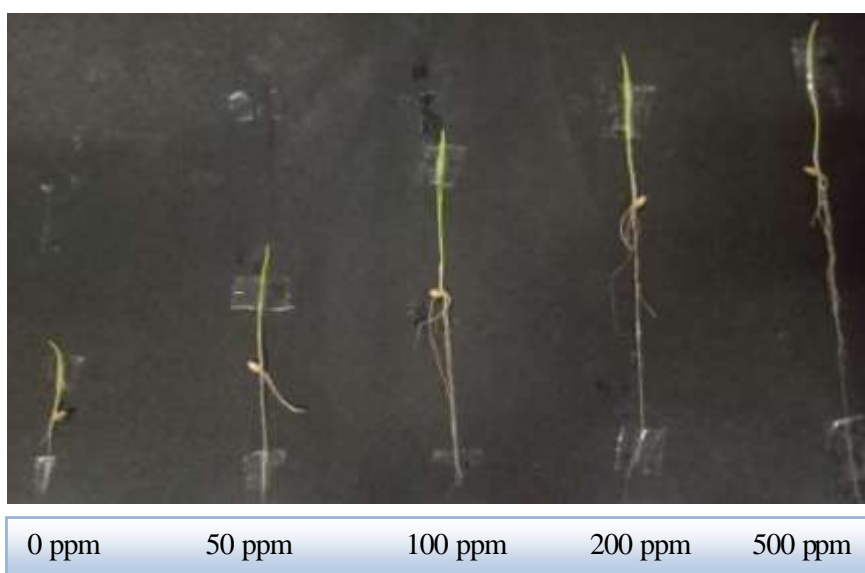


Fig 9: Effect of ZnO NPs on wheat seedlings

Table 8: Effect of different concentration of ZnO NPs on the growth of wheat

ZnO NPs (ppm)	Radicle (cm)	Plumule (cm)
0 ppm	28.9 ± 0.733	17.59 ± 0.738
50 ppm	31.39 ± 0.869	18.53 ± 0.531
100 ppm	33.01 ± 1.191	18.76 ± 0.532
200 ppm	33.9 ± 1.029	19.38 ± 0.742
500 ppm	35.29 ± 1.021	19.08 ± 0.982

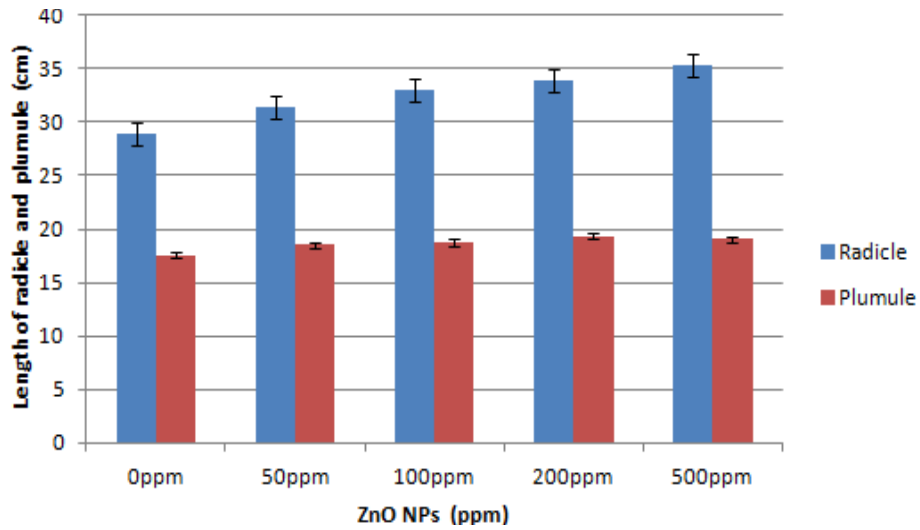


Fig 10: Effect of different concentration of ZnO NPs on the growth of wheat

Table 9: Percentage seed germination in wheat

ZnO NPs (ppm)	Percentage seed germination
0 ppm	96.66
50 ppm	96.66
100 ppm	100
200 ppm	100
500 ppm	100

