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REVIEW ARTICLE

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Green-Synthesized Zinc Oxide Nanoparticles as Nano-Zinc Fertilizers for Improving Crop Yield and Quality

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ABSTRACT

Green synthesis of metal oxide nanoparticles is an eco-friendly and pollution-free approach. It involves incubation of cell-free extracts of diverse living organisms such as prokaryotic eubacteria, and eukaryotic unicellular yeast to multicellular mold fungi and higher plants or their extracts. Zinc oxide nanoparticles (ZnONPs) are metal oxide nanoparticles that possess unique optical-chemical, and semi-conductor properties. On treatment of crop seeds, foliar sprays, and soil application, the ZnO NPs exhibit improvement in seed germination rates, seedling growth, yield attributing characters, and yield of the treated crop plants. This manuscript explores the biotechnological aspects of green synthesis of zinc oxide nanoparticles and their application as zinc micronutrient fertilizer to improve the Zn-content of the produce besides incentivizing the soil health.

Keywords: Green or Biogenic Synthesis, Micro-Nutrient, Nanotechnology and Zinc Oxide Nanoparticles.

INTRODUCTION

Zinc is the 23rd most abundant element in the Earth's crust. It exists in the form of an indivalent cation (Zn^{2+}) as the common stable oxidation state. Zinc plays a vital role as a component of enzyme/ co-enzyme molecules involved in several physiological processes and is desirable for ensuring proper growth and development of both plant and animals (Sidhu et al., 2016). For the crop plants, among the seventeen essential micronutrients, zinc is an important and secondary micronutrient required by the plants which are taken up by the root hair cells either as a divalent cation or through formation of organic ligand-complexes (Das et al., 2013). In plants, Zn plays many catalytical functions for several enzymes such as oxidases, dehydrogenases, anhydrases and peroxidases. It is also important for the biosynthesis of carbohydrates, proteins, growth hormones and maintenance of cell membrane integrity (Broadley et al., 2012).

In agricultural field, farmers face various kind of macronutrient (N,P,K) and micronutrient such as iron (Fe), manganese (Mn), cobalt (Co), copper (Cu), and zinc (Zn) deficiency symptoms in crops. Among them zinc has been identified as the fourth most yield-limiting micronutrient and its deficiency is the most worldwide deficiency problem in crops as well as in soils. The average level of Zn deficiency has been anticipated to be approximately 50% which may increase to 63% by 2025. Zn deficiency is influenced by a variety of factors such as pH, organic matter, precipitation or adsorption of zinc with other soil particle, redox potential and chemical applications (Kabir et al., 2014). Mostly, plants exhibit Zn deficiency as a bouquet of several complications such as stunted growth, chlorosis of leaves, shortened internodes and petioles, and clustering of small malformed leaves at top of the plant (classic rosette symptom of dicotyledons) (Brennan et al., 1993). It also leads to reduction in crop yields and quality, even without the appearance of Zn deficiency symptoms in plants which is hidden deficiency (Alloway, 2009). Therefore, the Zn deficiency manifested as stunted growth of plants, can lead to decrease in grain yields, reduction in ability of the plant to protect itself from diverse abiotic and biotic stresses, and lowering of the nutritional quality of the produce (Taheri et al., 2015).

Zn deficiency can be corrected by the application of Zn containing fertilizer such as zinc sulphate (ZnSO_4), zinc oxysulfates (ZnO), inorganic Zn complexes, synthetic chelates and natural organic complex (Mortvedt, 1992). These Zn-fertilizers can increase both yield and quality of several crops like wheat, rice and pea (Liu et al., 2003). Water soluble ZnSO_4 applied to soils undergoes loss and fixation as insoluble form due to several factors such as leaching, formation of complexes organic ligand with soil components and precipitation with different anions to form Zn salts thereby reducing nutrient use efficiency by being unavailable to plants (Ram et al., 2018). Therefore, novel Zn fertilizer formulations have to be developed by new emerging field of science and technology known as 'Nanotechnology'. Further, these novel Zn nanofertilizers should also be evaluated for features such as greater uptake efficiencies and sustained optimum release of micronutrient in an available form for longer durations (Kalia and Kaur, 2019a). Zinc oxide nanoparticles (ZnONPs) are metal oxide nanoparticles which have unique optio-chemical, semiconducting and catalytic, photochemical and antimicrobial activities. The use of zinc oxide nanoparticles for their fertilizer nutrient properties as source of zinc micronutrient are emerging as a possible alternative to conventional Zn fertilizer (Kalia and Kaur 2019b). Thenanoscale or nanostructured Zn nanofertilizers can provide Zn nutrient in an improved efficient manner leading to an enhancement in crop yield, decreased toxicity to the soil components and hence, decreasing the costs of environmental protection (Naderi and Danesh-shahraki, 2013).

