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Research Review

Plant Micronutrient Nanoparticles from Microalgae -Biosynthesis and their Applications – A Review

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ABSTRACT

Plant micronutrients play an important role in the growth and development of plants. Their deficiency causes reduction of yield and growth. Most of the plants derive their nutrition from the soil which is the major natural source of all nutrients. Inorganic nutrients get fixed in the soil as insoluble forms thus not being available in essential quantities to the roots. Micronutrient nanoparticles can substitute chemical fertilizers which can be used to increase agronomic yield, foliar sprays have proven to be easily absorbed and show high use of efficiency. Nanoparticles exhibit unique properties due to their small dimension between 1-100nm.They show significantly improved physical, chemical and biological properties. Biological synthesis is an ecofriendly process in contrast to other physical and chemical methods which makes use of toxic substances and leave residues which harm natural microflora of the soil. Microalgae based biosynthesis of nanoparticles is an emerging field known as "phyco-nanotechnology", which has a wide range of potential applications that are being explored. Algae contain bioactive molecules that act as reducing, capping and stabilizing agents for manufacturing nanoparticles. Biosynthesis of micronutrient nanoparticles is a boon as depletion of soil nutrients due to climatic changes causes nonavailability of nutrients to the crop plants.

***Key words*:** Biosynthesis, Micronutrients, Nanoparticles, Microalgae, Applications

Agriculture is the backbone of our country, with cultivable land of 159.7 million hectares and suitable weather we stand second in the production of various crops, fruits and vegetables [1]. Growing population and increasing demand for safe and healthy food makes improvement of crops with advance techniques the need of the hour. Droughts, floods, lack of irrigation facilities, pests, diseases and abiotic stresses are taking their toll on the agricultural sector. Management of agricultural practices from multiple fronts is very essential for India to achieve a GDP growth of 9-10% in the coming years. Extensive use of chemical fertilizers to meet the rising demand is taking its toll on the health of people already prone to various infections. Nanotechnology offers novel applications in the preparation of new formulations of fertilizers and pesticides which strengthens agricultural diversity and productivity. Nanofertilizers have the ability to release their nutrients at a slow and steady pace [2]. Nanotechnology is gathering information of atom in nano scale range, with considering the physical, catalytic, magnetic, optical properties [3]. Recent advancements in the fabrication of nanomaterials of different sizes and shapes have yielded their wide array of applications in medicine, food and feed, cosmetics, electronics, space industries, drug-gene delivery, environmental science, agriculture and food processing. Throughout history, agriculture has always benefited from these innovations [4]. Their small sizes, wide and reactive surface areas, makes them an excellent bactericide, fungicide, nano-fertilizer and are also used in the diagnosis of plant diseases and as agricultural chemicals.

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Micronutrients

Micronutritional imbalance in plants affect the plant's response to the surrounding environment, changes in growth pattern, chemical composition, antioxidant defense capacity, decrease in resistance to biotic and abiotic stresses thereby reducing productivity. Fourteen different mineral elements are required by green plants for their proper growth, it includes macronutrients nitrogen (N), phosphorous (P), potassium (K), calcium (Ca), magnesium (Mg) and sulphur (S). The micronutrients chlorine (Cl), boron (B), iron (Fe), manganese (Mn), copper (Cu), zinc (Zn), nickel (Ni) and molybdenum (Mo) are required in small quantities which are generally absorbed from the soil. The soil conditions like pH, concentration of organic matter, concentration of oxides and carbonates, water runoff, are some of the factors that cause low availability of micronutrients in required quantities to the plants, hence micronutrient nanoparticles when applied as foliar sprays have shown to make considerable changes in the yield. Two important strategies agronomic and genetic biofortifications are employed by scientists in recent years to increase bioavailability of minerals in agriculture produce thereby reducing the occurrence of mineral deficiency in human population [5-7]. Comprehensive knowledge on the bioavailability of micronutrients in the Crop-manure-soil system and how they meet requirements of human and animal nutrition are still lacking [8-9]. Manures are a rich source of micronutrients but excessive use causes salt and heavy metal accumulation in the soil along with a huge influx of pathogens.