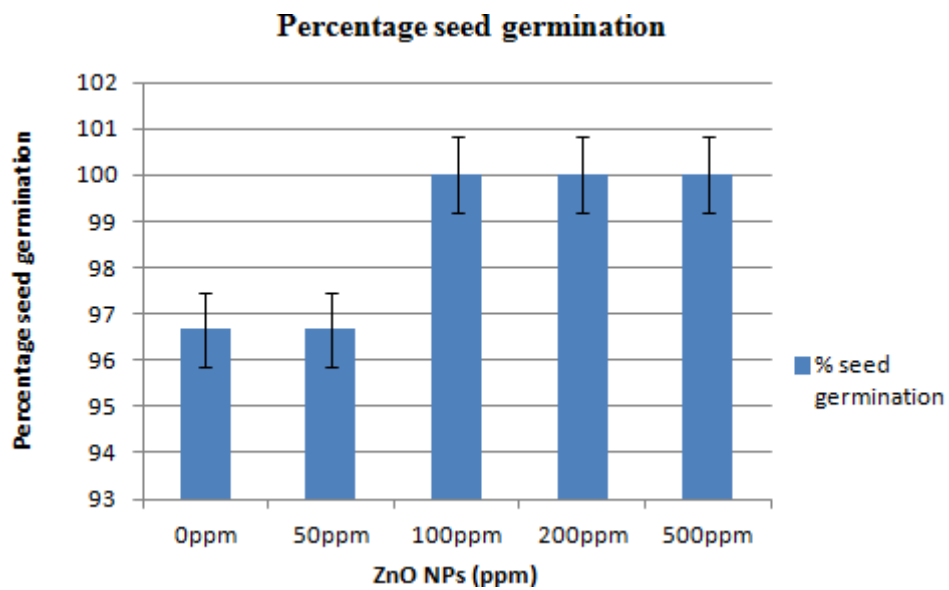


Fig 11: Percentage seed germination in wheat

The results are related to work done by Lee (2013) on buckwheat who observed that the growth of roots was inhibited at high concentration. Chemically synthesized nanoparticles at 2000 ppm and 4000 ppm, showed toxic effect on root length. On comparing results, green synthesized nanoparticles showed maximum growth at 1000 ppm indicating beneficial effect at high concentration and had some effect on seed germination in all treatments. Thus it can be concluded that green synthesized nanoparticles at high concentration (1000 ppm) are nontoxic, less hazardous and free of impurities (Agarwal *et al.*, 2017). So it can be used as a fertilizer for increasing the level of zinc in soil. As per the literature, the level of zinc in normal soil ranges between 10-300 ppm (Jala *et al.*, 2006).

4.3 Effect of different concentration of zinc oxide nanoparticles on the growth and percentage seed germination of rice (*Oryza sativa*)

Rice seeds used were surface sterilized with 1% bavistin and 0.1% HgCl₂ and transferred to petri plates having moist germination paper. The nanoparticles with different concentrations i.e. 0 ppm, 50 ppm, 100 ppm, 200 ppm, 500 ppm, 1000 ppm were applied and seeds were incubated for 14 days at 25°C in dark conditions. After 14 days of incubation, percentage seed germination, length of plumule and radicle were recorded (Table 14). It was found that, maximum radicle length was observed at 100 ppm concentration where as maximum plumule length was observed at 50 ppm concentration (Fig.14). 100% seed germination was observed at all concentrations as compared to control which indicated no significant effect (Table 15).

