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APPLICATION OF COPPER NANOPARTICLES IN AGRICULTURAL BIOTECHNOLOGY FOR PLANT PATHOGEN CONTROL, FOLLICULAR APPLICATIONS, MUTAGENESIS AND ELICITATION STUDIES

AFIFA ZAEEM[1*], MUHAMMAD YOUNAS[1] AND RAZIA KHURSHID[1,2]

[1]Department of Biotechnology, Quaid-i-Azam University, Islamabad, 45320, Pakistan
[2]Department of Biotechnology, Women University of Azad Jammu & Kashmir, Bagh, Pakistan
**Corresponding author's email: zaeem.afifa@gmail.com*

Abstract

The biotechnological applications of nanotechnology are flourishing tremendously in the past few years. Copper nanoparticles (CuNPs) are being produced at a large scale and have received great attention due to their unique properties and beneficial applications in various industrial and agriculture sectors. Biological- and/or green-based synthesis have an intrinsic eco-friendly nature and this is not a particularity for the production of CuNPs. In green biotechnology, the applications of CuNPs are at the initial stages. CuNPs are being successfully used to increase plant growth and productivity. CuNPs are also used in *In vitro* cell culture systems, foliar sprays and elicitation strategies to customize and optimize metabolite productions in plants in order to meet the excessive pharmacological and medicinal demands. Because of potential antimicrobial activity, CuNPs are also used to control (*In vitro*) contamination in plant tissue culture systems and (*In vivo*) plant pathogens causing plant diseases. Additionally, CuNPs are being employed as potential mutagens for site-targeted mutations in cultured plants but the research in this area is less explored. The current review highlights the applications of CuNPs in green biotechnology, with particular emphasis on its role on agriculture plants, control of different plant pathogens, foliar applications, plant tissue culture strategies as well as plant mutagenesis.

Key words: Copper nanoparticles (CuNPs); Nanotechnology; Green biotechnology; Agriculture; Plant tissue culture.

Introduction

The word "nano" is originated from the Greek word meaning "dwarf". The unit of a nanometer is equal to one billionth of a meter (10^{-9} m) which is very small and is equal to the length of ten hydrogen atoms or one hundred thousandths of the thickness of hair. Although scientists are using nanoscale materials for centuries and have underlined them in the field of Chemistry and Physics, it was until the introduction of electron microscopes such as the atomic force microscope (AFM) and scanning tunneling microscope (STM) in the 1980s, that the world of atoms at the molecular level could be seen, controlled and manipulated. This was for the first time when the words of nanotechnology and nanoscience were introduced for the science and engineering of nano-size materials. The general characteristics of nanomaterials are very different from bulk materials. Their physical, chemical, electronic, and magnetic properties change tremendously at the nanoscale size. This makes nanoscale materials more promising for industry and systems. The concept of nanotechnology has also its roots laid by the Noble Laureate Prof. Richard Feynman. In his lecture, he said: "There is plenty of room at the bottom". Furthermore, he also stated that nanoparticles (NPs) have three layers: the topmost layer which could be easily functionalized by small molecules, polymers, metal ions, or any surfactant; the shell layer with a completely different chemical composition than the core material; and the core, which is the real central portion of NP, referring to the NP itself (Feynman, 1960; Shin *et al*., 2016).

Nanomaterials and nanomachines are widely employed for biological use, such as in the field of gene, drug delivery and pharmaceutics (Mah *et al*., 2002; Pantarotto *et al*., 2004), biosensors for specific proteins and pathogens (Nam *et al*., 2003), mosquito-borne diseases and plasmodium control (Benelli, 2015; Benelli, 2016; Benelli, 2016), anticancer and antioxidant activity by green chemistry method (Rajan *et al*., 2015), fluorescent labeling (Bruchez *et al*., 1998; Chan & Nie, 1998), entry of NPs into the plants, targeted nutrition with the help of NPs, energy production by enzymatic nano-bioprocessing from agricultural waste products and advanced antimicrobial activity of NPs (Nair *et al*., 2010).

