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Biological Synthesis of Copper Nanoparticles and its impact - a Review

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ABSTRACT: *During the last decade, arrays of exploratory experiments conducted to gauge the solid impact of nanotechnology have comparatively proved its efficiency. Nanoparticles (Nps) are solid particles with dimensions between 1 to 100 nm. At present, Copper (Cu) nanoparticles find wide applications in agricultural, industrial engineering and technological fields. In agriculture much effort has been made in recent years to ascertain the necessity of certain minor elements in the economy of plants. Bionanotechnology combines biological principles with physical and chemical approaches to produce nano-sized particles with specific functions. Though the use of nanoscience in agriculture has been predominantly theoretical, yet effective antibacterial activities shown by Cu Nps for agriculture has fascinated the researches in the arena of nanotechnology, leading to the development of intensively clean, cost-effective and efficient biosynthesis techniques of Cu Nps. Global emphasis is given to the importance of biological synthesis either extracellularly or intracellularly. The impact of biosynthesized Cu has been studied on a few plant species at their very early growth stages. This manuscript reviews the biosynthesis, characterization and risks of Cu Nps.*

KEYWORDS: *Agriculture, Biosynthesis, Bionanotechnology, Copper Nanoparticles.*

I. INTRODUCTION

Nanomaterials (NMs) are defined as materials with at least one external dimension in the size range from approximately 1-100 nanometers. A focused integration of bio and nano techniques for biological synthesis of NMs, known as bionanotechnology, has emerged from nanotechnology [1-3]. Biological synthesis combines biological principles (i.e., reduction/oxidation) by microbial enzymes or plant phytochemicals with physical and chemical approaches to produce nano-sized particles. Nanoparticles (Nps) are solid particles with all three external dimension at the nano scale [3-10] that can drastically modify physico-chemical properties compared to the bulk material. It can explicate actions depending on the chemical composition, biological actions, size and shape [9, 11-13]. Since, NMs show large surface area to volume ratio, it finds wide range of applications in industries [5, 9, 14-17]. In recent years, NMs have received particular attention for its positive impact in improving many sectors of economy including consumer products, energy, transportation, cosmetics, pharmaceuticals, antimicrobial agents and agriculture. Presently, synthesis of inorganic Nps has been demonstrated by many physical and chemical means [18]. The chemical methods have a low productivity, non eco-friendly, capital intensive and toxic. Therefore, biological synthesis either extracellularly or intracellularly from higher plants or microbes have gained an upper hand [19-21]. The importance of biological synthesis is being emphasized globally.

The use of nanoscience in agriculture has been predominantly theoretical. Since it has begun, it will continue to have a significant effect in the areas of disease detection, enhancement of nutrient absorption and delivery. It can also enhance yield by delivering pesticides for targeted treatment to fight diseases. Thereby improving our understanding of “the biology of different crops” [22-25]. During the last decade, arrays of exploratory experiments have been conducted to gauge the solid impact of nanotechnology on crop improvement [24]. Recent research on Nps in a number of crops like corn, wheat, ryegrass, alfalfa, soybean, tomato, radish, lettuce, spinach, onion, pumpkin, bitter melon and cucumber have provided evidence of enhanced seedling growth, germination, photosynthetic activity, nitrogen metabolism, protein level, mRNA expression and changes in gene expression indicating their potential use for crop improvement [24, 26-27]. Much effort has been made in recent years to ascertain the necessity of certain minor elements in the economy of plants.Metals like Silver, Titanium, Palladium, Aluminum, Zinc, Gold, Carbon, Iron, Fullerenes and Copper have been routinely used for the synthesis of nanoparticles. Till date, the research in the field of biosynthesis mainly focused Ag Nps [7,699 papers, 59 %], Au Nps followed by ZnO [4,640 papers, 36 %] and finally CuO Nps [690 papers, 5 %] [2, 29-30]. Copper-based nanoparticles are of great interest because of low cost, availability and properties possessed are similar to that of other metallic Nps [31-35]. It finds applications in heat transfer systems as super strong materials, sensors [36-38], antimicrobial, bactericidal agents used to coat hospital equipments and also as catalysts [39-45].

