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BIOLOGICAL SYNTHESIS OF COPPER NANOPARTICLES AND THEIR ANTIMICROBIAL PROPERTIES: A REVIEW

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ABSTRACT

Copper nanoparticles (CuNP) due to their unique structural and effective biological properties find use in number of fields. Biologically synthesized copper nanoparticles have gained special attention recently in the field of agriculture. This review article enlists all the literature on green synthesis of copper nanoparticles using microbes and plant sources and their antimicrobial properties. Various aspects of green synthesis, the factors influencing the synthesis procedure, the effect of nanopartice treatment on the host plant as well as the pathogen are also discussed. Here copper nanoparticles are being looked upon as an alternative to routine antibiotics or chemical fungicides for crop plant disease management.

KEYWORDS: Copper nanoparticles, green synthesis, antimicrobial properties and crop plant disease management.

INTRODUCTION

World population is increasing everyday and with it is increasing the demand for food. The destruction of crop plants due to infection by bacterial and fungal pathogens is a major challenge faced by the farmers worldwide. Widespread use of agrochemicals has certainly decreased the outbreak of diseases but at the same time has contributed to the development of resistant pathogens (Lamsal *et al*., 2011). Growing microbial resistance against metal ions, antibiotics, agrochemicals has raised a major concern (Gong *et al*., 2007; Rai *et al*., 2009). Pesticides/ insecticides enter food chain and cause several deleterious effects on biosphere, contributing to significant declines in populations of beneficial soil organisms, soil acidification, compaction etc (Aktar *et al*., 2009) Thus researchers are now trying to develop safer methods of disease control. Employment of metal nanoparticles as antimicrobial agent is one such approach.

A nanoparticle is a microscopic particle with at least one dimension less than 100nm. Nanoparticle research is currently an area of intense scientific interest due to a wide variety of potential applications in biomedical, optical and electronic fields. Currently the main thrust of research in nanotechnology focuses on applications in the field of electronics (Feiner *et al*., 2006), energy (Chen, 2007), medicine and life sciences (leiber *et al.*, 2006; Caruthers *et al*., 2007). The development of nanodevices and nonmaterial can open up novel applications in plant biotechnology and agriculture (Scrinis & Lyons, 2007). In the long term nanotechnology intends to be able to manipulate individual atoms and molecules for application in different fields of industry (Tanaka, 1999).

Traditionally nanoparticles have been synthesized by two methods.
1) Physical
2) Chemical.

Both physical and chemical methods of nanoparticle synthesis follow one of the two routes which are:
1) The top down or
2) Bottom up

The top down approach relies on reducing the size of a suitable starting material until the nano dimension is attained. Bottom up or self assembly means moving from lower to higher that is smaller units assembled to form a definite structure or the construction of a structure atom by atom, molecule by molecule or cluster by cluster. It has been reported that bottom up process is advantageous as it enhances possibility of obtaining metallic nanoparticles with comparatively lesser defects and more homogenous chemical composition.

Copper

Among all metals, Copper (Cu) which is an element of block D of periodic table, has the best antimicrobial properties and is used as an antimicrobial agent since ancient times. Cu is relatively non-toxic to mammals, (Flemming & Trevors, 1989) but is toxic towards many micro-organisms and this offers new prospects for antimicrobial treatments (Hsiao *et al*., 2006). There are a number reports available on anti microbial properties of Copper nanoparticles (Lee *et al*., 2013; Sutradhar *et al*., 2014; Ahamed *et al*., 2014; Awwad *et al*., 2014; Prabhu *et al*., 2015). CuNPs have been shown to exhibit strong antibacterial activity against strains of *E.coli, Pseudomonas aeruginosa, Staphyllococcus aureus, Bacillus subtilis, Salmonella choleraesuis* (Rai *et al*., 2009; Deryabin *et al*., 2013; Usman *et al*., 2013) and antifungal activity against several pathogenic fungi like *Candida albicans, Fusarium oxysporum, Phoma destructive, Curvularia lunata, Alternaria alternata* (Usman *et al*., 2013; Kanhed *et al*., 2014).

The US Environmental Protection Agency (EPA) has approved registration of copper as an antimicrobial agent which is able to reduce specific harmful bacteria linked to potentially deadly microbial infections (European Copper Institute, 2008). The copper nanoparticles are more important here as antimicrobial agent as nanoparticles of other metals such as gold, platinum, iron oxide, silica and its oxides and nickel have not shown significant bactericidal effects in studies with E.Coli (Park *et al*., 2005; Williams *et al*., 2006; Kapoor *et al*., 2006).