Industries have produced different type of engineered NPs at a large scale, of which the production of copper nanoparticles (CuNPs) is increasing because of unique thermal and electrical properties and cost-effectiveness as compared to gold and silver NPs (Han *et al*., 2006). Moreover, because of its optical, catalytic, and electrical properties, CuNPs are extensively used in commercial and industrial level applications in electronics, films, ceramics, inks and coating metallics (Nasibulin *et al*., 2000; Yang *et al*., 2006). There are different physical and chemical strategies for producing CuNPs involving high temperatures, inert atmospheres, organic solvents and surfactants (Dhas *et al*., 1998; Joshi *et al*., 1998; Cheng *et al*., 2006; El-Nour *et al*., 2010; Thakkar *et al*., 2010). They are most commonly produced by various methods such as electrochemical, chemical reduction, sonochemical, microwave, microemulsion, sol-gel, pulsed wire discharge, solvothermal decomposition, mechanochemical, pulse laser, and biological procedures (Tamilvanan *et al*., 2014). Especially, in the field of plant biotechnology, biological/green synthesis is more attractively used for CuNPs production because of its less toxic and more eco-friendly nature (Shende *et al*., 2015). The role of CuNPs in plants is not completely understood. The current review focuses on the recent applications of CuNPs in green biotechnology on agriculture crops, foliar applications, controlling plant pathogens as well as its role in plant mutagenesis and plant tissue culture experiments.

Copper nanoparticles in agri-biotechnology: Agri-biotechnology is promoted by the most recent developments in nanotechnology. Nanomaterials are being employed to enhance plant growth, development, and nutrients together with improved tolerance to diseases with the help of site-targeted delivery systems. The site-targeted delivery systems of NPs in plants are efficient enough to cross biological barriers with eco-friendly characteristics but, as compared to the nano-drug delivery procedures in humans, this innovation is not efficiently developed (Yang *et al*., 2007; Cañas *et al*., 2008). Particularly, copper is an essential micronutrient for plants and is required for numerous physiological and biological functions in plants such as antioxidant activity, hormone signaling, cellular transportation, mitochondrial respiration, and protein trafficking (Javed *et al*., 2017; Javed *et al*., 2017). Copper is also vital for photosynthetic reactions because it activates many enzymes contributing to RNA synthesis and the performance of photosystems (Adhikari *et al*., 2012). However, there exists a research report which suggests that the effect of copper ions isolated from copper oxide molecules is different from the effect of copper ions isolated from copper salts (sulfates or nitrates) at the same soluble concentrations (Gunawan *et al*., 2011). That's why CuNPs have leverage over their salts. CuNPs can have stimulating as well as retarding effects in plants in a dose-dependent manner (Hong *et al*., 2015; Javed *et al*., 2018). Depending on the exposure time, CuNPs enter plant cells and affect their growth by altering the process of photosynthesis, transpiration, lipid peroxidation, and chromatin condensation (Karlsson *et al*., 2009; Rajput *et al*., 2018). For instance, Zafar *et al*., (2017) reported that CuNPs inhibited the seed germination and seedling growth in Mustard, but root induction was observed in the leaf and stem explants, which shows that CuNPs have stimulatory effects (Zafar *et al*., 2017). Therefore, Cu ions, when supplied in small, controlled concentrations, help in stimulating plant growth by playing the role of essential micronutrients (Wierzbicka & Obidzińska, 1998; Karlsson *et al*., 2009; Jain *et al*., 2017). Peng *et al*., (2015) outlined the fate of nanoparticles. It was demonstrated that CuNPs are taken by the roots, transported inside, and then biotransformed. After biotransformation, NPs move towards the epidermis, reaching the cortex and stele. However, it is difficult for CuNPs to pass through the casparian strip (Peng *et al*., 2015). Particularly, the green synthesized CuNPs have more importance in the agriculture and medical industry than the chemically synthesized ones (Kumar *et al*., 2015; Kasana *et al*., 2017). This is because chemically synthesized nanoparticles have hazardous chemical binding to surfaces and they are more toxic to the environment than the green synthesized ones (Gade *et al*., 2008). In another study on *Triticum aestivum* L., CuNPs promoted lateral root formation by inducing the genes involved in auxin transmembrane transport (Zhang *et al*., 2018). Moreover, the germination of lettuce seeds in CuNPs also enhanced the shoot and root ratio as compared to control (Shah & Belozerova, 2009). (Fig. 1) elucidates different ways of how CuNPs influence plant species. In agri-nanotechnology, both positive and negative effects are cumulatively exploited for the betterment of the agricultural industry. The potential effects of CuNPs on agricultural species are briefly summarized in (Table 1).