Various physical and chemical methods have been extensively used to synthesize Cu nanoparticles. Literature available on Cu nanoparticles synthesis reported that it is susceptible to oxidation [46-48]. Therefore most successful chemical synthesis of metallic copper nanoparticles is either carried out in organic or aqueous phase synthesis to avoid potential oxidation of Cu [49]. The biosynthesis of pure metallic copper nanoparticles in aqueous phase is still an open challenge for bionanotechnologists.

II. IMPORTANCE OF COPPER IN PLANTS

Copper (Cu) of block D and period 4 of the periodic table is a microelement required for the development of plant. It exists as Cu^{2+} and Cu^{+} under physiological condition. Concentration required for normal development of plant is from 10^{-14} to 10^{-16} M [28], below which deficiency occurs. However higher concentration than optimum showed toxicity in uptake of nutrients [28]. It acts as a structural element in protein regulation, participates in photosynthetic electron transport, mitochondrial respiration, cell wall metabolism, hormone signaling, oxidative stress response, cofactor for many enzymatic reactions carried out by enzymes such as polyphenol oxidase, amino oxidase, plastocyanin, laccase, super oxide dismutase. At the cellular level, it plays an important role in oxidative phosphorylation, signal trafficking machinery and iron mobilization. The Cu deficiency in plants is expressed as curled leaves, petioles bent downwards and light chlorosis along with permanent loss of turgor in the young leaves. Chronic Cu deficiency develops a rosette form of growth [28, 50 - 51]. Fig 1 shows the effect of Cu on plant.Higher concentration of Cu leads to toxicity, growth inhibition, photosynthesis interferences, photo respiration and increases oxidative stress. Diagnosis of Cu deficiency in plants is an important as it results in yield losses, with little evidence of the characteristic symptoms [52-53]. Cu deficiency may become more prevalent in coming future, the applications made 10 to 30 years ago would be running out and increased use of nitrogenous fertilizers will lead to severity of Cu deficiency.

III. BIOSYNTHESIS OF COPPER NANOPARTICLE

Today's nanotechnology needs to develop alternative method to chemical synthesis which is reliable, non-toxic, clean and eco friendly. One such method is biological synthesis, were reducing and capping agents like bacteria, fungi, actinomycetes, yeast and plants are used [18, 19, 29, 54-55]. Very few literature is available on the biosynthesis of Cu Nps and other metal Nps compared to chemical synthesis [17, 56-58]. Fig 2 represents a flowchart for nanomaterial biosynthesis.

Preparation of Biological extracts for Copper Nano particle synthesis

Preparation of Microbial extracts : Studies have shown the general method which includes culturing microorganism on suitable broth medium, incubating on a rotary shaker at suitable rpm and temperature specific for microorganism for set number of days. The cultures were than centrifuged at required rpm and time. The supernatants obtained were used for the synthesis of Cu Nps. Fungi is incubated on a rotary shaker at 200 rpm for ten days at 28°C [58] and bacteria is incubated at 37 °C for 24 hours in an incubator shaker at 1550-200 rpm [59].

Preparation of Botanical extracts : It is reported by the researchers that plant of interest was collected from the available place, were washed & cleaned thoroughly with tap water and distilled water to remove debris. Shade-dried for two weeks and then powdered using domestic blender. The plant broth preparation was made by a known gram of the dried powder boiled at 70-80^{0}C for 2-3 minutes with known volume of distilled water. The resulted infusion is filtered and used as a reducing agent and stabilizer [60].

Fabrication of Nanoparticles Using Microorganisms : Survey studies have shown that prokaryotic organisms such as bacteria is used as an agent for the synthesis of nanoparticles due to ease of culturing, short generation time, mild experimental conditions (pH, pressure, and temperature), extracellular production and easy downstream processing. Hence, it has gained importance in nanoparticle synthesis [61]. A gram-negative bacterium belonging to the genus *Serratia* was used to synthesize CuO Nps isolated from the midgut of *Stibara sp*., an insect reported by Hasan et al. [46]. Silver resistant bacteria *Morganella morganii* RP42 and *Morganella psychrotolerans* are attributed to the formation of Cu Nps in range of 15-20 nm [62]. Varshney et al [59] showed that the spherical Cu Nps in the size range of 8-15 nm was synthesized by a rapid biological synthesis technique using non-pathogenic *Pseudomonas stutzeri.* Usha et al [63] presented that CuO Nps synthesized from *Streptomyces Sp.* can be used for the development of antimicrobial textiles for hospital use to prevent or to minimize infection with pathogenic bacteria. Singh et al [64] reported biological synthesis of CuO Nps using *Escherichia coli* with variable size and shapes. *Pseudomonas stutzeri,* present in the soil was used to synthesize cubical Cu Nps sized 50-150 nm from electroplating waste water [65].