Green synthesis of zinc-oxide nanoparticles

The ZnONPs can be synthesized by following various chemical and physical methods including sol-gel, micro-emulsion, sonochemical and chemical reduction techniques. All these methods involve use of toxic chemicals, requirement for higher working temperatures and high pH conditions culminating to heightened biological and environmental risks (Ong et al., 2018). Therefore, to avoid the use of toxic chemicals, researchers have developed alternative methods collectively termed as 'Green synthesis or biogenic synthesis'.

These methods involve utilization of pollutant-free chemicals for the synthesis of ZnO NPs and the use of ecofriendly and safe solvents such as water, natural extracts for the synthesis of ZnO NPs (Table 1). The green synthesis of ZnO NPs can be performed by two ways: first one is the use of cell free extracts of various types of unicellular too multicellular microorganisms such as bacteria, fungi, actinomycetes, yeast, algae and second one is with the use of plants or their extracts (Lakshmi et al., 2017). Therefore, the ZnO NPs can be generated from renewable biological components derived from diverse living organisms (Mohamedy et al., 2019). These green synthesis techniques have an edge over the conventional chemical and physical methods due to their cost effectiveness, and negligible to lesser toxicity because of coating of synthesized ZnO NPs with biological moieties (Figure 1) (Kaur et al., 2016a).

Synthesis of ZnONPs using microorganisms

The synthesis of zinc oxide nanoparticles by various types of microbes may involve combination of several activities, including the basic cell biochemistry, the mechanisms involved in transportation of the ionic metal both in and out of the microbial cells, interplay of diverse microbial resistance mechanisms to toxic/ heavy metals and intra-cellular accumulation of the metallic ions leading to nucleation of metal-oxides (Hussein et al., 2009). Therefore, microorganisms exhibit enormous diversity at various hierarchies of cellular biochemistry, physiology, and macromolecular entities which ensure the synthesis of ZnO NPs possessing different morphologies and hence, functionalities. Moreover, a specific biomolecule may perform dual function of being the template for the synthesis of the ZnO NPs besides acting as a capping agent such that the synthesized ZnO NPs get coated by the biomolecule. Shamsuzzaman et al. (2017) explained the simple green process for biosynthesis of ZnO NPs by using *Candida albicans* as a capping and reducing agent. These nanoparticles exhibited rapid and efficient production of steroidal pyrazolines when used as a catalyst. Such phenomena of biotemplating and coating of the NP surface may affect the ZnO NP dissolution kinetics, release and availability of Zn^{2+} ions by the ZnO NPs, zeta potential and ability to trespass intact membranes and other barriers.

Spherical shaped ZnONPs with average particle diameters of 7-19 nm were achieved by using a probiotic bacterium, *Lactobacillus plantarum* VITESO7 (Salvarajan and Mohanasarinivasam, 2013). Likewise, stable and roughly spherical ZnONPs with particle size of about 100-120 nm have been reported to be synthesized from *Rhodococcus pyridinivorans* NT2 (Kundu et al., 2014). However, stable but hexagonal to spherical shaped ZnONPs with particle size range within 7-19 nm have been reported to be obtained with the help of *Bacteria subtilis* (Alkaim et al., 2017). Contrarily, Kalpana and Rajeswari (2018) have reported synthesis of complex ZnO NP morphologies i.e. raspberry and plate-like structures by simple thermal decomposition of zinc acetate soluble salt in the presence of *Bacillus cereus* as a biotemplating agent.

Fungus-mediated synthesis of metallic nanoparticles has been rather lucrative approach as fungal cultures exhibit high metal tolerance capabilities, besides production of a greater number of enzymes and proteins which are generally secreted extracellularly, economic viability, and convenient downstream processing (Sangappa et al., 2013).

Therefore, fungus-mediated NP synthesis ensures considerably high NP productivity. It further ensures coating of the surface of the synthesized NPs with the secreted biomolecules thereby imparting high stability through prevention of the agglomeration of the nanoparticles (Jain et al., 2011). Kadam et al., (2019) have reported synthesis of ZnONPs by use of mycelia free extracts of endophytic fungus *Cochltobolus geniculatus*. The synthesized ZnONPs exhibited quasi-spherical morphologies with high polydispersity. Baskar et al., (2013) obtained spherical ZnO NPs with a size range of 54.8 to 82.6 nm from *Aspergillus terreus* filtrate which were synthesized extracellularly.