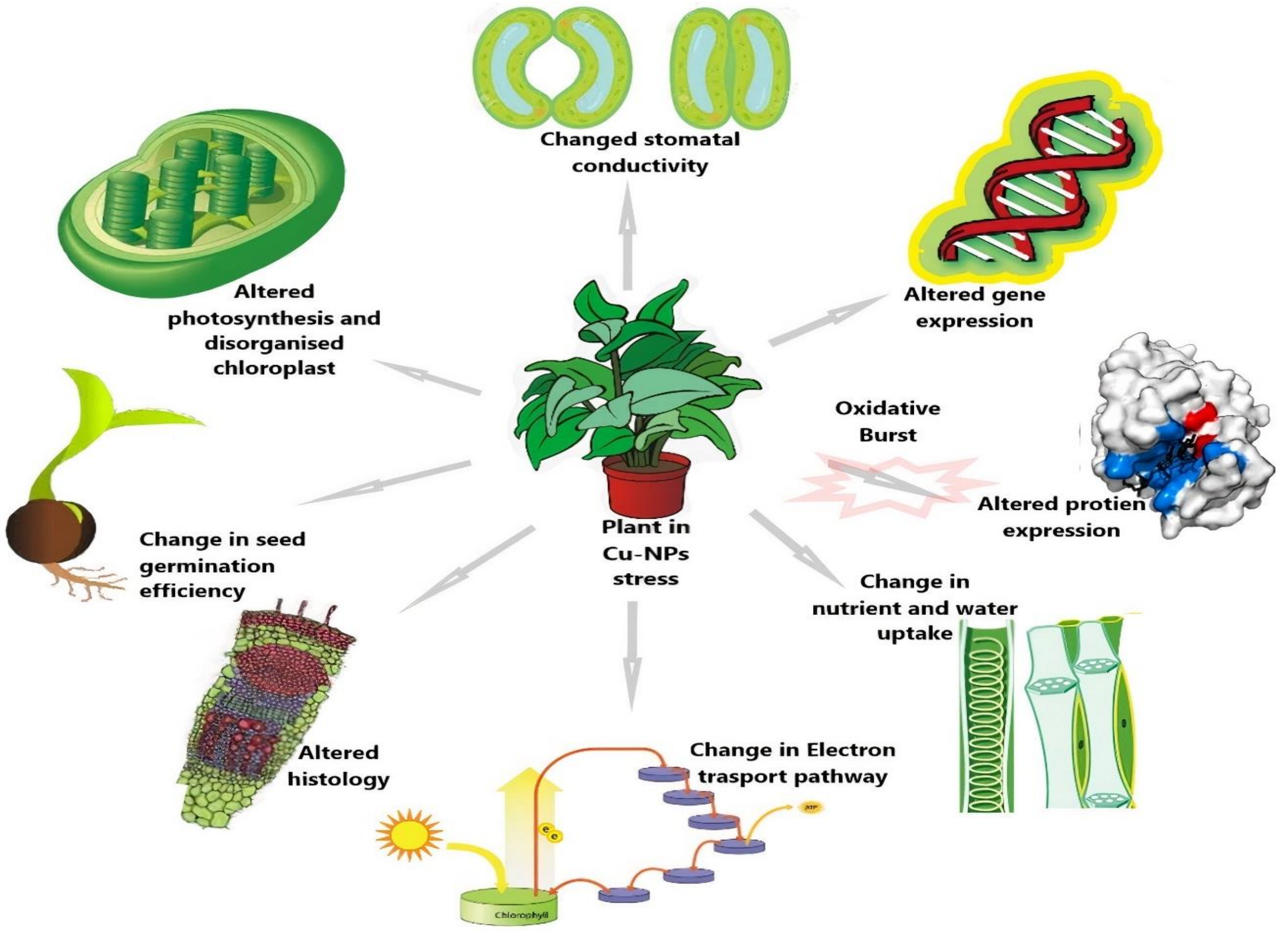

Fig. 1. Potential effects of copper nanoparticles on agricultural plants.