Physical and chemical methods of synthesis of copper nanoparticles

Physical methods

(i) Pulse laser ablation/deposition: Marine *et al*., 2000; Saito and Yasukawa (2008)

(ii) Mechanical/ ball milling: McCormick,1995

(iii)Pulsed wire discharge method: Lisiecki *et al*., 2000; Barrabes *et al*., 2006).

(iv)Photolytic: Scaiano *et al*., 2010

(v) Evaporation and condensation of metal vapor on a cold surface: Martra *et al*.,2002; Ponce and Klabunde, 2005

(vi)Thermal Decomposition: Sun and Zeng, 2002; Robinson *et al*., 2004; Serna *et al*.,2006; Roca *et al*., 2007; Betancourt *et al*.,2014.

Chemical methods

(i) Chemical reduction method

A copper salt is reduced by a reducing agent such as sodium borohydride (Lisiecki *et al*., 1996; Clavee *et al*., 1999; Tarasankar *et al*., 2000; Mukherjee *et al*., 2002; Aslam *et al*., 2002; Kobayashi *et al*., 2009; Zhang *et al*., 2009; Dang *et al*., 2011).

Chemical reduction of copper salts using ascorbic acid (Vitamin C) is a new approach wherein ascorbic acid is used both as the reduction and capping agent (Umer *et al*., 2012).

(ii) Microemulsion/colloidal method

Purely metallic nanoparticles (Cu, Ag, Co, Al), oxides (TiO_2, SiO_2), metal sulfides (CdS, ZnS) and various other nanomaterials are prepared using this technique (Robinson *et al*., 1990; Komasawa *et al*., 1994; Komasawa *et al*., 1995; Khilar *et al*., 2000; Cason *et al*., 2001, Wang *et al*., 2012).

(iii) Sonochemical method

Wonpisutpaisana *et al*., 2011.

(iv) Microwave method

The microwave-assisted synthesis of copper nanoparticles has become popular due to its simplicity, ease of operation and increasing yield of products compared to the conventional heating methods (Yin *et al*., 2005; Blosi *et al*., 2011).

(v) Electrochemical method

Copper sulphate and Sulphuric acid as the electrolytic solution and supplied 4 V, 5 A for 30 min with the resulting 406 nm sized copper nanoparticle (Raja *et al*., 2008).

(vi) Solvothermal decomposition

The chemical reaction takes place in a sealed vessel such as bomb or autoclave, where solvents are brought to temperatures well above their boiling points. (Cansell *et al*., 1999; Yu, 2001; Rajamathi and Seshadri, 2002).

There are other reports on chemical synthesis of copper nanoparticles. Usman *et al*., (2013) reported the formation of pure copper nanoparticles using chitosan stabilizer, acetic acid solution (0.1 M), ascorbic acid (0.05 M) etc. The antibacterial as well as antifungal activity of the nanoparticles was investigated using several microorganisms of interest, including methicillin-resistant Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa, Salmonella choleraesuis, and Candida albicans.

The synthesis of CuNPs has been reported by reduction of copper (II) nitrate with isopropyl alcohol (IPA) in the presence of the cationic surfactant Cetyl trimethyl ammonium Bromide (CTAB). The antifungal activity of CuNPs was studied for selected plant pathogenic fungi such as *P*. destructiva, C. lunata, A. alternata and F. oxysporum. (Kanhed et al., 2014).

Kobayashi *et al*., (2013) reported the formation Cu particle of size 60nm using 0.01 M $Cu(NO_3)_2$), 0.2 - 1.0 M hydrazine as reducing agent, and 0.5×10^{-3} M citric acid and 5.0×10^{-3} M Cetyl trimethyl ammonium bromide as stabilizers.

Liu *et al*., (2012) reported the synthesis of copper nanoparticles by reducing Cu^{2+} ions with ascorbic acid through aqueous solution reduction method. It was found that $Cu(OH)_2$ was initially formed as a precursor, followed by the formation of Cu_2O, which was finally reduced to Cu particles.