Boron: Boron is an essential micronutrient which plays an important role in cell structure, function and growth. It forms cis-diol groups with glycoproteins and glycolipids. Its deficiency causes cessation of root growth and decreases CO2 assimilation due to reduction of stomatal conductance [10]. Depression of growing points and deformity of organs which also affects vascular tissues causing suppression of water intake and decrease in metabolic processes is reported [11]. Boron is required in very small quantities but is very essential.

Copper: Copper is essential for photosynthesis where it is an active component of plastocyanin. It catalyzes various reactions and activates enzymes in plant-growth processes [12]. It plays a very important role in seed production. Deficiency of copper causes chlorosis, chloroplast deformation and drastic reduction of grain yield as a result of reduced photosynthesis.

Iron: Iron is required to produce chlorophyll and participates in various physiological processes including respiration and other redox reactions [13]. Its deficiency causes chlorosis of young leaves usually in the interveinal region. It reduces antioxidant enzyme activity, which reduces yield drastically. Iron deficient plants tend to over accumulate heavy metals. Iron deficiency is a major nutritional problem affecting crop trees in calcareous soils. Iron (Fe) content in the soil is usually high but is not easily available as it is fixed in aerobic soils and soils with high pH as insoluble $Fe3^{+}$, whereas plants generally absorb $Fe2^{+}$ [14].

Manganese: Manganese is a critically essential element in plants which forms a part of the metalloenzyme cluster. In photosystem II (PS II) Mn is an essential part of the oxygen-evolving complex (OEC) thus plays a significant role in photosynthesis. Mn fertilizer application to the soil can be effective only if the pH of the soil is also corrected which is impractical to farmers and hence there is a need to substitute with foliar sprays [15]. Manganese does not remobilize from older leaves to young leaves which have Mn deficiency as the foliar sprays are effective only for a limited amount of time [16]. It accelerates germination, onset of maturity, increases the availability of Phosphorous and calcium in the soil.

Molybdenum: Molybdenum is a trace element which is available to plants as molybdate anion in the soil. They play a significant role in the enzymes which help in the geochemical cycles of Nitrogen, Sulphur and Carbon It is vital for the process of symbiotic nitrogen fixation in synthesis and activity of the enzyme nitrate reductase. Its deficiency causes paleness of leaves, improper flower formation and withering. Alteration in the morphology of leaves and lesions are also caused due to deficiency of Molybdenum [17].

Zinc: Zinc is an important micronutrient which plays an important role in the formation of chlorophyll, protein, lipid, carbohydrate, a cofactor in DNA and RNA polymerase enzymes and hormones. Abnormal roots, mottled leaves or chlorosis are a few symptoms of Zinc deficiency [18-19]. It is taken up by plants as the divalent Zn+2 cation. Zinc is found to be the only metal present in all the major six enzyme classes, viz. oxidoreductases, lyases, isomerases, transferases, hydrolases and ligases [20].

Chlorine: Chlorine plays an essential role in physiological functions such as osmotic stomatal regulation, evolution of oxygen, disease resistance and tolerance. It affects the crop yield and quality. Deficiency symptoms are leaf spots, brown edges, wilting leaves and leaf mottling, especially at margins, shriveling and necrosis of leaves, frond fracture and stem cracking in coconut [21].

Nickel: Nickel is a component of Urease enzyme and is essential in Nitrogen metabolism. Deficiency causes stunted growth, chlorosis, and deformation of various plant parts, leaf spots and foliar necrosis [22].

Nanofertilizers

Fertilizers are sprayed in many ways either to the soil or through leaves, even to the aquatic environments where these inorganic fertilizers are supplied in order to provide three main components, nitrogen, phosphorous and potassium in equal ratios or proportion as required. Nano-fertilizers are new generation of the synthetic fertilizers which contain readily available nutrients in nano scale range. Nano fertilizers are preferred largely due to their efficiency and environment friendly nature compared to conventional chemical fertilizers. The initial studies performed on nano materials have shown serious health hazards and showed toxic effects to human body. Nanoparticles synthesized chemically used for the delivery of fertilizers to plants poses serious threat to the microbial fabric of the soil as ecosystem causing membrane damage, reducing the annual growth of grass, depletion photosynthesis in various algae in routine waste water treatment plants, results showed serious impacts on plant - microbe interaction, affecting N2 fixing symbiosis for which metals used for developing nano fertilizers are considered highly sensitive [23]. Biosynthesized nano fertilizers overcome the drawbacks of these chemical nano fertilizers. Biological fertilizers are the newest and most technically advanced way of supplying mineral nutrients to crops. A biological process with the ability to strictly control the shape of the particles would be a considerable advantage. The application of foliar spray SiO2 at the rates of (15, 30, 60 and 120 mg) increased plant height (cm), number of leaves/plant and fresh and dry weights of leaves/plant (g), number of fruits / plant, mean weight of fruit, fruit length, fruit diameter and yield kg/plant and total yield as compared with the untreated plants under agricultural drainage water [24].