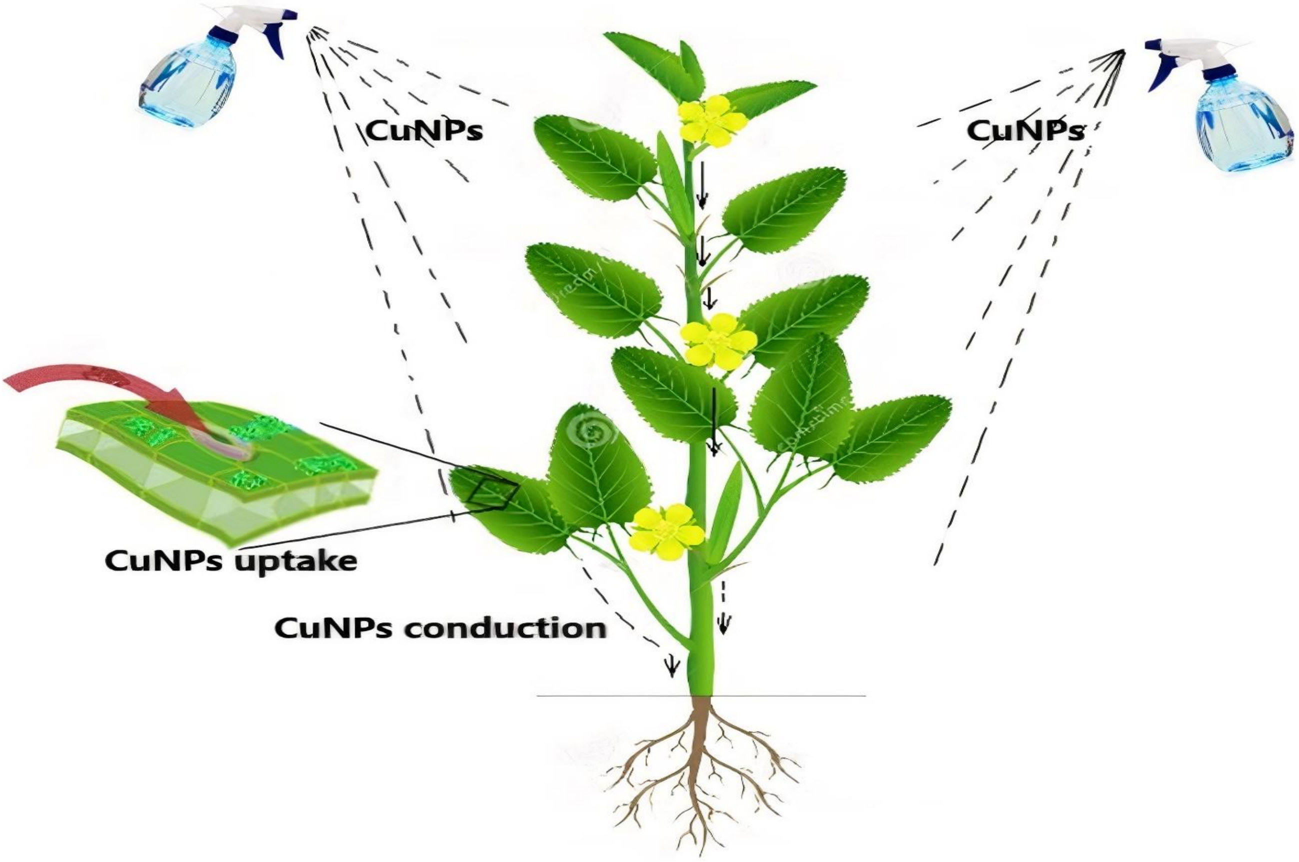

Fig. 2. An illustration of foliar applications of CuNPs.

Foliar application of copper nanoparticles: Foliar application is the process of spraying important nutrients or chemicals such as CuNPs on significantly grown plants. Leaves are most exposed to sprayed NPs which transport these NPs through phloem conduction pathways to the rest of the plant. In this case, the conduction direction is reversed as that of normal plants, i.e., from leaves to the rest of plant parts (Fig. 2) (López-Vargas *et al*., 2018). One of the benefits of foliar application is that completely grown plants can be optimized for enhancements, without the need for seedling care and soil preparation. In bean seedlings, for instance, the fruit quality has been improved by foliar spray because the lignifying enzyme, phenylalanine ammonia-lyase, accumulation in cell wall increased in proportion to the application of CuNPs. This accumulation ultimately catalyzed the phenylpropanoid pathway by transforming L-phenylalanine to cinnamic acid for the ultimate formation of lignin (Bouazizi *et al*., 2011; Wang *et al*., 2013). The foliar application of CuNPs also stimulated bioactive metabolites formation such as vitamin C, lycopene, flavonoids, phenolic and antioxidant compounds (López-Vargas *et al*., 2018). These bioactive compounds are of interest to different pharmaceutical and medical industries (Vanisree *et al*., 2004). Moreover, foliar application of CuNPs has also enhanced the tolerance to salt stress by activating plant antioxidant mechanisms (Pérez-Labrada *et al*., 2019). Several studies have reported the foliar applications of CuNPs for different plants species including *Lactuca sativa* (lettuce) (Laughton *et al*., 2019), *Solanum lycopersicum* (López-Vargas *et al*., 2018), *Mentha piperita* (Lafmejani *et al*., 2018), *Oleifera Lam* (Juárez-Maldonado *et al*., 2018), *Ocimum basilicum* (Tan *et al*., 2018), *Camellia sinensis* (Gnanamangai *et al*., 2017), *Moringa oleifera* (Juárez-Maldonado *et al*., 2018) and *Triticum aestivum* (Al-juthery *et al*., 2019).