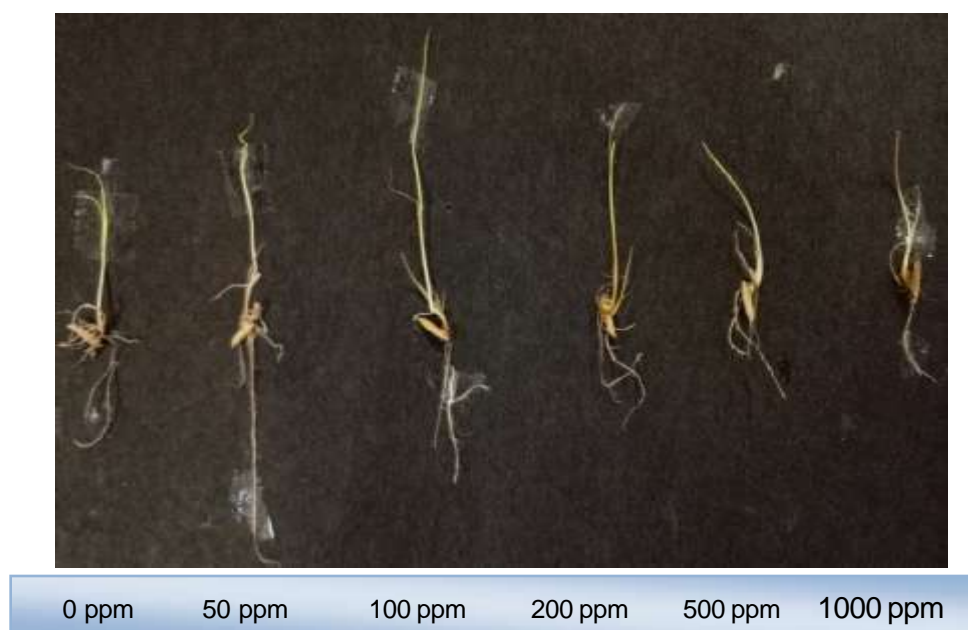


Fig 12: Effect of ZnO NPs on rice

Table 10: Effect of ZnO NPs on rice

ZnO NPs (ppm)	Radicle (cm)	Plumule (cm)
0 ppm	16.32 ± 0.63	18.14 ± 0.58
50 ppm	17.58 ± 0.768	22.63 ± 0.918
100 ppm	18.79 ± 0.429	19.55 ± 0.918
200 ppm	16.26 ± 0.547	19.48 ± 1.104
500 ppm	12.19 ± 0.507	18.98 ± 0.252
1000 ppm	12.00 ± 0.251	16.99 ± 0.803

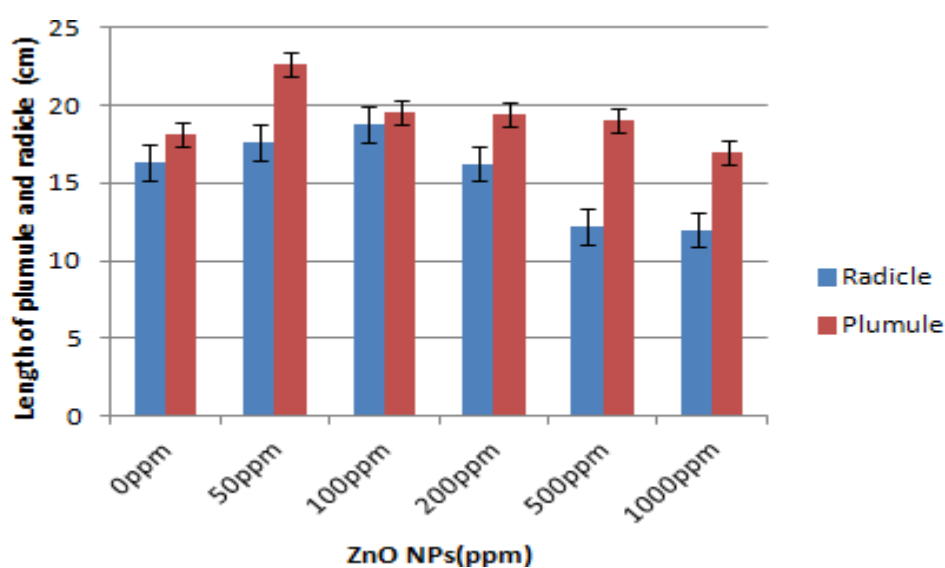


Fig. 13: Effect of ZnO NPs on the growth of plumule and radicle

Table 11: Percentage seed germination of rice

ZnO NPs (ppm)	Percentage seed germination
0ppm	96.66
50ppm	96.66
100ppm	100
200ppm	100
500ppm	100
1000ppm	100