There are certain advantages and disadvantages of both physical and chemical methods. Chemical methods for nanoparticle synthesis suffer from drawbacks like use of toxic solvents, noxious precursor chemicals, and harmful byproducts (Thakkar *et al*.,2010) which make these choices eco-hazardous and preclude their applications in biology, medicine and clinical applications (Awwad *et al*.,2014).

The production rate through physical methods is low and importantly the expense is very high. A further drawback of physical process is high energy consumption to maintain high temperature and pressure used in synthesis procedure. Therefore in search for safer technology researchers have developed biological methods of nanoparticle synthesis which are environment friendly and cheap. Most bioprocesses occur under normal temperature and pressure resulting in vast energy savings (Sharma *et al*., 2009; Singh *et al*., 2010).

Biological methods

A number of researchers have reported biological or green approach for nanoparticle synthesis. Green synthesis is the synthesis of nanoparticles using living organisms or their products (Mohanpuria *et al*., 2008). There are a number of reports on biological synthesis of nanoparticles using bacteria, algae, fungi, plant and plant products.

From bacteria

The need for simple, cost effective and reliable methods for synthesis of nanomaterials led to the employment of bacteria for nanoparticle synthesis. Biosynthesis methods, employing microorganisms, have emerged as an eco-friendly, clean and viable alternative to chemical and physical methods. Specific bacteria can be used for the synthesis of specific bacteria-based nanoparticles. For instance, some of well known examples of bacteria include magnetic bacteria for magnetic nanoparticles, S-layer bacteria for gypsum and calcium carbonate layer and silver mine-inhabiting Pseudomonas spp. that reduces silver ions to form silver nanoparticles.

CuNPs from Bacteria

Table 1.1: Synthesis of copper nanoparticles using Bacteria.

Sr. No.	Name of the Bactreium	Author	Size of the nanoparticle	Feature tested
1	Morganella morganii	Ramanathan et al., 2013	15-20nm	
2	Phormidium cyanobacterium	Rahman et al., 2009	50-150nm,	
3	Pseudomonas stutzeri	Varshney et al., 2011		
4	Enterococcus Faecalis	Ashajyothi., 2014	20-90nm	Antibacterial effect against E. coli, Klebsiella pneumoniae, and Staphylococcus aureus
5	Streptomyces species	Usha et al; 2010		Antibacterial and anti fungal activity against E.coli, S.aureus and A.niger
6	E.Coli	Singh et al., 2010		
7	Pseudomonas fluorescens	Shantikrati and Rani, 2014	49nm	

Rahman *et al*. (2009) reported the synthesis of copper oxide nano particles using the bacteria *Phormidium cyanobacterium*. They hypothesized that synthesis of copper oxide nanoparticles occured by extracellular hydrolysis of copper by certain metal chelating anionic proteins/reductases secreted by the bacterium.

Usha *et al*. (2010) demonstrated the synthesis of copper and Zinc oxide nanoparticles using *Streptomyces* species. The antibacterial and antifungal activity of nanoparticle coated fabric was evaluated. 100% reduction of viable *E.Coli*, *A. niger* and *S. aureus* was observed in coated fabric material after 48h of incubation. This proves the efficacy of Nanoparticles as antimicrobial agent when applied as a coating to the surface of clothes, reducing the risk of transmission of infectious agents.

Singh *et al*. (2010) have reported extracellular synthesis of copper oxide nanoparticles (CONPs) using Escherichia coli (E. coli) protein. The trichloroacetic acid (TCA) precipitated protein fraction of E. coli was used for synthesis procedure under simple experimental conditions like aerobic environment, neutral pH and room temperature.

In another study copper nanoparticles (CuNPs) were synthesized using cell-free culture supernatant of non-pathogenic bacteria *Pseudomonas fluorescens*. The influencing parameters such as pH, concentration of copper, volume of cell-free supernatant used and reaction time were studied. Neutral pH, 318.4 ppm Cu and 10 ml supernatant were optimal for NP production. Well-defined CuO NP formation occurred after 90 min incubation. NPs remained stable in aqueous solution with increasing time. It was established that the presence of some heat labile molecules released by bacteria in the cell-free supernatant leads to the reduction and formation of CuNPs which have to be identified in future (Shantkriti and Rani, 2014).

From Fungus

Eukaryotic organisms such as fungi may be used to grow nanoparticles of different chemical composition and sizes. A number of different genera of fungi have been investigated in this effort and it has been shown that fungi are extremely good candidate for the synthesis of metal nanoparticles. In addition to good monodispersity, nanoparticles with well defined dimensions can be obtained by using fungi (Mukherjee *et al*., 2001).