Control of plant pathogens using copper nanoparticles: Crops have many fungal and bacterial pathogens which reduce their potential yield. In 1971, the first report was published regarding the use of copper as a potential fungicide. It was observed that the seeds soaked in copper sulfate were less prone to seed-borne fungi (García *et al*., 2003). CuNPs have been known for their antimicrobial activity which could be exploited to treat different plant pathogens. For this, the experiments are first carried out using *In vitro* conditions to evaluate the antimicrobial potential of CuNPs before being applied in the fields. In a study, maximum antifungal activity of CuNPs was recorded against the fungal plant pathogen *Curvularia lunata*, followed by *Alternaria alternata*, *Fusarium oxysporum* and *Phoma destructive* (Kanhed *et al*., 2014). Another report indicated the antifungal activity of green synthesized CuNPs against pathogenic *Fusarium culmorum, F. oxysporum and F. graminearum* fungal species (Shende *et al*., 2015). Moreover, CuNPs also demonstrated antifungal activity against *F. equiseti, F. oxysporum* and *F. culmorum* with a maximum inhibition zone of 25 mm (Bramhanwade *et al*., 2016). This biocidal activity of copper has been used in treating plant species against microbial diseases as a fungicide (Borkow & Gabbay, 2005). Similarly, under controlled conditions, various field studies have been conducted against the *Phytophthora infestans* pathogen using *Lycopersicon esculentum* (Tomato). The research used three different types of CuNPs (Cu_2O, CuO, and Cu/Cu_2O). Results of the research indicated that CuNPs are more efficient than copper-based chemicals. Additionally, CuNPs also had less deleterious effects on plants as compared to copper-based chemicals (Giannousi *et al*., 2013). This is just the beginning of CuNPs role as bactericidal and fungicidal agent and, therefore, further detailed exploration is required to fully elucidate the role of CuNPs in agriculture practices.

Copper nanoparticles as plant mutagen: Nanobiotechnology offers a new set of tools to manipulate genes using nanoparticles, nanofibres, and nanocapsules. Properly functionalized nanomaterials serve as a platform to transport a large number of genes as well as chemicals that trigger gene expression in plants (Xia *et al*., 2009). In a previous study, copper nanoparticles were used to induce phenotypic mutation in *Macrotyloma uniflorum* as a potential novel mutagen (Halder *et al*., 2015). These macromutations are very useful in agriculture fields to widen the gene pool of target plant species (Vecchio *et al*., 2012). Induction of mutation forms an integral part of the breeding program as it widens the gene pool through the creation of genetic variability. This methodology could be successfully adapted for crop improvement and release of elite "plant type" mutants (Mandal & Datta, 2014). There are various reports which support the fact that copper nanoparticles prove to be potential mutagen in different plant species including *Lathyrus sativus, Macrotyloma uniflorum, Nigella sativa* and *Allium cepa* (Halder *et al*., 2015; Nagaonkar *et al*., 2015; Kumbhakar *et al*., 2016; Ghosh *et al*., 2017). (Fig. 3) outlines a brief overview of how DNA mutation is caused by copper nanoparticles and how this mutation could be channelized for good purpose.