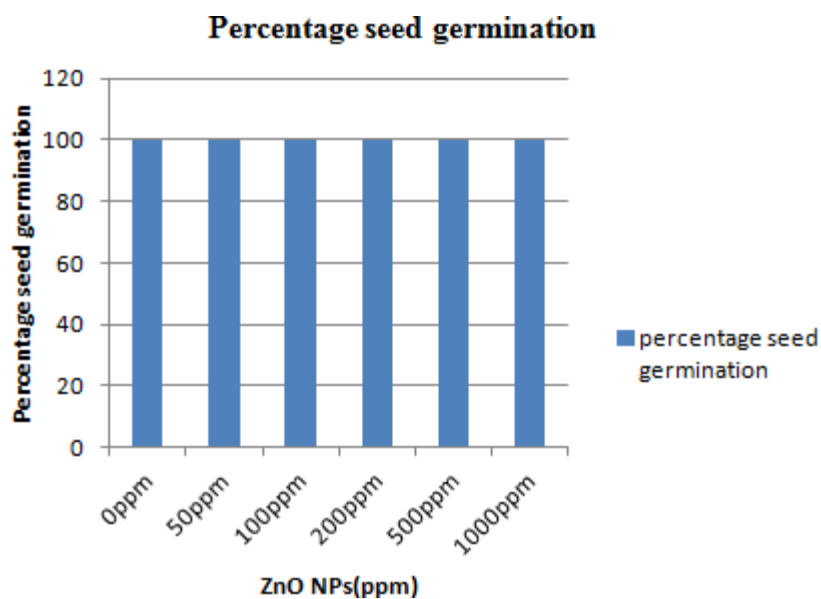


Fig. 14: Percentage seed germination in rice

The results are related to the work done by Boonyanitipong (2011) on rice, who observed that toxicity of ZnO NPs increased with increase in its concentration. Chemically synthesized nanoparticles at higher concentration i.e. 1000 ppm showed toxic effect on root length. The above study done on rice explained that our green synthesized nanoparticles show best results at less concentration i.e. 50 ppm and are nontoxic, free of impurities. In both studies, 100% seed germination was observed at all treatments which show that ZnO nanoparticles had no effect on rice seed germination.

It was concluded that, different seeds gave different response to ZnO NPs which is primarily a micronutrient. Improvement in seedling growth in presence of ZnO NPs as compared to control clearly shows its impact as one of essential micronutrient requirement for plant growth. ZnO NPs as such had no effect of seed germination of rice and mung where as in wheat, increased percentage seed germination with increase in nanoparticle concentration as compared to control was observed.

4.4 Synthesis of Zinc oxide nanoparticles from *Haematococcus*

Haematococcus was inoculated in BG 11 medium with pH 7.0-7.3 and was incubated for 30 days at $25\pm 2^\circ\text{C}$ with light condition of 1500-3000 lux. After 30 days, algal biomass was harvested and allowed to dry for 48 hrs at 37°C . The dried biomass was mixed with distilled water and heated at 80°C on hot plate for 5 hrs at stirring condition. Filtrate was collected in order to synthesize ZnO nanoparticles. During the synthesis, 0.1M zinc acetate was added as

precursor and the reaction was kept on continuous stirring at 500 rpm for 6 hrs at 80°C. The formation of ZnO NPs was confirmed through change in colour of the reaction mixture from green to pale white colour. Pellets were obtained and allowed to dry at 80°C overnight. There are some bioreducing agents like enzymes, proteins, flavanoids, terpenoids, cofactor etc which help in reducing metal oxide to form nanoparticles. These green synthesized nanoparticles take less time for synthesis, are less toxic, ecofriendly and free of impurities. Light green ZnO NPS were obtained. The ZnO NPs sample was characterised by XRD, SEM-EDS and FTIR.