Meanwhile, use of fungi to synthesis nanoparticles has been reported in literature. A number of different genera of fungi have been investigated in this effort and was shown that fungi are extremely good candidate since they secrete large amounts of enzymes and are simpler to deal in the laboratory [2]. Extracellular synthesis of Cu Nps was observed in *Penicillium aurantiogriseum, Penicillium citrinum* and *Penicillium waksmanii* [58]. Majumder [66] reported the synthesis of Cu Nps using *Fusarium oxysporum* at ambient temperature, were screened for extracting copper from integrated circuits and obtained it in nano form. Spherical Cu Nps with an average size of 24.5 nm were synthesized extracellularly by dead biomass of *Hypocrea lixii* isolated from the metal mine and infrared spectroscopy [IR] study, they revealed that the amide groups in proteins was accountable for the stability and capping agents surrounding the Cu Nps [18]. Abboud et al [17] investigated the synthesis of Cu Nps from brown algae *Bifurcaria Bifurcata*, the diterpenoids present in extract perform dual functions of reduction and stabilization of Cu Nps.

Fabrication of Nanoparticles Using Higher Angiospermic Plants : Use of plants for the fabrication of Nps have fascinated the workers of its rapid, economical, eco-friendly protocol, broad variability of metabolites that aid in reduction. It provides a single step technique for the biosynthesis process [19, 38, 67]. Nps produced by plants are more stable, are of various sizes and shapes. The rate of production is faster than in the case of microorganisms. Recently, several researchers exploited inactivated plant tissue, plant extracts, exudates, gums and other parts of plants for the synthesis of Cu Nps [38, 68-70]. Guajardo-Pacheco et al [71] reported a method of producing metallic nanoparticles of Cu by using soybeans extract as a chelating agent. Lee et al [72] reported biologically synthesized Cu Nps ranged in size from 40-100 nm using plant leaf extract *Mangolia* as reducing agent. Bioreduction activity of leaf extracts of *Brassica juncea, Medicago sativa* and *Helianthus annus* and *Tridax procumbens* resulted in the synthesis of Cu Nps in the presence of Cu^{+2} ions [19, 43]. Majumder [66] investigated the synthesis of Cu Nps using the leaf extract of weeds namely, *Lantana camara.* A rapid synthesis of the Cu Nps has been reported when the 0.001M aqueous of $CuSO_4$ solution was contacted with *Zingiber officinale* and *Syzygium aromaticum* [60, 73]. Natural plant materials such as *Magnolia* leaf extract and stem latex of *Euphorbia nivulia* have been used for the synthesis of Cu Nps [74]. The stable Cu Nps were synthesized using *Magnolia kobus* leaf extract on treatment with aqueous solution of $CuSO_4 \cdot 5H_2O$. Electron microscopy analysis revealed the average size was between 37 to 110 nm [74]. *Calotropis procera L. latex* were used to fabricate Cu Nps from copper acetate and transmission electron microscopy (TEM) revealed the average size was found to be 15 ± 1.7 nm. Cysteine proteases present in the latex act as capping agent and contributed to long term stability of Cu Nps (6 months) in aqueous medium [75]. Černík [38] reported the synthesis of Cu Nps of smaller size [4.8 ± 1.6 nm] from gum karaya, natural hydrocolloids, act both as reducing and capping agents in nanoparticle synthesis. *Ocimum sanctum*, a traditional medicinal plant of India have been used as a source of bio-reduction and stabilizers for synthesis of Cu Nps and the constituents such as alkaloids, glycosides, tannins, saponins and aromatic compounds may be responsible for the synthesis of nanoparticle [21]. Table 1 summarizes the biological agents used in the biosynthesis of Copper nanomaterial and Fig 3 Shows the TEM and SEM images of Cu nanoparticles.