Pesticides used in agriculture are not very efficient; they pollute both terrestrial and aquatic environments as a result of their widespread use. Therefore, in agriculture, a positive effect on ecology can be made by using nano-agrochemicals instead of pesticides. Nano-agrochemicals containing polymeric nanoparticles, silver ions, gold nanoparticles and iron oxide nanoparticles are used as pesticides. Most of the pesticides are conventional resistant to biodegradation and are found to be carcinogenic in nature even at trace levels. Conventional methods of pesticide removal are disadvantageous due to their inherent time consumption or expensiveness. Nanoparticles alleviate both of these drawbacks and more so if it is biosynthesized. Nanoparticles synthesized by agriculturally important microorganisms provide a cost-effective and eco-friendly alternative for plant disease management.

Synthesis and characterization

Biological synthesis involves the use of natural living organisms and their metabolites which act as reducing and capping agents to convert bulk material to nanoparticles with unique properties. Microalgae can be used to synthesis nanoparticles extracellularly or intracellularly. Intracellular synthesis involves culturing the targeted microalgae, allowing it to interact with the precursor solution, separation and purification. Physicochemical procedures are used for characterization of the nanoparticle synthesized. While working on *Plectonema boryanum* silver nanoparticles were synthesized by reduction of silver nitrate solution, carried out by intracellular electron donor or exported by a membrane transporter system [25]. Reduction by nitrogenases, macromolecules, thylakoids poly saccharides and biomolecules has been reported [26].

Extracellular synthesis makes use of exudates like pigments, hormones, ions, proteins, antioxidants and enzymes. Cellular biomass extracts and cell free culture media, biomolecules and secondary metabolites are used for this purpose. Characterization of nanoparticles is done using SEM, TEM, UV-Vis Spectrophotometer, X-ray based analysis (Spectroscopy and Diffraction), FTIR, and magnetic techniques. Illumination, time of exposure, pH, temperature, nature of the microalgae, nutrient medium and precursor used play an important role in the synthesis of nanoparticles.

Table 1 Biosynthesis of nanoparticles from microalgae

Microalgae	Nanoparticle synthesised	Morphology / Mode of synthesis	Size (nm)	References
Anabaena flos-aquoe	β-FeOOH	Nanorods	100	[27]
Anabaena doliolum	Ag	Extracellular	10-15	[28]
Anabaena strain L 31	Zn	Spherical	70-100	[29]
Chlamydomonas reinhardtii	Ag	Rectangular and round extracellular	1-15	[30]
Chlamydomonas reinhardtii	Au	Rectangular and rounded	1-15	[31]
Chlorella pyrenoidosa	Au	Icosahedral and spherical	25-30	[32]
Chlorella vulgaris	Au	Spherical extracellular	2-10	[33]
Chlorella vulgaris	Ag	Triangular	28	[34]
Chlorococcum humicola	Ag	Spherical	16	[35]
Diatoms	Si, Au	Extracellular	-	[36]
Enteromorpha flexuosa	Ag	Circular	15	[37]
Galdieria sp.	Iron (II), Zn	-	-	[38]
Klebsormidium flaccidum	Au	Extracellular	10-20	[39]
Nostoc sp. EA03	ZnO	Star	50-80	[40]
Phormidium cyanobacterium	CuO	Quasi-Spherical	10-40	[41]
Pithophora oedogonia	Ag	Cubical and hexagonal,	24-55	[42]
Plectonema boryanum UTEX 485	Ag	Octahedral platelets	10-6	[43]
Scenedesmus sp	Ag	-	15-20	[44]
Spirulina platensis	Ag	Spherical	30-40	[45]
Tetraselmis kochinensis	Au	Triangular, FCC, and spherical, Intracellular	5–35	[46]