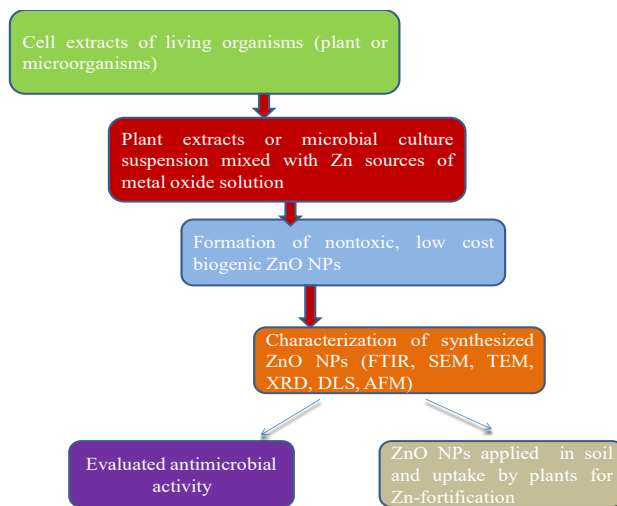


Figure 1. Different steps for green synthesis of zinc oxide nanoparticles.

Biosynthesis of ZnONPs through plant extracts

The use of a variety of plants for the synthesis of nanoparticles is a rapid, cost-effective, and eco-friendly option ensuring safety for the environment as well as human health from harsh chemical wastes produced on chemical synthesis of ZnO NPs industrially or at mass scale (Rajkumar et al., 2017). Extracts of *Vitex negundo* were utilized for production of ZnO NPs with zinc nitrate hexahydrate as a precursor. The biosynthesized ZnO NPs showed antimicrobial activities against *E.coli* and *S. aureus* bacteria (Ambika et al., 2015). Dobrucka and Dugaszewska (2016) reported synthesis of hexagonal-shaped ZnO NPs with size ranging from 60-70 nm by using *Trifolium pratense* extracts. Nava et al., (2016) reported low cost, nontoxic green synthesis of ZnO NPs by using *Camellia sinensis* extract. Likewise, green tea leaf extract synthesized ZnO NPs exhibited proved capacitance behavior on evaluation of their supercapacitor applications (Dhanemozhi et al., 2017). The aqueous peel extract of *Aloe vera* led to synthesis of hexagonal shaped ZnO NPs with particle size ranging between 50 to 220 nm which possessed appreciable antimicrobial activities against pathogenic bacteria like, *Staphylococcus epidermidis*, *Klebsiella pneumonia* and *E.coli* and fungi such as *Aspergillus niger* and *A. oryzae* (Chawdhary et al., 2018).

Table 1. Green synthesis of Zinc Oxide nanoparticles by incubation of soluble zinc salts with cell or cell free extracts of different types of organisms.

Organism	Size and shape of nanoparticles	Potential activity studied	Reference
Bacteria			
<i>Bacillus subtilis</i>	Stable hexagonal to spherical shapes, particle size ranging from 7-19 nm	Antimicrobial activity against methicillin-resistant <i>Staphylococcus aureus</i> and <i>E. coli</i>	Alkaim et al., 2017
<i>Bacillus circulans</i> MTCC 7906	Spherical shape, particle size 50 to 85 nm	Antimicrobial activity against gram positive and negative bacteria	Kaur et al., 2016b
<i>Rhodococcus pyridini</i> voransNT2	Spherical shapes, particle size ~100-200nm	Antimicrobial activity	Kundu et al., 2014
Fungus			
<i>Pleurotus florida</i>	Spherical shaped, particle size 10-20 nm	Antimicrobial activity	Kaur et al., 2016a
<i>Aspergillus fumigatus</i>	Spherical with particle size range within 60-80 nm.	Antibacterial activity	Rajan et al., 2016
<i>Aspergillus terreus</i> VIT	Spherical shape, particle size ranging from 28-82 nm	Antifungal activity against fungal food pathogens such as <i>Micrococcus</i> sp, <i>Bacillus</i> sp, <i>Penicillium</i> sp.	Sangappa et al., 2013
<i>Aeromonas hydrophila</i>	Spherical to oval morphology, particle size 57 nm.	Antimicrobial activity	Jayseelan et al., 2012
Plants			
<i>Lycopersicon esculentum</i>	Spherical shape, particle size ranging from 40-100nm	Used for photovoltaic application	Saha et al., 2016
<i>Azadirachta indica</i>	Spherical shaped particles	Antibacterial and photocatalytic activity against gram negative bacteria	Bhuyan et al., 2015
<i>Trifolium pretense</i>	Hexagonal morphology, particle size about 60-70 nm	Antimicrobial efficacy against <i>S. aureus</i> and <i>P. aeruginosa</i>	Dobrucka and Dlugaszewska, 2015
<i>Allium cepa</i> (bulb), <i>Allium sativum</i>	Hexagonal wurtzite, particle size 70 nm	Photodegradation of methylene blue	Elumalai et al., 2015
<i>Solanum nigrum</i> (leaves)	Quasi-spherical morphology, particle size 29 nm	Antibacterial activity against pathogenic <i>Salmonella paratyphi</i> , and <i>Vibrio cholera</i>	Ramesh et al., 2015
<i>Citrus aurantifolia</i>	Wurtzite hexagonal morphology, particle size 50-200 nm		Samat et al., 2012