CuNP from Fungus

Honary *et al*. (2012) reported the synthesis of copper nanoparticles using *Penicillium aurantiogriseum, Penicillium citrinum* and *Penicillium waksmanii* isolated from soil. Addition of fungal supernatant to copper sulphate solution led to change in colour from pale yellow to brownish color. SEM and AFM confirmed the formation of copper nanoparticles.

Salvadori *et al*. (2013) reported the biosynthesis of nanoparticles from *Hypocrea lixii*. Spherical nanoaprticles with an average size of 24.5 nm were formed. Nanoparticles were synthesized extracellularly. These studies demonstrate that dead biomass of *Hypocrea lixii* provides an economic and technically feasible option for bioremediation of wastewater and is a potential candidate for industrial-scale production of copper NPs.

From Algae

Abboud *et al*. (2013) have studied the use of brown alga (*Bifurcaria bifurcata*) in the biosynthesis of copper oxide nanoparticles. These nanoparticles were found to exhibit high antibacterial activity against two different strains of bacteria *Enterobacter aerogenes* (Gram negative) and *Staphylococcus aureus* (Gram positive).

From Plants

A lot of work has been done on bio reduction of metal ions to form nanoparticles by phytochemicals and compounds found in plant extracts like enzymes, proteins, amino acids, vitamins, polysaccharides, and organic acids etc. The phytochemicals responsible for facilitating/ catalyzing the reduction reaction are terpenoids, flavones, ketones, aldehydes, amides and carboxylic acids etc. Amongst them the water soluble phytochemicals such as flavones, organic acids and quinones are found responsible for immediate reduction. Recently natural gums obtained from trees have been used for the production of nanomaterials which act both as reducing and capping agents in nanoparticle synthesis (Umer *et al*., 2012).

The advantage of using plants for the synthesis of nanoparticle is that they are easily available, safe to handle and possess a broad variability of metabolites that may aid in reduction. Some plants synthesize nanoparticles while assimilating metal ions which are present in higher concentration than what is tolerable. For example *Brassica juncea* (Bali *et al*., 2006). Table 1.2 enlists plant mediated synthesis of copper nanoparticles.

Awwad *et al*. (2015) reported the synthesis of copper oxide nanoparticles using *Malva sylvestris* leaf extract. An important outcome of their research was their observation that the morphology of the copper oxide nanoparticles could be controlled by adjusting the amount of *Malva sylvestris* leaf extract and copper ions.

Harne *et al*., (2012) used the extacts of *Calotropis procera* L. to fabricate CuNP from copper acetate. According to their study the Cysteine proteases present in the latex acted as capping agent and contributed to long term stability of CuNPs (6 months) in aqueous medium.

The effect of temperature on particle size of the nanopartlce were studied by Lee *et al*. (2013). As the reaction temperature increased, both synthesis rate and conversion to copper nanoparticle increased. With the increase in reaction temperature, reaction rate increases and thus most copper ions are consumed in the formation of nuclei stopping secondary reduction process on the surface of preformed nuclei. The nanoparticles prepared were used for coating foams to impart antimicrobial properties to them. Foams that were coated with biologically synthesized copper nanoparticles showed higher antibacterial activity as compared to foams untreated and foams treated with chemically synthesized copper nanoparticles. The antibacterial activity was found to be inversely proportional to the average nanoparticle size.

They also reported that in case of copper nanoparticles maximum absorbance occurs at 560nm and steadily increases in intensity with reaction time.

Padil *et al*. (2013) reported the synthesis of CuO nanoparticles using Gum Karaya as a biotemplate by a colloid-thermal synthesis process. The results showed that increase in precursor concentration enhances an increase in particle size, and affects the morphology of synthesized CuO nanoparticles.