CONCLUSION

Micronutrient deficient soils will produce crops deficient in micronutrients which in turn affects man and animals dependent on it. Deficiency not only affects crop yield and quality but also exhibits visible symptoms of physiological stress depending on the severity. Foliar and soil applications of micronutrient nanoparticles and their effective management could enhance crop production, quality and reduce malnutrition in man and animals. Nanoparticles play an important role in stress management of crops in nutrient deficient soils, increase yield, improve germination and nutrient uptake. Foliar sprays of biosynthesized nanoparticles are more advantageous than chemically synthesized fertilizers as they do not leave any residue on the plants or soil. Biosynthesized micronutrient nano fertilizers are the need of the hour as it increases nutrients in plants thereby reducing malnutrition in man.

LITERATURE CITED

1. Saxena A, Suna T, Saha D. 2021. Application of Artificial Intelligence in Indian Agriculture. *Researchgate.net* 2021: 2.
2. Seleiman Mahmoud F, Almutairi KF, Alotaibi M, Shami A, Alhammad BA, Battaglia ML. 2021. Nano-fertilization as an emerging fertilization technique: why can modern agriculture benefit from its use? *Plants* 10(1): 2.
3. Sadik OA, Zhou AL, Kikandi S, Du N, Wang Q, Varner K. 2009. Sensors as tools for quantitation, nanotoxicity and nanomonitoring assessment of engineered nanomaterials. *Journal of Environmental Monitoring* 11(10): 1782-1800.
4. Chen YW, Hwei VL, Juan JC, Phang SM. 2016. Production of new cellulose nanomaterial from red algae marine biomass *Gelidium elegans*. *Carbohydrate polymers* 151: 1210-1219.
5. Çakmak I, Torun AYFER, Millet E, Feldman M, Fahima T, Korol A, Nevo E, Braun HJ, Özkan H. 2004. *Triticum dicoccoides*: An important genetic resource for increasing zinc and iron concentration in modern cultivated wheat. *Soil Science and Plant Nutrition* 50(7): 1047-1054.
6. Cakmak I. 2008. Enrichment of cereal grains with zinc: agronomic or genetic biofortification? *Plant and Soil* 302(1): 1-17.
7. White PJ, Martin R, Broadley R. 2009. Biofortification of crops with seven mineral elements often lacking in human diets–iron, zinc, copper, calcium, magnesium, selenium and iodine. *New Phytologist* 182(1): 49-84.
8. Pfeiffer, Wolfgang H, McClafferty B. 2007. Harvest plus: breeding crops for better nutrition. *Crop Science* 47: S-88.