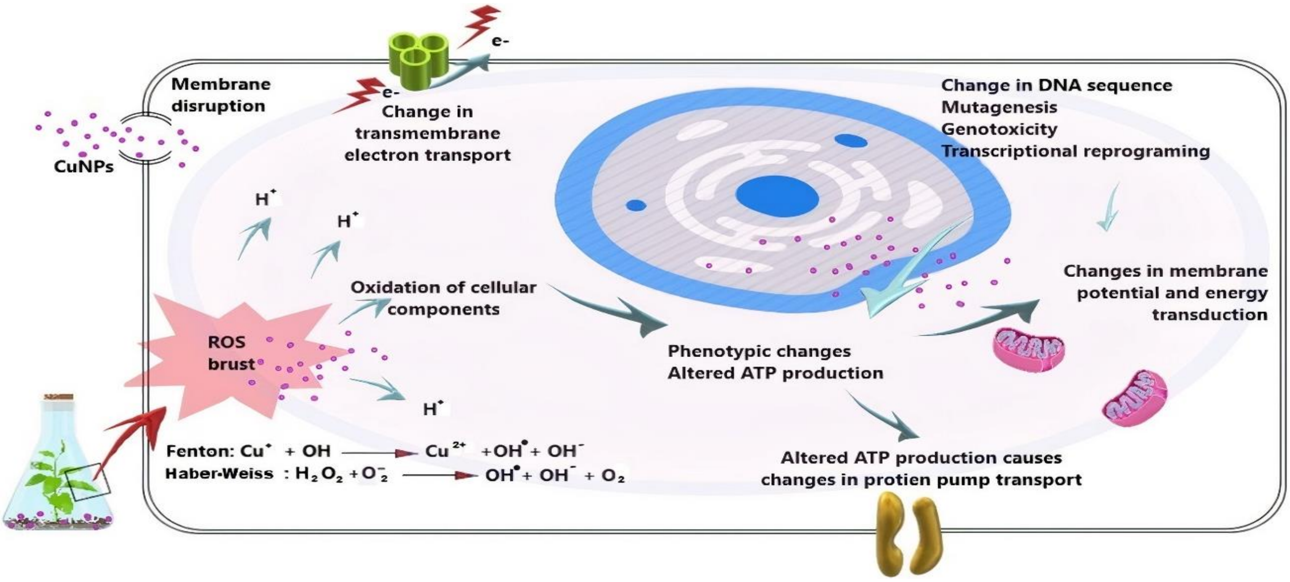

Fig. 3. A comprehensive diagram illustrating CuNPs-induced mutagenesis and changes in phenotypic expressions.

Copper nanoparticles and nano priming: Seed priming is a process of hydrating and dehydrating seeds before sowing to promote uniform germination and enhance seed quality and crop yield. There are different ways of seed priming such as osmopriming, hydropriming, nutrient priming, chemical priming, or biopriming (with plant growth regulators). Recently, seeds are also being primed with NPs termed as nanopriming (Hasanuzzaman & Fotopoulos, 2019). In nanopriming, seeds are primed with zinc oxide, iron oxide and copper oxide NPs etc. When seeds are primed with fertilizers, the seedling cannot use fertilizer because they are easily drained away with light and water, but on the other hand, NPs could be utilized by seedlings to promote their growth (Pawar & Laware, 2018). In most cases, seed priming with NPs is used to increase drought tolerance in plants. As drought tolerance is one of the biggest threats to food security resulting in significant yield loss of crops, in such a scenario seed priming with CuNPs is the best way to rescue crop yield. Seed priming with CuNPs helps plants to produce more secondary metabolites and ROS species which result in the maintenance of photosynthesis to deal with drought stress. The priming of CuNPs not only helps the plant to combat drought but also increases productivity (Van Nguyen *et al*., 2021). Nowadays, priming is done by NPs loaded with nutrient polymers. Chitosan is one of the most widely used nutrient polymer. CuNPs have been loaded with chitosan to increase the biocompatibility and biodegradability of CuNPs. These loaded NPs, if used for seed priming, could better distribute Cu in seeds to enhance the productivity. Also, chitosan loaded CuNPs have induced the defense response of seeds against fungi. Chitosan-loaded CuNPs encourage stress-free initial development of seedlings to promote electron transport and shoot growth. Chitosan-loaded CuNPs also induce enzymatic antioxidative responses to promote drought tolerance (*Gomes et al*., 2021). Seed priming with CuNPs helps to protect seeds from insects such as *Sitophilus granaries* and *Rhyzopertha dominic*. Every year, huge amount of seeds are lost because of insect infestations in seed storage. Pest infestation in grain storage could be easily controlled with nanopriming. Using insecticide is a good way to protect seeds in storage but it is not eco-friendly and affordable. As experimented on wheat, using CuNPs as a priming agent to protect seeds in storage is more affordable and eco-friendly (Badawy *et al*., 2021). Table 2 summarizes the potential effects of seed priming with CuNPs on plants.