Mechanism of Copper nanoparticle formation : Much work has been done in bioreduction of metal nanoparticles by a combination of biomolecules found in plant extracts (enzymes, proteins, amino acids, vitamins, polysaccharides, and organic acids such as citrates) and the respective role of phytochemicals [38, 43-44]. The phytochemicals responsible have been identified as terpenoids, flavones, ketones, aldehydes, amides and carboxylic acids by IR spectroscopic studies. The main water soluble phytochemicals are flavones, organic acids and quinones which are responsible for immediate reduction [19]. Natural hydrocolloids (gums) isolated from trees are a new class of biomaterial used for the production of nanomaterials which act both as reducing and capping agents in nanoparticle synthesis [38].Different microorganisms have different mechanisms of forming nanoparticles. It grabs target ions from the environment on the surface or inside of the microbial cells and then reduces the metal ions to nanoparticles in the presence of enzymes generated by the cell activities. The electrostatic interaction between the ions and negatively charged cell wall from the carboxylate groups in the enzymes reduces the metal ions which subsequently grow through further reduction and accumulation [76].

IV. CHARACTERIZATION OF COPPER NANOPARTICLE

The synthesized nanomaterial is characterized by UV–visible absorption spectroscopy, Fourier transform infrared spectrum analysis, X-ray diffraction and microscopic techniques such as transmission electron microscopy [TEM], scanning electron microscopy [SEM], and atomic force microscopy.

UV-Visible Spectra Analysis : The research results have shown the formation of various nanoparticles from different salt that gives characteristic peaks at 24hrs time interval at different absorptions using UV-visible spectroscopy. Cu Nps show characteristic absorption peaks at the range of 200-800 nm [77]. A progressive increase in the characteristic peak with increase in reaction time and concentration of biological extracts with salt ions is a clear indicator of nanoparticle formation. UV-vis absorption spectrum shows peaks characteristics of the surface plasmon resonance of nanosized particles [17, 78-80].

The X-ray diffraction (XRD) Analysis

Technique used to establish the metallic nature of particles gives information on translational symmetry size and shape of the unit cell from peak positions and information on electron density inside the unit cell, namely where the atoms are located from peak intensities [77].

XRD patterns were calculated using X'per Rota flex diffraction meter using Cu K radiation and λ =1.5406 A°.

Crystallite size is calculated using Scherrer equation

$CS= K\lambda /\beta \cos \theta$

Where CS is the crystallite size

Constant [K] = 0.94

β is the full width at half maximum [FWHM]

Full width at half maximum in radius [β] = FWHM x $\pi/180$

λ = 1.5406 x 10-10, Cos θ = Bragg angle.

Fourier Transform Infrared [FTIR] Spectroscopy : Measures infrared intensity *vs* wavelength [wave number] of light, it is used to determine the nature of associated functional groups and structural features of biological extracts with nanoparticles. The calculated spectra clearly reflect the well-known dependence of nanoparticle optical properties, *viz.* the resonance wavelength, the extinction cross-section, and the ratio of scattering to absorption, on the nanoparticle dimensions.

Microscopic techniques : Mainly used for morphological studies of nanoparticles. Their image reveals the shape and size distribution of colloidal particles in the range [73]. SEM image shows that the synthesized nanoparticles were more or less uniform in size and shape [77].