9. Pfeiffer Wolfgang H, McClafferty B. 2007. Biofortification: breeding micronutrient-dense crops. *Breeding Major Food Staples* 2007: 61-91.
10. Han S, Chen LS, Jiang HX, Smith BR, Yang LT, Xie CY. 2008. Boron deficiency decreases growth and photosynthesis, increases starch and hexoses in leaves of citrus seedlings. *Journal of Plant Physiology* 165(13): 1331-1341.
11. Lu YB, Yang LT, Li Y, Xu J, Liao TT, Chen YB, Chen LS. 2014. Effects of boron deficiency on major metabolites, key enzymes and gas exchange in leaves and roots of *Citrus sinensis* seedlings. *Tree Physiology* 34(6): 608-618.
12. Yamasaki H, Marinus P, Shikanai T. 2008. How do plants respond to copper deficiency? *Plant Signaling and Behavior* 3(4): 231-232.
13. Mimmo T, Buono DD, Terzano R, Tomasi N, Vigani G, Crecchio C, Pinton R, Zocchi G, Cesco S. 2014. Rhizospheric organic compounds in the soil–microorganism–plant system: their role in iron availability. *European Journal of Soil Science* 65(5): 629-642.
14. Ye L, Lin L, Lu W, Shoudong W, Sen L, Juan D, Shuqun Z, Huixia S. 2015. MPK3/MPK6 are involved in iron deficiency-induced ethylene production in *Arabidopsis*. *Frontiers in Plant Science* 6: 953.
15. White PJ, Greenwood DJ. 2013. Properties and management of cationic elements for crop growth. *Soil Conditions and Plant Growth* 2013: 160-194.
16. Li C, Peng W, Neal WM, Enzo L, Kopittke PM. 2017. Effects of changes in leaf properties mediated by methyl jasmonate (MeJA) on foliar absorption of Zn, Mn and Fe. *Annals of Botany* 120(3): 405-415.
17. Rana M, Bhantana P, Sun XC, Imran M, Shaaban M, Moussa M, Saleem MH. 2020. Molybdenum as an essential element for crops: an overview. *International Journal of Scientific Research and Growth* 24: 18535.
18. Clarkson DT, Marschner H. 1995. Mineral nutrition of higher plants. London: Academic Press. *Annals of Botany* 78(4): 527-528.
19. Soetan KO, Olaiya CO, Oyewole OE. 2010. The importance of mineral elements for humans, domestic animals and plants-A review. *African Journal of Food Science* 4(5): 200-222.
20. Auld DS. 2001. Zinc coordination sphere in biochemical zinc sites. *Zinc Biochemistry, Physiology, and Homeostasis* 2001: 85-127.
21. Alloway BJ. 2008. Micronutrients and crop production: An introduction. *In*: *Micronutrient Deficiencies in Global Crop Production*. Springer, Dordrecht, 2008: 1-39.
22. Patra A, Dutta A, Jatav SS, Choudhary S, Chattopadhyay A. 2019. Horizon of nickel as essential to toxic element. *Int. Jr. Chem. Stud.* 7: 1185-1191.
23. Nguyen, Nhung HA, Vellora V, Padil T, Slaveykova VI, Miroslav Č, Alena Š. 2018. Green synthesis of metal and metal oxide nanoparticles and their effect on the unicellular alga *Chlamydomonas reinhardtii*. *Nanoscale Research Letters* 13(1): 1-13.
24. Tian, L, Jupei S, Guoxin S, Bin W, Rong J, Zhao L. 2020. Foliar application of SiO_2 nanoparticles alters soil metabolite profiles and microbial community composition in the Pakchoi (*Brassica chinensis L.*) rhizosphere grown in contaminated mine soil. *Environmental Science and Technology* 54(20): 13137-13146.
25. Lengke, Maggy F, Fleet ME, Southam G. 2007. Biosynthesis of silver nanoparticles by filamentous cyanobacteria from a silver (I) nitrate complex. *Langmuir* 23(5): 2694-2699.
26. Zhang R, Kelong F Yan X. 2020. Nanozymes: created by learning from nature. *Science China Life Sciences* 2020: 1-18.
27. Brayner R, Claude Y, Chakib D, Thibaud C, Frederic H, Jacques L, Fernand F, Alain C. 2009. Photosynthetic microorganism-mediated synthesis of akaganeite (β-FeOOH) nanorods. *Langmuir* 25(17): 10062-10067.
28. Singh G, Babele PK, Shahi SK, Sinha RP, Tyagi MB, Kumar A. 2014. Green synthesis of silver nanoparticles using cell extracts of *Anabaena doliolum* and screening of its antibacterial and antitumor activity. *Journal of microbiology and Biotechnology* 24(10): 1354-1367.
29. Singh G, Babele PK, Kumar A, Srivastava A, Sinha RP, Tyagi MB. 2014. Synthesis of ZnO nanoparticles using the cell extract of the cyanobacterium, *Anabaena strain* L31 and its conjugation with UV-B absorbing compound shinorine. *Journal of Photochemistry and Photobiology B: Biology* 138: 55-62.
30. Barwal I, Ranjan P, Kateriya S, Yadav SC. 2011. Cellular oxido-reductive proteins of *Chlamydomonas reinhardtii* control the biosynthesis of silver nanoparticles. *Journal of Nanobiotechnology* 9(1): 1-12.
31. Rao D, Gautam P. 2014. A facile one-pot synthesis of gold nanoparticles by *Chlamydomonas reinhardtii. Asian Jr. Microbiol. Biotechnol. Environment Science* 16: 633-639.
32. Oza G, Pandey S, Mewada A, Kalita G, Sharon M, Phata J, Ambernath W, Sharon M. 2012. Facile biosynthesis of gold nanoparticles exploiting optimum pH and temperature of fresh water algae *Chlorella pyrenoidusa*. *Advances in Applied Science Research* 3(3):: 1405-1412
33. Annamalai J, Thangaraju N. 2015. Characterization of biosynthesized gold nanoparticles from aqueous extract of *Chlorella vulgaris* and their anti-pathogenic properties. *Applied Nanoscience* 5(5): 603-607.
34. El-Moslamy S, Kabeil S, Hafez EJO. 2016. Bioprocess development for *Chlorella vulgaris* cultivation and biosynthesis of anti-phytopathogens silver nanoparticles. *Journal of Nanomaterials and Molecular Nanotechnology* 9: 2.
35. Agrawal S, Rathore P. 2014. Nanotechnology pros and cons to agriculture: a review. *International Journal of Current Microbiology and Applied Sciences* 3(3): 43-55.
36. Schröfel A, Gabriela K, Markéta B, Edmund D, Ivo V. 2011. Biosynthesis of gold nanoparticles using diatoms—silica-gold and EPS-gold bionanocomposite formation. *Journal of Nanoparticle Research* 13(8): 3207-3216.
37. Yousefzadi M, Zohreh R, Vahid G. 2014. The green synthesis, characterization and antimicrobial activities of silver nanoparticles synthesized from green alga *Enteromorpha flexuosa* (wulfen) J. Agardh. *Materials Letters* 137: 1-4.
38. Salas-Herrera G, González-Morales S, Benavides-Mendoza A, Castañeda-Facio AO, Fernández-Luqueño F, Robledo-Olivo AJJoAP. 209. Impact of microalgae culture conditions over the capacity of copper nanoparticle biosynthesis. *Journal of Applied Phycology* 2019 1-11.