Fakhari et al., (2019) have reported synthesis of spherical shaped ZnONPs with average particle size of 21.49 and 25.26 nm diameter by use of *Laurus nobilis* L. leaf extracts in presence of two different zinc salts (zinc acetate, and zinc nitrate) as precursors, respectively. There are two fundamental problems related to green synthesis of ZnO NPs; foremost is polydispersity of the nanoparticles and the other is requirement of longer durations (atleast 24 hours) for the synthesis of ZnO NPs. Both of these constraints regarding synthesis of uniform size and shaped ZnO NPs can be over-ruled by following the thermal methods with use of microwave irradiation.

Fertilizer action of zinc oxide nanoparticles

The applications of chemical fertilizers are leads to enhanced food grain production. However, negative impacts of continuous application of conventional synthetic fertilizers have dramatically resulted in cropping up of stern issues in crop plants such as decreased fertilizer response rate, development of soils ailing with multi nutrient deficiencies, imbalanced fertilization, decrease in crop productivity and yield. Therefore, the sustainable agriculture dogma demands development and application of innovative fertilizers, 'nanofertilizers' or novel fertilizer application modules/ techniques. These approaches will be most promising techniques to significantly increase crop productivity (Subramanian and Karsu, 2017). Nanofertilizers can be formulated as nano-scaled nutrients particularly micronutrients like ZnO NP fertilizers or soluble nutrients can be loaded on/ encapsulated with the nanoparticles to deliver nutrient(s) to crops in a use-efficient manner (De Rosa et al., 2010). Nanofertilizer due to their small size, high surface to volume ratios are more effective than conventional fertilizers. These fertilizers are anticipated to provide sustainable nutrient delivery systems which will exhibit improved nutrient uptake through the crop on account of exploitation of nanoporous surface of the plant parts on plant surfaces (Kalia and Kaur, 2019a). These fertilizers can be formulated as nutrient encapsulating nanoparticles, nutrient adsorbed or intercalated in nanoclays/nano-zeolites and nano-scale engineered nanoparticles like ZnONPs (Elemike et al., 2019). ZnONPs are one of the most commonly used metal-oxide nanoparticles, which possess ultra-small size and exhibit high reactivity and photocatalytic properties compared to the bulk ZnO. Thus, nano-sizing will affect the Zn solubility, and diffusion of Zn nutrient and hence higher availability to plants for better growth and development (Milani et al., 2010).

There are plenty of reports advocating application of ZnONPs as fertilizer in various crops for improving productivity and quality of the produce (Table 2). Adhikari et al., (2011) reported that application of ZnONPs in maize at lower concentration (0.5 ppm) increased plant parameter such as plant height, root length and volume, dry matter compared to bulk ZnSO₄ (0.5ppm). Prasad et al., (2012) reported that seed treatment with zinc oxide (ZnO) at 1000ppm enhanced seed germination, seedling vigor, early flowering and chlorophyll content of leaf in groundnut. Zhao et al.,(2013) have reported that application of 400-800 mg kg⁻¹ZnONPs to a soil mixture enhanced plant dry weight mass, dry weight of fruits, starch content, gluten and Zn content in the harvested cucumber fruits. Davarpanah et al.,(2016) studied the foliar application of nano-Zn fertilizer at different concentration 0.6, and 120 mg ZnL⁻¹. They have observed improved fruit yield due to an increased number of fruits per tree at lower dose.

While at higher dose (120 mg ZnL⁻¹) significant improvement in fruit quality parameters such as total soluble sugars (4.4-7.6%), titrable acidity (20.6-46.1%) and juice in pomegranate (*Punica granatum*). Seed priming with ZnONPs at the rate of 400 and 800 ppm increased germination percentage, root and shoot length and seedling vigour index compared to the same concentration of ZnSO₄ in tomato (Khanm et al., 2018).