Antimicrobial Activity : Copper used as an antimicrobial agent for decades has revealed a strong antibacterial activity and was able to decrease the microorganism concentration by 99.9% [3, 60]. The US Environmental Protection Agency [EPA] has approved registration of copper as an antimicrobial agent that is able to reduce specific harmful bacteria linked to potentially deadly microbial infections [81]. Cu Nps are known to exhibit wide range of antibacterial activity against different strains of gram positive and gram negative bacteria.Copper oxide (CuO) nanoparticles acts as potential antimicrobial agent against infectious organisms such as *E. coli*, *Bacillus subtilis*, *Vibria cholera*, *Pseudomonas aeruginosa*, *Syphillis typhus*, and *Staphylococcus aureus* [38, 82- 84]. The Cu Nps synthesized using plant extract of *Magnolia*, *Syzygium aromaticum*, *Tridax procumbens* were tested against *Escherichia coli*, they showed higher antibacterial activity on cells after 24 h growth [72-73, 85]. Cu Nps synthesized by *Zingiber officinale* extract showed the zone of inhibition against *E.Coli* of 15 mm [60]. Highly stable CuO Nps formed from gum karya showed significant antibacterial action on *E. coli* MTCC 443 and *S. aureus* MTCC 737. The smaller size of the CuO nanoparticles [4.8 ± 1.6 nm] was found to yield a maximum zone of inhibition compared to the larger size synthesized CuO nanoparticles [7.8 ± 2.3 nm] [38]. CuO Nps produced using brown alga extract of dimensions 5–45 nm showed potential antibacterial activity against *Enterobacter aerogenes* and *Staphylococcus aureus* with radial diameter of inhibition zone 14 and 16 mm, respectively [17]. The CuO Nps of size [4.8 ± 1.6 nm] formed from microorganisms *Fusarium oxysporum* and *Pseudomonas* are highly stable and showed significant antibacterial action on both the gram classes of bacteria compared to larger size synthesized CuO [7.8 ± 2.3 nm] nanoparticles. Lee et al. [74] showed that the synthesized nanoparticles using *Magnolia kobus* leaf extract has higher antibacterial effect on *E. coli* cells after 24 h growth in shake flasks.

Copper colloidal solutions synthesized by polyol method using copper oxalate as a precursor resulted in Nps of average size of 6nm as confirmed by TEM images. It showed efficacy as anti pink disease drug for rubber tree which was infected by the fungus *Corticium salmonocolor* [86]. Yoon et al [87] demonstrated that the antibacterial effects of silver and copper nanoparticles using single representative strain of *E. coli* where the Cu Nps showed superior antibacterial activity compared to the silver. Cu Nps synthesized by chemical reduction of Cu^{2+} in the presence of cetyl trimethyl ammonium bromide and isopropyl alcohol having particle size of 3–10 nm showed significant antifungal activity against plant pathogenic fungi, *Fusarium oxysporum, Alternaria alternata, Curvularia lunata* and *Phoma destructive* [88]. Cu Nps synthesized by polyol method by the reduction of copper acetate hydrate in the presence of tween 80 showed the antimicrobial activity against *Micrococcus luteus, Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, on fungi like Aspergillus flavus, Aspergillus niger* and *Candida albicans* [89-90]. Antimicrobial effects of Cu nanoparticles on microorganisms are listed in Table 2.

Mechanism of antimicrobial activity of Cu Nps : The antimicrobial activity of Cu Nps is linked with ions that are released from nanoparticles. The activity is further enhanced by its small size and high surface area to volume ratio that it allows them to interact closely with microbial membranes [81].Antimicrobial activity is due to its tendency to alternate between its cuprous - Cu [I], and cupric - Cu [II], oxidation states. Differentiating Cu from other trace metals, results in the production of hydroxyl radicals that subsequently bind with DNA molecules and lead to disorder of the helical structure by cross-linking within and between the nucleic acid strands and damage essential proteins by binding to the sulfhydryl amino and carboxyl groups of amino acids. This denatures the protein makes the enzymes ineffective [87]. It inactivates cell surface proteins necessary for transport of materials across cell membranes, thus affecting membrane integrity and membrane lipids [91]. Copper ions inside bacterial cells also disrupt biochemical processes. The exact mechanism behind is not known and needs to be further studied. Based on all of these studies, the denaturing affect of Cu ion on proteins and enzymes in microbes gives Cu its antimicrobial characteristics.

Agronomical impact : The propensity of the Nps to cross cell barriers and its interactions with intracellular structures owing to its small size and high surface reactivity, contribute to potential phytotoxicity, genotoxicity, as well as cytotoxic effect [92-93]. In spite of increasing research on the toxicity of various Nps, limited studies are available in higher plants. Very few Nps and plant species have been studied, mainly at its very early growth stages of the plants and dried biomass [9]. Literature survey on the toxicity of Cu Nps available is very less [94]. The toxicity of Cu Nps depends on the combination of several factors such as concentration of Nps, pH, temperature, aeration and concentration of bacteria. The higher temperature, aeration, low pH decrease the agglomeration and provide more surface area for interaction with bacterial membranes for solubilization of copper ions that leads to toxicity.Lee et al. [95] analysed toxicity and bioavailability of Cu Nps of the plants *Phaseolus radiates* [mung bean] and *Triticum aestivum* [Wheat]. The bioavailability of copper was found to be 8 mgkg–^{1} for mung bean and 32 mgkg^{-1} for wheat plants treated with 1000 mg Cu Nps L^{-1}. Cu Nps tested on *Cucurbita pepo and zucchini* plants showed the reduced emerging root length [96].