Sr. No.	Plant Source	Plant part utilized	Size of Nanoparticle	Reported by	Feature tested
1.	Acalypha indica	Leaves	26-30nm	Sivaraj et al., 2014	Antibacterial and antifungal effect against Escherichia coli, Pseudomonas fluorescens and Candida albicans. The cytotoxicity activity copper nanoparticles against MCF-7 breast cancer cell lines
2.	Aegle marmelos	Leaves	≈50nm	Kulkarni et al., 2013	
3.	Artabotrys odoratissimus	Leaves	135 nm	Kathad and Gajera, 2014	
4.	Albizia lebbeck		<100nm	Jayakumarai et al., 2015	
5.	Aloe barbadensis	leaves	15-30nm	Gunalan et al.,2012	
6.	Aloe vera	leaves	20nm	Kumar et al.,2015	Antibacterial activity against fish pathogens
7.	Bifurcaria bifurcata		5-45nm	Abboud et al.,2013	Antibacterial activity against Enterobacter aerogenes and Staphylococcus aureus
8.	Calotropis procera	latex	15± 1.7nm	Harne et al., 2012	Cytotoxicity studies on HeLa, A549 and BHK21 cell lines
9.	Capparis Zeylanica	leaves	50-100nm	Saravanyadevi 2014	Antimicrobial study of both gram positive and gram negative pathogens.
10.	Carica papaya	leaves	140nm	Sankar et al., 2013	Degradation of Coomassie brilliant blue dye
11.	Centella asiatica	leaves	2 to 5 µm	Devi and singh, 2014	Phtocatalytic degradation of methyl orange
12.	Citrus medica	Fruit juice	10-60nm abs at 630nm	Shende et al., 2015	Inhibitory activity against E. coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Propionibacterium acnes, Salmonella typhi. Fusarium culmorum

					F.oxysporum and F. graminearum.
13.	Curd, lime juice, cow milk, tamarind juice, soap nut, indian gooseberry	Varoius source	37-162 nm	Sastry et al., 2013	
14.	Curcumin and lemon extract		60- 100nm	Haneefa et al., 2015	Antimicrobial activity against Staphylococcus aureus, Bacillus subtilis, Escherichia coli Staphylococcus Bacillus, Candida albicans, Curvularia lunata, Aspergillus niger and Trichophyton simii
15.	Eclipta prostrata	leaves	≈ 31nm	Chung et al., 2017	Anit cancer and antioxidant properties
16.	Euphorbia esula	Leaves		Nasrollahzadeh et al.,2014	Catalytic activity for ligand-free Ullmann-coupling reaction and reduction of 4-nitrophenol
17.	Euphorbia nivula	latex		Valodkar et al., 2011	Toxicity on A549 cell line
18.	Garcinia mangostana	leaves	26.51nm	Prabhu et al., 2015	Antibacterial activity against E.coli and S. aureus
19.	Ginko biloba	leaves		Nasrollahzadeh and Sajadi,2015	Catalytic activity for the Huisgen[3+2] cycloaddition of azides and alkynes at room temperature.
20.	Gloriosa superba	Leaves	5-10nm	Naika et al., 2015	Antibacterial activity against Klebsiella aerogenes, Pseudomonas desmolyticum, and Escherichia coli, Gram +ve bacteriaStaphylococcus aureus
21.	Gum acasia		3-9nm	Dong et al., 2014	
22.	Gum karaya		≈5nm	Padil and Cernik 2013	Antibacterial acitivity against E. coli and S. aureus
23.	Gymnema sylvestre	leaves	65-184nm	Heera et al., 2015	
24.	Ficus religiosa	leaves	577nm spherical	Sankar et al., 2014	Anticancer agent
25.	Lemongrass		2 nm	Brumbaugh et al., 2014	
26.	Lemon juice		10- 60 nm	Shende et al., 2016	
27.	Malva sylvestris	leaves	14nm	Awwad et al., 2014	Antibacterial activity against Shigella and Listeria
28.	Magnolia kobus	leaves	37-110 nm	lee et al., 2013	Antibacterial activity against E. coli
29.	Nerium oleander	leaves	50-100nm	Gopinath et al.,	Antibacterial activity