Fig 15: Pellets of Zinc oxide nanoparticles synthesized from *Haematococcus*.

4.5 XRD

The X-ray diffraction patterns of ZnO nanoparticles synthesised from *Haematococcus sp.* are shown in Fig. 16. Sharper and stronger diffraction peaks were observed from Fig. 16 at 31.1°, 32.1°, 33.33°, 35.1°, 36.8°, 38.0°, 42.2°, 45.1°, 68.1°, 72.2°, 75.1°. The appearance of signals was likely due to X-ray emission from carbohydrates/proteins/enzymes present in the cell wall of the biomass (Parial *et al.*, 2012). The shift in the 2θ peak values of ZnO nanoparticles may be due to the presence of protein molecule from algal culture filtrate (Parial *et al.*, 2012). The average crystallite size of the nZnO synthesised from *Hematococcus* was 31 nm as calculated by Debye sherrer formula (West, 1974).

Debye sherrer formula $D = K\lambda / \beta_{1/2} \cos\theta$

Where, K is the Sherrer constant (K=0.89 for spherical particle)

λ is the X-ray wavelength ($\lambda=1.00 \text{ \AA}$)

$\beta_{1/2}$ is the width of the XRD peak at half height

θ is the Bragg diffraction angle

After substituting these values,

$$D = 0.9 (1.54060) / (0.451) (\cos 0.3170) = 30.76 \text{ nm}$$

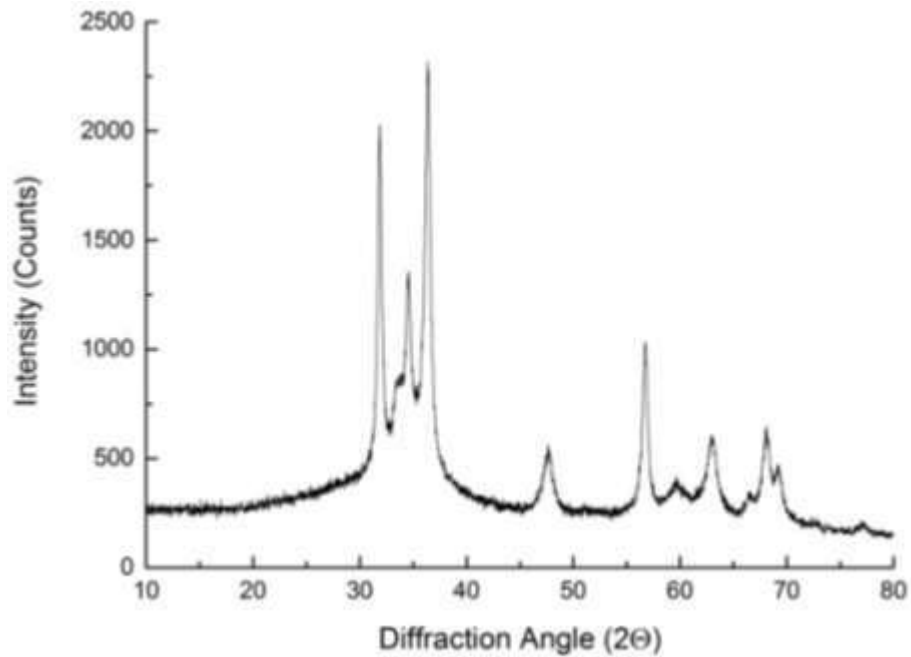


Fig. 16: X-ray diffraction patterns of ZnO nanoparticles synthesized from *Haematococcus*

4.6 SEM-EDS

SEM studies were done in order to examine the morphology of synthesized ZnO NPs. Fig. 17 shows the SEM image showing morphology and topology of ZnO nanoparticles which are almost spherical in shape with agglomeration. This agglomeration is due to polarity and electrostatic attraction of ZnO NPs (Azizi *et al.*, 2014). The size of nanoparticle cannot be estimated due to compaction in arrangement.