In most cases, Cu toxicity considerably reduces the uptake of Fe by tomato plant, thereby imposing visible symptoms similar to those of Fe deficiency. Exposure to 0.1 to 0.4 g/L CuO Nps for 48h, induced strong inhibition of photosynthetic processes resulting in a decrease of plant growth in Lemna *gibba* has been reported [97]. The radishes and the two ryegrasses exposed to CuO Nps and large sized cupric oxide particles [< 100 nm] showed many DNA lesions. The cellular uptake of Cu Nps was significantly greater than the larger particles when plants were treated with Nps. The cellular uptake resulted in difference in DNA damage profiles of ryegrasse from the radish, indicating the damage is dependent on the plant species and on the nanoparticle concentration.CuO Nps, having a core of CuO Nps and a shell of polyacrylic acid [PAA], were found to be very toxic to unicellular algae, causing inhibition of PSII electron transport capacity in *Chlamydomonas reinhardtii* cultures [98]. Cytotoxicity studies of latex of *Calotropis procera* Cu Nps was carried out on HeLa, A549 and BHK21 cell lines by MTT dye conversion assay. HeLa showed excellent viability even at 120 μM concentration of copper nanoparticles. This shows that copper nanoparticles synthesized by the above method hold excellent biocompatibility [75]. Atha and collaborators [99] observed for the first time that CuO Nps damaged DNA in some agricultural and grassland plants [*Raphanus sativus*, *Lolium perenne*, and *Lolium rigidum*].However, the germination of lettuce seeds in the presence of Cu Nps 0.013% [w/w] showed an increase in shoot to root ratio compared to control plants [100].The Nps CuO showed beneficial effects on microbes, *Pseudomonas putida*, has bioremediation potential and is a strong root colonizer [101-103]. Nekrasova et al. [104] reported the effect of copper ions and copper oxide nanoparticle on lipid peroxidation rate, anti oxidant enzyme activities [superoxide dismutase, catalase and peroxidase] and photosynthesis with *Elodea densa planch.* The results show nanoparticles that are accumulated by plants and activate lipid peroxidation rate from 120 to 180% of the control level by copper ions and nanoparticles, respectively. Catalase and Superoxide dismutase activity increased by a factor of 1.5 to 2.0 when plants were treated with Nps. Copper ions suppresses photosynthesis at a concentration of 0.5 mg/l, where as nanoparticles produce such an effect only at 1.0 mg/l.