				2014	against Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae, Salmonella typhi and Bacillus subtilis
30.	Ocimum Sanctum	leaves	77nm	Kulkarni and Kulkarni, 2013	
31.	Punica granatum	Fruit peel	50-100nm	Kaur et al., 2016	Comparison of Antibacterial activity with standard antibiotics
32.	Tea leaf extract	leaves	50-100nm	Mohindru and Garg, 2017	
33.	Tea leaf and coffee powder extract		50 -100nm,	Sutradhar et al., 2014	Antibacterial activity against Shigella dysenteriae, Vibrio cholerae, Streptococcus pneumoniae, Staphylococcus aureus and Escherichia coli.
34.	T. arjuna	Bark	23nm	Yallapa et al., 2013	Anitmicrobial activity against Staphylococcus aureus, Salmonella typhi, Escherichia coli and Pseudomonas aeruginosa and fungus like Candida albicans, Trichopyton rubrum and Chrysosporium indicum
35.	Terminalia bellirica	Fruit extract	2-7nm	Viswadevarayalu et al., 2016	Antimicrobial activity against E. coli. And Candida tropicalis
36.	Tinospora cordifolia	Leaves	6-8nm	Udayabhanu et al.,	Degradation of methylene blue and antibacterial activity against klebsiella aerogenes, Pseudommonas aeruginosa, E. coli,S. aureus
37.	Tridax procumbens	leaves		Gopalkrishnan et al., 2012	Antibacterial activity against E. coli
38.	Syzigium aromaticum	Flower buds	5- 40nm, 570nm	Subhankari and Nayak, 2013	
39.	Vitis vanifera	leaves		Angrasan and subbaiya, 2014	Antibacterial activity against E. coli, S. aureus, B. subtilis, Salmonella typhi and Klebsiella pneumoniae
40.	Tabernaemontana divaricate	leaves	48nm	Sivaraj et al., 2014	Anibacterial activity against E.coli
41.	Zingiber officinale	fruit	10.13nm	Chitra et al., 2015	Anibacterial activity against Escherichia coli, Klebsiella pneumonia and Staphylococcus aureus.

Antimicrobial activity of biologically synthesized copper nanoparticles

Nanoparticles (NPs) are being increasingly used to target microbes as an alternative to antibiotics. Nanotechnology may be particularly advantageous in treating bacterial and fungal infections. Application of NPs in antibacterial coatings for implantable devices and medicinal materials to prevent infection and promote wound healing, in antibiotic delivery systems to treat disease, in antibacterial vaccines to control bacterial infections and plethora of other fields has proved the potential of nanoparticles as antimicrobial agent.

The CuNPs synthesized using plant extract of *Magnolia kobus, Syzygium aromaticum, Tridax procumbens* etc were tested against *Escherichia coli*, they showed higher antibacterial activity on cells after 24 h growth (Lee *et al*., 2011; Gopalakrishnan *et al*., 2012) Cu NPs synthesized by Zingiber officinale extract showed the zone of inhibition of 15 mm against E.Coli.

Copper oxide (CuO) nanoparticles showed significant antimicrobial activity against *Bacillus subtilis, Vibrio cholerae, Pseudomonas aeruginosa, Syphillis typhus,* and *Staphylococcus aureus* (Perelshtein *et al*., 2009; Akhavan and Ghaderi, 2012; Hassan *et al*., 2012).

Highly stable CuO NPs formed from gum karaya showed significant antibacterial action on *E. coli* and *S. aureus* 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).

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. The CuO NPs of size [4.8 ± 1.6 nm] formed using the fungi *Fusarium oxysporum* and the bacteria *Pseudomonas* were 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. (Majumder, 2012).

Copper nanoparticles have shown efficacy as anti pink disease drug for rubber tree which was infected by the fungus *Corticium salmonocolor* (Phong *et al*.,2012).

Yoon *et al*. (2007) 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 Cu2+ in the presence of cetyl trimethyl ammonium bromide (CTAB) 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 destructiva* (Kanhed *et al*.,2014).

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* (Jeyaraman *et al*., 2012; Usman *et al*., 2013).

Silver and copper nanoparticle supported on various suitable materials such as carbon, polyurethane foam, polymers and sepiolite have also been used for bactericidal application (Cubillo *et al*., 2006; Son *et al*., 2006; Rubner *et al*., 2006).

(Karthik and Geetha, 2013) report, the novel synthesis of Copper and Copper oxide nanoparticles using Chemical reduction method using Copper (II) succinate as precursor. Copper nanoparticles formed had an average diameter of about 45nm. The antimicrobial properties of copper nanoparticles were investigated using *Streptococcus pyogenes*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Staphylococcus aureus*. The copper nanoparticles showed excellent activity against *Escherichia coli* and *Staphylococcus aureus*, with inhibition zones of 14 mm and 10 mm, respectively.