Table 2. Application of ZnONPs as nano-micronutrient fertilizer and its effects on different crop plants

Crop	Method of synthesis of ZnO NPs	Application	Type of study	Parameters observed	Reference
Cereal grain crops					
Rice (<i>Oryza sativa</i>) cv. PR-124	Chemical synthesis	Foliar spray @ 0.5, 1.0 and 5.0 g L ⁻¹ after every 15 days	Pot experiment	Reversal of Zn deficiency symptoms, improved growth and yield at 5.0g L ⁻¹ concentration	Bala et al., 2019
Wheat (<i>Triticum aestivum</i>)	Evaporation method	Seed priming using different concentrations (0,25,75, and 100ppm)	Pot experiment	Enhanced plant height, number of tillers per plant, spike length and improved grain yield with high Zn concentration in root, shoot and grain at 100 ppm	Munior et al., 2018
Maize (<i>Zea mays</i>)	Aqueous precipitation method	Seed treatment @ 0.16 mgseed ⁻¹	Lab experiment	Enhanced physiological quality of seed (higher germination %) and sanitary quality (low contamination of seeds.	Urbina et al., 2018
Pulse crops					
Chickpea (<i>Cicer arietinum</i>)		Foliar spray with 1.5 or 10 ppm concentrations	Pot experiment	At 1.5 ppm of ZnONPs increased shoot dry weight and overall biomass accumulation improved.	Burma et al., 2013
Chickpea (<i>Cicer arietinum</i>)	Hydrothermal method	Seed soaked (0.2 gm per g seed)	Petri plate experiment	Increased the level of IAA in the root of chickpea	Pandey et al., 2010

Oil-seed crops					
Sunflower (<i>Helianthus annus</i>)		Foliar application at 2gL^{-1}	Pot experiment	Enhanced shoot dry weight, leaf area, photosynthesis parameters and Zn concentration	Torbian et al., 2016
Peanut (<i>Arachis hypogea</i>)	Oxalate decomposition method	a) Seed treated with various concentration (400, 1000 and 2000 ppm) b) Foliar spray at $2\text{g } 15\text{L}^{-1}$	a) Pot culture b) Field experiment	a) At the 1000 ppm of ZnONPs improving both root volume and root dry weight b) Significantly increased pod yield, shelling percentage and other biometric parameters of peanut	Prasad et al., 2012
Vegetable crops					
Brinjal (<i>Solanum melongena</i>)		Soil application @ 4500 mg ha^{-1}	Field experiment	Increased yield (91%) and biomass (45.3%) respectively	Kale et al., 2016
Spinach (<i>Spinacia oleracea</i>)		Foliar spray of graded concentrations at 0, 100, 500 and 1000 ppm	Pot experiment	Improved physical and nutritional properties of spinach leaves Enhanced protein and dietary fibers contents at 1000 ppm	Kisan et al., 2015
Onion (<i>Allium cepa</i>)	Chemical method	Onion bulbs treated with $20\text{-}30\text{ }\mu\text{g mL}^{-1}$	Pot experiment	Improved flowering and seed productivity such as higher seed fruit / umbel, seed weight / umbel and 1000 seed weight.	Laware et al., 2014
Cucumber (<i>Cucumis sativus</i>)		Soil treatment at 400, and 800 mg kg^{-1}	Pot experiment	Increased starch and globulin content and no reduced micronutrient concentration in cucumber fruits on application of 400 mg kg^{-1} of ZnONPs	Zhao et al., 2013
Tomato (<i>Lycopersicon esculentum</i>)		Foliar spray at 20 mg mL^{-1}	Pot studied	Improved growth and biomass production of tomato plants.	Panwar, 2012

CONCLUSION

Biosynthesis of metal oxide nanoparticle such as zinc oxide nanoparticles, is a nontoxic, green reusable and pollution free technique. It harnesses the biochemical, morphological and catalytic diversity of cellular biomolecules obtained from different types of living organisms for the synthesis of zinc oxide nanoparticles. The synthesized nanoparticles exhibit strict size dependent activities. However, the use of nano-scale zinc oxide particles as micronutrient fertilizer delivery vectors has been documented in the present decade. Different formulations of ZnO NPs developed so far can be applied to crops in varied manners to effectively enhance both productivity and Zn-nutritional quality of diverse crop plants. It could be an imperative technique for tackling the conventional Zn-fertilizer losses incurred due to mobilization and complexation of the applied Zn-fertilizer. Further, the zinc fortification of the produce will improve the Zn-nutrition and therefore, growth and development of human and livestock.

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Conflict of Interest Statement

The authors declare that there is no conflict of interest.

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