Copper nanoparticles in plant tissue culture medium: Plant tissue culture is a modern technique of regenerating plant tissues, organs and whole plants by scientifically controlling environmental and nutritional supply under *In vitro* conditions. Nowadays, this method of large-scale plant multiplication is very commonly used. Explants, known as the small pieces of plant tissues, are excised from the mother plant and are then used for producing hundreds and thousands of plantlets in a continuous supply. In plant tissue culture experiments, explants may be a root tip, a stem part, a shoot tip, a leaf part, an axillary bud, an anther, an ovary, or an endosperm. These excised parts are used as seed material for plant tissue culture experiments. In a typical experiment, a sterile explant is inoculated onto the surface of a solidified medium in aseptic conditions. The contained explant is then incubated between 25 to 28°C for a defined period to form an undifferentiated mass of cells called callus. There are also certain cases in which the explant directly develops into roots and shoots. In case of callus, new media is provided for the induction of roots and shoots. The newly produced plants are then transferred to a greenhouse or field for further development. In this way, a small piece of explant could be used to produce thousands of plants in a very less period in *In vitro* conditions without the impact of seasonal variations (Idowu *et al*., 2009; Chun *et al*., 2020). CuNPs in this case can offer several advantages ranging from contamination control to elicitation (increasing the yield of useful metabolites), which are explained as follow:

Copper nanoparticles for contamination control: One of the problems in plant tissue culture is the chronic contamination by microorganisms. The bacterial and fungal growth on plant tissue culture media is a big barrier in culture establishments. Usually, conventional antibiotics and chemotherapeutic agents are used to control contamination in plant cultures but they somehow tend to be phytotoxic to plant growth (Dodds & Roberts, 1981). In such settings, NPs could be used as an effective treatment to reduce contamination (Sarmast *et al*., 2011; Hajipour *et al*., 2012). Particularly, there are various reports concerning the antibacterial properties of CuNPs (Theivasanthi & Alagar, 2011; Bajpai *et al*., 2012; DeAlba-Montero *et al*., 2017). Because of antibacterial and other properties, CuNPs could be used in plant tissue culture systems to eliminate unnecessary contamination. Moreover, the biosynthesized CuNPs also showed good antimicrobial activity against the growth of pathogenic fungi and pathogenic bacteria, making them a good antimicrobial candidate in plant tissue culture media (Giannousi *et al*., 2013; Abboud *et al*., 2014; Bramhanwade *et al*., 2016). Recently, Cu containing nano-pesticides are used commercially in agriculture because of antimicrobial properties (Huang *et al*., 2018). To achieve the antimicrobial function, NPs interact with microbial cells in a different manner such as Van der Waals forces, electrostatic attractions, hydrophobic and receptor-ligand interactions. These NPs then cross the microbial cell membrane and gather between the metabolic pathways to affect the function and shape of cell membrane. NPs also interact with DNA, ribosomes and enzymes by creating oxidative stress which ultimately kills the microbe (Wang *et al*., 2017). Previously, tea plants cultured *In vitro* showed antimicrobial activity against fungus in the presence of CuNPs as a potent contamination control agent (Ponmurugan *et al*., 2016). Nevertheless, a detailed investigation in this area is still needed.

Copper nanoparticles as elicitors: Elicitors are substances used on plants in small quantities for enhanced biosynthesis of various classes of important bioactive compounds including phytoalexins. These could be classified into biotic (biological) and abiotic (non-biological) elicitors. Abiotic elicitors are usually inorganic salts and physical factors such as Cu and Cd ions (Ahmad *et al*., 2021). Copper is an essential metal for plant growth and development when applied in controlled concentrations, otherwise, its excess amount can damage the plant in different ways (Fig. 4) (Shabbir *et al*., 2020). CuNPs have been employed as abiotic elicitors in plant tissue culture medium for enhanced production of secondary metabolites with significant industrial and pharmacological potentials (Hendawey *et al*., 2015). Plants are considered an unlimited source of secondary metabolites. External stimuli are used to trigger changes in a plant cell to initiate the cascade of various metabolic pathways for enhanced metabolite production (Sudha & Ravishankar, 2002). The presence of CuNPs in controlled concentrations trigger metabolism in plant tissues and result in antioxidative activities because of excessive oxidative stress. This stress, when optimized, could be used for triggering metabolic pathways to elicit secondary metabolite productions (Choi & Hu, 2008). Plant receptors receive signals of elicitors to activate effectors; GTP binding proteins, ion channels, and protein kinases. These effectors then transfer the elicitor signals to downstream reactions (Ebel & Mithöfer, 1998). There are sequential reactions to the elicitor action, outlined as; 1) elicitor perception, 2) phosphorylation and dephosphorylation of plasma membrane proteins, 3) cytosolic spiking, 4) depolarization of plasma membrane, 4) potassium and chloride ions efflux and hydrogen ions influx, 5) acidification of cytoplasm and alkalinization of extracellular spaces, 6) activation of mitogen-activated protein kinase, followed by NADPH activation, 7) ROS production, 8) gene expression activation of key early defense genes involved in signaling including jasmonate and ethylene biosynthetic genes, 9) late defense gene expression, and 10) secondary metabolites production (Zhao *et al*., 2005). Plant cell culture systems are established for excessive metabolite productions when the culture conditions are manipulated and then optimized cultures are grown on large scale bioreactors for industrial level productions (Sudha & Ravishankar, 2002). Different species have been exploited for elicitation such as *Stevia rebaudiana* (Javed *et al*., 2018), *Gymnema sylvestre* (Chung *et al*., 2019), *Withania somnifera* (Singh *et al*., 2018), *Brassica rapa* (Chung *et al*., 2019), *Glycyrrhiza glabra* (Oloumi *et al*., 2015), *Bacopa monnieri* (Lala, 2019), *Lens culinaris* (Sarkar *et al*., 2020), *Trigonella foenum-graecum* (ul Ain *et al*., 2017) and *Artemisia absinthium* (Hayat *et al*., 2021) etc. (Table 3).