FIGURES AND TABLES

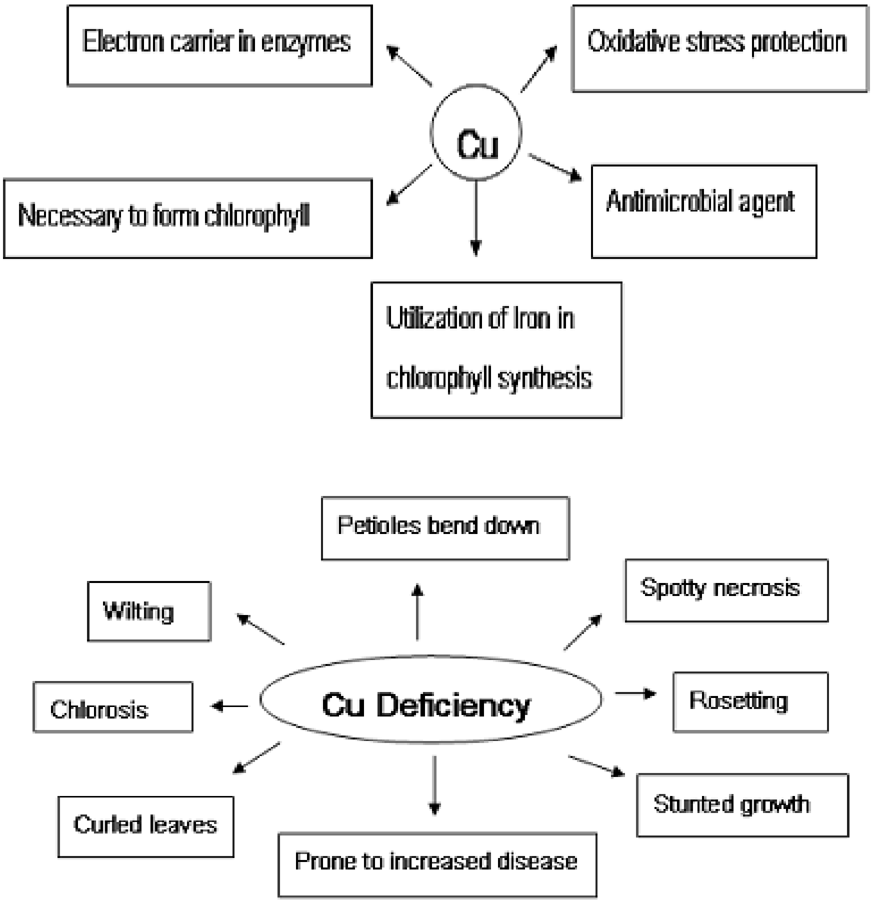

Fig 1: The role of Copper particle in plants

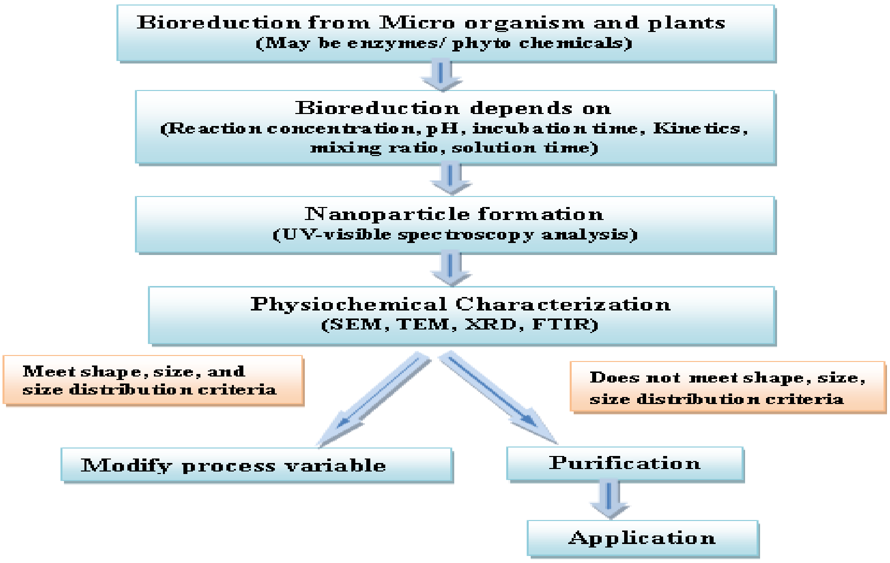

Fig: 2. Generalized flow Chart of the biosynthesis of nanoparticles

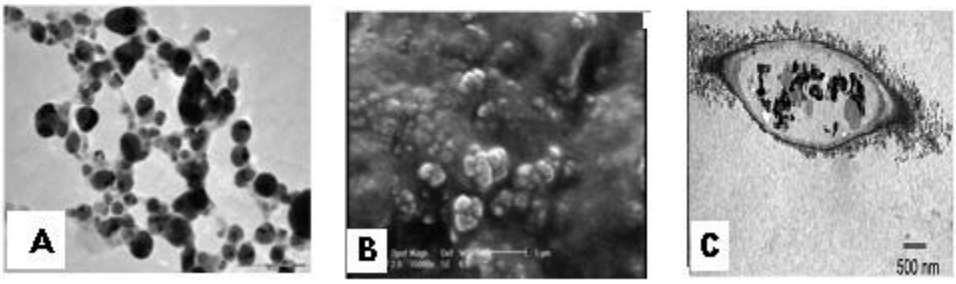

Fig 3: TEM and SEM images of Cu Nps synthesized from the: A) Fungal extract *Penicillium waksmanii* B) The fungus *H. lixii* C) Algal extract *Bifurcaria bifurcate* showing extracellular localization of copper nanoparticles (Courtsey: Honary et al.[58], Abboud et al.[17], Salvador et al. [18])