Mechanism Of Action of nanoparticles

Antimicrobial activity of nanoparticles is due to its tendency to alternate between its cuprous - Cu [I], and cupric - Cu [II], oxidation states resulting in the production of hydroxyl radicals which bind with DNA molecules destroying their helical structure by cross-linking within and between the nucleic acid strands. They also damage essential proteins by binding to the sulfhydryl amino and carboxyl groups of amino acids. Similarly surface proteins necessary for transport of materials across cell membranes are destroyed affecting membrane integrity and membrane lipids (Aslam *et al*., 2002). Copper ions inside bacterial cells also disrupt biochemical processes.

Chaterjee *et al*. (2009); Kanhed *et al*. (2009) studied the mechanism of antibacterial activity of CuNP and found that CuNP treatment caused multiple toxic effects such as generation of ROS, lipid peroxidation, protein oxidation and DNA degradation in E.Coli cells.

In order to visualize the microscopic effect of the three nanoparticles treatments on *Botrytis cinerea*, healthy fungal hyphae grown on PDA plates were compared with other plates that were supplemented with 15 mg L - 1 of Ag, copper and Ag/Cu nanoparticles separately and then observed under an electron microscope. Hyphae in control appear to have remained intact, while there were damage to the surface of the fungal hyphae was observed, which could have caused the release of internal cellular materials, resulting in shrinkage of the hyphae (Ouda, 2014).

Effect of Nanoparticle treatment on crop plants

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 (Yang *et al*., 2007; Canas *et al*., 2008; Sanghamitra *et al*., 2011; Kole *et al*., 2013).

The effects of SWCNTs (single walled carbon nanotubes) on root elongation of 6 different crop species: *Brassica oleracea*, *Daucus carota*, *Cucumis sativus*, *Lactuca sativa*, *allium cepa and solanum lycopersicum* were studied to understand their toxicity to crops. CNTs enhanced root elongation in onion and cucumber. Cabbage and carrot were not affected, root elongation in lettuce was inhibited and tomatoes were found to be most sensitive (Canas *et al*., 2008).

The influence of magnetic nanoparticles coated with tetra methyl ammonium hydroxide on the growth of maize plants in early growth stages was studied (Pariona, 2017). chlorophyll ‘a’ level was increased at low level and inhibited at high level of nanoparticles.

The effects of nano-TiO_2 on the germination and growth of spinach seeds were studied. These nanoparticles improved light absorbance and promoted the activity of rubisco activase thus accelerated spinach growth (Zheng *et al*., 2005).

Aluminium nanoparticles (13nm) reduced root elongation in *Zea mays, Cucumis sativus, Glycine max, Daucus carota,* and *Brassica oleracea*. However when nanoparticles were loaded with phenanthrene, their toxicity significantly decreased (Yang *et al*., 2005).

The seed germination and root growth study of Zucchini seeds in hydroponic solution containing ZnO nanoparticles showed no negative effects (Stampoulis *et al*., 2009). whereas the seed germination of rye grass and corn was inhibited by nanoscale zinc (Lin and Xing, 2007).

The effect of CuNPs on the seedling growth of Mung bean and wheat were studied. Mung bean was found to be more sensitive to CuNPs than wheat. However the germination of lettuce seeds in the presence of CuNPs showed an increase in shoot to root ratio (Lee *et al*., 2008).

Applications of copper nanoparticles

Metallic nanoparticles have possible applications in diverse areas such as electronics, cosmetics, coatings, packaging and biotechnology. For example NP can be induced to merge into a solid at relatively lower temperature often without melting, leading to improved and easy to create coatings for electronic application eg. Capacitors (Thakkar *et al*., 2009).

NP possess wavelength below the critical wavelength of light, this renders them transparent, a property that makes them very useful for applications in cosmetics, coatings and packaging (Luo *et al*., 2006).

Copper oxide nanoparticles (CuO NPs) are used to provide antimicrobial properties to coatings, plastics, textiles and food packaging (Abramova *et al*., 2013; Llorens *et al*., 2012).

Metallic nanoparticles can be attached to single strands of DNA nondestructively this opens up avenues for medical diagnostic applications. Nanoparticles can traverse through the vasculature and localize any target organ. This potentially can lead to novel therapeutic, imaging and biomedical applications (Zhang *et al*., 2008) there are a number of reports on anti tumor activity of biologically synthesized copper nanoparticles.