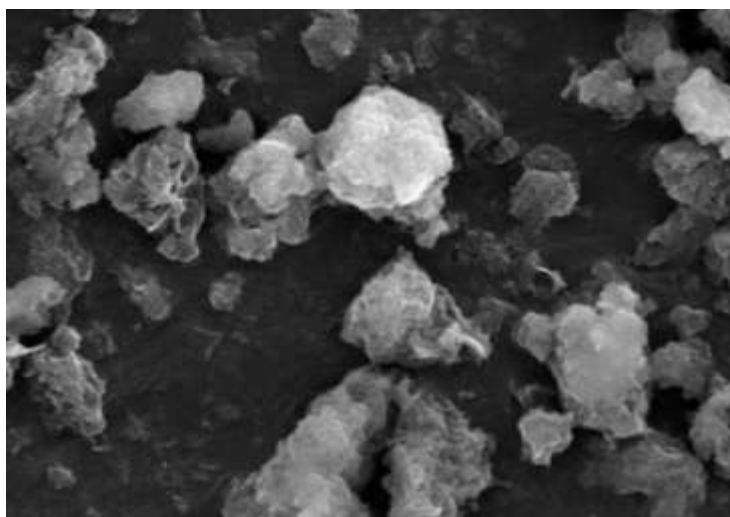


Fig. 17: SEM image of ZnO nanoparticles synthesized from *Haematococcus*

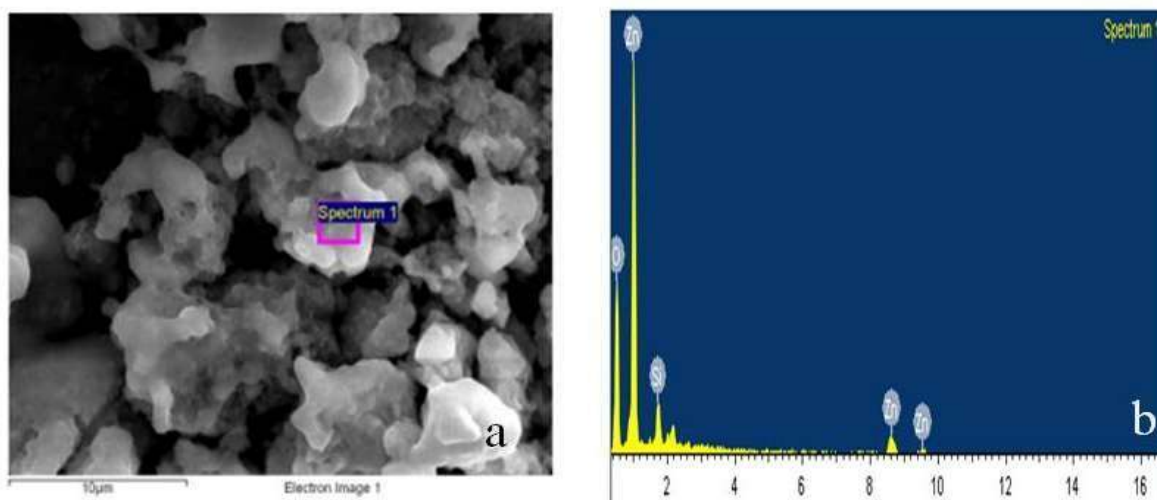


Fig. 18: EDS image of ZnO nanoparticles synthesized from *Haematococcus*

EDS micrograph shown in Fig. 18 indicate elemental composition of nanoparticle synthesised, thus indicating the purity of ZnO compound and it was found to be 99% pure. However there were some residues of silica (SiO_2) which were also found.