Table 1. Use of various biological entities in the synthesis of Copper nanoparticles

Biological entity		**Parts Used**	**Size**	**Citations**
Microorganism	*Escherichia coli*	-		Singh et al. [64]
Bacteria	*M.psychrotolerans* and *M. morganii* RP42	-	15-20 nm.	Ramanathan et al. [62]
	Pseudomonas sp.	-	84-130 nm	Majumder [66]
	Pseudomonas stutzeri	-	8-15 nm	Varshney et al. [59]
	Pseudomonas stutzeri	-	50-150 nm	Varshney et al. [65]
	Serratia			Hasan et al.[46]
	Streptomyces sp.	-		Usha et al. [63]
Fungi	*Fusarium oxysporum*	-	93-115 nm	Majumder [66]
	Hypocrea lixii	-	average of 24.5 nm	Salvador et al. [18]
	Penicillium aurantiogriseum, *Penicillium citrinum* *Penicillium waksmanii*	-	-	Honary et al. [58]
Algae	*Bifurcaria bifurcata*	-	5–45 nm.	Abboud et al. [17]
Angiospermic Plant				
	Calotropis procera L.	Latex	15 ± 1.7 nm.	Harne et al. [75]
	Euphorbia nivulia	Latex	-	Lee et al.[74]
	Gum karaya	Gum	4.8 ± 1.6 nm	Černík [38]
	Helianthus annus	-	-	Hameed et al. [19]
	Lantana camara	-	Average of 20 nm	Majumder [66]
	Magnolia	Leaves	40-100 nm	Lee *et al.*, [72]
	Magnolia kobus	Leaves	average size 37 -110 nm	Lee et al.[74]
	Medicago sativa	-	-	Hameed et al. [19]
	Ocimum sanctum	Leaves	77 nm	Kulkarni and Kulkarni, [21]
	Soy beans	Seed	-	Guajardo-Pacheco et al., [71]
	Syzygium aromaticum	Flower Buds	40-45 nm	Ipsa subhankari and Nayak [73]
	Tridax procumbens	-	-	Hameed et al. [19]
	Zingiber officinale	Rhizome	40 and 25 nm	Ipsa subhankari and Nayak [60]

Table 2. Antimicrobial effect of biologically synthesized Cu Nps on various microbes

Biological entity	**Test microorganisms**	**Method**	**References**
Bifurcaria bifurcata	*Enterobacter aerogenes* and *Staphylococcus aureus*	Disc diffusion	Abboud et al. [17]
Brassica juncea	*Fusarium oxysporum, Alternaria alternate, Curvularia lunata and Phoma destructive*	Agar disc-diffusion	Umer et al. [43]
Fusarium oxysporum	*E.coli*	Agar disc-diffusion	Majumder [66]
Gum karaya	*E. coli* MTCC 443 and *S. aueus* MTCC 737	Well diffusion method	Černík [38]
Lantana camara	*E. coli*	Agar disc-diffusion	Majumder [66]
Magnolia kobus	*E. coli*	Agar disc-diffusion	Lee et al. [74]
Pseudomonas sp.	*E. coli*	Agar disc-diffusion	Majumder [66]
Syzygium aromaticum	*E. coli* 2065	Agar disc-diffusion	Ipsa subhankari and Nayak [73]
Tridax procumbens	*E.coli*	Agar disc-diffusion	Hameed et al. [19]
Zingiber officinale	*E. coli*	Zone of inhibition assay	Ipsa subhankari and Nayak [60]

V. CONCLUSION

This paper provides an overview of the current research activities that center the biological synthesis and the study of copper nanoparticles, which have shown positive and negative impact on the micro-organism and the plants. Researches on the toxicity of nanomaterials are still emerging. There are ongoing researches to discover the rich potential effect of Copper nanoparticles application in the field of agriculture and effectively meeting its challenges.

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