Sivaraj *et al*. (2014) tested the cytotoxicity activity of *A. indica* mediated CuNP by MTT assay against MCF-7 breast cancer cell lines and confirmed that copper oxide NP have cytotoxic activity.

Longano *et al*. (2012) developed a new type of nanomaterial which can be used as antibacterial additive for food packaging applications. This nanocomposite is composed of copper nanoparticles embedded in polylactic acid, combining the antibacterial properties of copper nanoparticles with the biodegradability of the polymer matrix.

Nasrollahzadeh *et al*. (2015) reported the use of immobilized copper nanoparticles on natural natrolite zeolite, as environmentally benign catalyst. These nanoparticles were synthesized biologically using the *Anthemis xylopoda* flower aqueous extract as a reducing and stabilizing agent. The catalytic performance of the prepared catalyst was investigated for N- formylation of amines at room temperature. In an another report by the same authors copper nanoparticles synthesized using the leaf extract of *Ginko biloba* showed very high catalytic activity for the Huisgen[3+2] cycloaddition of azides and alkynes at room temperature. Furthermore, the catalyst can be simply recovered and reused several times with almost no loss in activity.

Renu Sankar *et al*. (2014) Copper nanoparticles synthesized using FIcus religiosa extract were tested for their anticancer properties. The apoptotic effect of copper oxide nanoparticles is mediated by the generation of reactive oxygen species (ROS) involving the disruption of mitochondrial membrane potential ($\Delta\psi m$) in A549 cells. The observed characteristics and results obtained in *in vitro* assays suggest that the copper nanoparticles might be a potential anticancer agent.

CuS nanoparticles find use in a wide range of applications, including solar cells, solar controllers, solar radiation absorbers, catalysts, high capacity cathode materials in lithium secondary batteries, superconductors at low temperature, chemical sensor Nanoparticles have been recently acknowledged as a novel class of materials having the capability to induce autophagy. CuONP induced autophagy in MCF7 cells in dose and time dependent manner.

Inhibition of autophage resulted in induction of apoptosis suggesting autophagy may be a survival strategy for MCF7 cells in response to oxidative stress induced by CuONPs(Laha *et al*., 2013).

Harne *et al*.(2012) showed that Cysteine proteases present in the latex of Calotropis procera cause the reduction of copper ions to its nanoparticulate form. Cytotoxicity studies of latex stabilized copper nanoparticles were carried out on HeLa, A549 and BHK21 cell lines. HeLa, A549 and BHK21 cells showed excellent viability even at 120 M concentration of copper nanoparticles. This shows that copper nanoparticles synthesized by above method hold excellent biocompatibility.

The effect of ZnO nanoparticles (spherical, 16–30 nm) on production of polysaccharides, phosphatases and phytase was studied on two fungi Aspergillus terreus CZR1 (JF 681300) and Aspergillus flavus CZR2 (JF 681301). The fungi were tested for the yield of polysaccharides as well as phosphatases (acid and alkaline) and phytase in presence of identical concentration of ZnO compound. The result suggested 8 to 9 folds improvement in extracellular polysaccharide secretion due to ZnO nanoparticle. The intracellular polysaccharide content also improved between 5 to 8 folds. ZnO nanoparticles also enhanced 51 to 108% acid phosphatase and 80 to 209% alkaline phosphatase activity. The phytase activity improved between 137 and 174%.(Tarafdar *et al*.,2012)

CONCLUSION

Nanotechnology can play key role in sustainable agriculture and second green revolution. This technology affects, and has potential to revolutionize, every sector of agricultural and food industry. Nanosensors for contaminant detection and disease diagnosis; Nanomembranes for purification, desalination and detoxification; nanofertilizers, nanopesticides and robust water tanks to prevent seepage, liquid and gaseous fuels-based lighting, cooking materials, pesticides, hormones, vaccines, solar cell panels; Nano catalysts for hydrogen generation etc.; Nanozeolites for efficient release of water, slow release of fertilizer particles; Nanomagnets for soil health testing, removal of soil contaminants; Nano-emulsions for enhancing shelf-life; Quantum dots for diagnosis; Nanoscale formulations of different food products for flavoring, refining catalytic devices in oils, dairy, meat, poultry products; Nanocomposite particles in packaging materials; Nanocapsules for better nutrient delivery, bioavailability. Nanotechnology gives strong impact on food preparation and conservation. It has great promise of sustainable development in long term and second green revolution.

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