4.7 FTIR

Fig.18 shows FTIR spectra of green synthesized ZnO NPs from *Haematococcus*. FTIR analysis detects various peaks which indicate different characteristics functional groups associated with the synthesized nanoparticles. It is inferred from Fig.18 that absorption peaks in the range 3397.42 cm^{-1} , 2930.98 cm^{-1} , 1562.61 cm^{-1} , 1397.07 cm^{-1} , 1249.33 cm^{-1} , 972.41 cm^{-1} and 464.20 cm^{-1} represents various functional groups. The absorption peak at

3397.42 cm^{-1} corresponds to stretching vibration of O-H bond of hydroxyl group. The peak at 2930.98 cm^{-1} , 1397.07 cm^{-1} and 972.41 cm^{-1} corresponds to stretching vibration of C-H bond. The absorption peak at 1249.33 cm^{-1} indicates bond stretch of C-N bond of primary amine. The absorption peak at 972.41 cm^{-1} corresponds to C-O bond of primary alcohol. The absorption peak at 1562.62 cm^{-1} corresponds to N-O bond. The absorption peak at 464.20 cm^{-1} corresponds to metal oxide i.e. ZnO finger print region which confirms the synthesis of ZnO NPs. These characteristic functional groups indicate the presence of some proteins, polysaccharides, biomolecules which act as capping agents or stabilizing agents present in the extract of *Haematococcus* (Sharma *et al.*, 2015).