39. Sicard C, Roberta B, Jérémie M, Miryana H, Alain C, Claude Y, Chakib D, Aubard J, Fiévet F, Livage J, Coradin T. 2010. Nano-gold biosynthesis by silica-encapsulated micro-algae: a "living" bio-hybrid material. *Journal of Materials Chemistry* 20(42): 9342-9347.
40. Ebadi M, Zolfaghari MR, Aghaei SS, Zargar M, Shafiei M, Zahiri HS, Noghabi KA. 2019. A bio-inspired strategy for the synthesis of zinc oxide nanoparticles (ZnO NPs) using the cell extract of cyanobacterium *Nostoc sp.* EA03: from biological function to toxicity evaluation. *RSC Advances* 9(41): 23508-23525.
41. Rahman A, Ismail A, Jumbianti D, Magdalena S, Sudrajat H. 2009. Synthesis of copper oxide nano particles by using *Phormidium cyanobacterium*. *Indonesian Journal of Chemistry* 9(3): 355-360.
42. Sinha SN, Paul D, Halder N, Sengupta D, Patra SK. 2015. Green synthesis of silver nanoparticles using fresh water green alga *Pithophora oedogonia* (Mont.) Wittrock and evaluation of their antibacterial activity. *Applied Nanoscience* 5(6): 703-709.
43. Lengke MF, Fleet ME, Southam G. 2007. Biosynthesis of silver nanoparticles by filamentous cyanobacteria from silver (I) nitrate complex. *Langmuir* 23(5): 2694-2699.
44. Jena J, Pradhan N, Nayak RR, Dash BP, Sukla LB, Panda PK, Mishra BK. 2014. Microalga *Scenedesmus sp*.: a potential low-cost green machine for silver nanoparticle synthesis. *Journal of Microbiology and Biotechnology* 2(4): 522-533.
45. Satyavani K, Selvaraj G, Çakmak ZE, Çakmak T. 2016. Production and characterization of spherical thermostable silver nanoparticles from *Spirulina platensis* (Cyanophyceae). *Phycologia* 55(5): 568-576.
46. Senapati S, Syed A, Moeez S, Kumar A, Ahmad A. 2012. Intracellular synthesis of gold nanoparticles using alga *Tetraselmis kochinensis*. *Materials Letters* 79: 116-118.