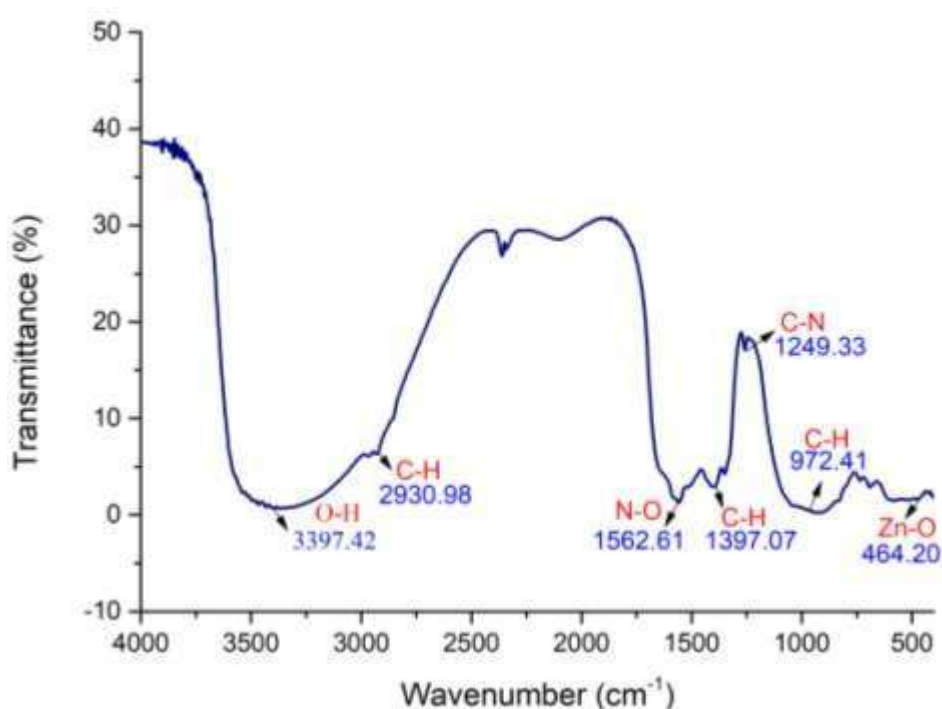


Fig. 19: FTIR spectra of ZnO nanoparticles synthesized from *Haematococcus*

CONCLUSIONS

- 1) The present study demonstrated effect of ZnO nanoparticles on the growth and percentage seed germination of plant seedlings of mung (*Vigna radiate*), wheat (*Triticum aestivum*) and rice (*Oryza sativa*) under *in vitro* conditions. The study was carried out in triplicates with different concentrations of ZnO nanoparticles in suspension form under dark conditions at 25°C-35°C.
- 2) In wheat, at 500 ppm of ZnO NPs promoted maximum radical growth and 96.6% seed germination was recorded at 0ppm and 50 ppm followed by 100% seed germination at 100-500 ppm of ZnO NPs after 7 days of incubation.
- 3) In mung, after 3 days of incubation in presence of 1000 ppm of ZnO nanoparticle, there was increase in radical growth and 100% seed germination was observed at all tested concentrations. In rice maximum radical growth was observed at 100 ppm followed by maximum plumule length at 50 ppm. 100% seed germination was observed after 14 days of incubation at all tested concentrations of ZnO NPs.
- 4) Results indicated that Zinc oxide (ZnO) nanoparticles induce beneficial changes in wheat and mung, where as exhibits somewhat toxicity on rice roots as indicated by root length at high concentration.
- 5) Zinc oxide (ZnO) nanoparticles using zinc acetate as metal precursor were synthesized from algae *Hematococcus sp.* Nanoparticles are most versatile materials, due to their diverse properties, functionalities, and large surface area. ZnO NPs as nanofertilizers for crops can affect percentage seed germination and improves plant growth.
- 6) Zinc oxide nanoparticles synthesised from *Hematococcus* were characterized by FTIR, XRD and SEM. Sharper and stronger diffraction peaks were observed in XRD analysis and the average crystallite size of the nZnO synthesised by *Hematococcus* was 31 nm as calculated by Debye sherrer formula.
- 7) The FTIR spectra of ZnO NPs synthesized from *Haematococcus* indicated various characteristic peaks representing different functional groups. The absorption peak at 464.20 cm^{-1} corresponds to metal oxide i.e. ZnO finger print region which confirms the synthesis of ZnO NPs.

8) SEM image showing morphology and topology of ZnO nanoparticles which are almost spherical in shape with agglomeration. Size cannot be detected due to compact arrangement. EDS micrograph indicate the purity of ZnO compound and it was found to be 99% pure. However there were some residues of silica (SiO_2) which were also found.

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APPENDIX 1

Table 12: BG11 Medium composition

Constituents	Quantity (mg/l)
NaNO ₃	1.5g
K ₂ HPO ₄	0.04g
MgSO ₄ .7H ₂ O	0.075g
CaCl ₂ .2H ₂ O	0.056g
Citric acid	0.006g
Ferric ammonium citrate	0.006g
EDTA (disodium salt)	0.001g
Na ₂ CO ₃	0.02g

Table 13: Trace metal mix A5

Constituents	Quantity (mg/l)
H ₃ BO ₃	2.86g
MnCl ₂ .4H ₂ O	1.81g
ZnSO ₄ .7H ₂ O	0.39g
CuSO ₄ .5H ₂ O	0.079g
CO(NO ₃) ₂ .6H ₂ O	49.4mg

Table 14: Dilution of ZnO nanoparticle (ppm) used in mung seeds

Stock of ZnO nanoparticles = 30mg ZnO + 30ml dis. H₂O (1000ppm)

Nanoparticle conc. (ppm)	dH ₂ O (ml)	Amount of stock (ml)
0ppm	10	0
50ppm	9.5	0.5
100ppm	1	9
200ppm	2	8
500ppm	5	5
1000ppm	10	0

Table 15: Dilution of ZnO nanoparticle (ppm) used in rice seeds

Stock of ZnO nanoparticles = 30mg ZnO + 30ml dis. H₂O (1000ppm)

Nanoparticle conc. (ppm)	dH₂O (ml)	Amount of stock (ml)
0ppm	15	0
50ppm	14.25	0.75
100ppm	13.5	1.5
200ppm	12	3
500ppm	7.5	7.5
1000ppm	15	0

Table 16: Dilution of ZnO nanoparticle (ppm) used in wheat seeds

Stock of ZnO nanoparticles = 30mg ZnO + 30ml dis. H₂O (1000ppm)

Nanoparticle conc. (ppm)	dH₂O (ml)	Amount of stock (ml)
0ppm	10	0
50ppm	9.5	0.5
100ppm	1	9
200ppm	2	8
500ppm	5	5