Table 1. Effect of different concentrations of CuNPs on agricultural plant species.

Plant species	Media used to grow plants	CuNPs Conc.	Potential effects of CuNPs	Ref.
Triticum aestivum	Soil in pots	0-50 ppm	Increased leaf area, chlorophyll content, fresh & dry weight, and root dry weight	(Hafeez *et al*., 2015)
Zea mays	Water culture method for growing plants without soil	0-0.02 ppm	Increased plant growth	(Adhikari *et al*., 2016)
Maize hybrid hema	Seeds treated with CuNPs suspension before germination	0-1500 mg/kg seed	Increase in germination, shoot & root length	(Maithreyee & Gowda, 2015)
Quercus robur	NPs applied to foliage of plants grown in soil	0-50 ppm	Enhanced mycorrhization	(Olchowik *et al*., 2017)
Lycopersicon esculentum	NPs applied to foliage of plants grown in soil	0-250 mg/L	Increased stress tolerance to salinity	(Pérez-Labrada *et al*., 2019)
Phaseolus radiatus	Agar culture media	0-1,000 mg/L	Phytotoxicity, bio-availability, bioaccumulation	(Lee *et al*., 2008)
Triticum aestivum	Agar culture media	0-1,000 mg/L	Phytotoxicity, bio-availability, bioaccumulation	(Lee *et al*., 2008)
Allium cepa	Onion bulbs placed directly in CuNPs suspensions	0-20 µg m/l	Increased mitotic index	(Nagaonkar *et al*., 2015)
Oryza sativa	Hydroponic system	0-1,000 mg/L	Phytotoxicity	(Da Costa & Sharma, 2016)
Oryza sativa	CuNPs suspension saturated cotton pad	0-1.5 mm	Phytotoxicity	(Shaw & Hossain, 2013)
Oryza sativa	Plastic crate with germination paper	0-5 mg/L	Phytotoxicity	(Wang *et al*., 2015)
Cucumis sativus	Seeds are soaked in petri plates with filter paper	0-600 ppm	Phytotoxic effect	(Moon *et al*., 2014)
Zea mays	Hydroponic culture	0-100 mg/L	Bioaccumulation & biotransformation	(Wang *et al*., 2012)
Lycopersicon esculentum	NYA culture media	0-250 mg/L	Increased tolerance to *Clavibacter michiganensis*	(Cumplido-Nájera *et al*., 2019)
Spirodela polyrhiza	ISO culture media	0-2.5 mg/L	Phytotoxicity	(Song *et al*., 2015)
Lemna minor	ISO culture media	0-2.5 mg/L	Phytotoxicity	(Song *et al*., 2015)
Wolffia arrhiza	ISO culture media	0-2 mg/L	Phytotoxicity	(Song *et al*., 2015)
Elsholtzia splendens	Hydroponic cultures	0-2000 mg/L	Phytotoxicity & bioaccumulation	(Shi *et al*., 2014)
Lens culinaris	NPs solution in petri plates	0-0.05 mg/mL	Elicitation	(Sarkar *et al*., 2020)
Trigonella foenum-graecum	Callus cultures	0-400 mg/L	Elicitation	(ul Ain *et al*., 2017)

Table 2. Effects of CuNP seed priming on plants.

Plant species	Type of NPs	Potential Effect of Priming	Ref.
Zea mays	CuONPs loaded with chitosan	Promoted early growth and enzymatic antioxidant defense	(Gomes *et al*., 2021)
Phaseolus vulgaris	CuONPs	NPs with low concentrations and larger nanoparticles resulting in a higher biomass	(Duran *et al*., 2017)
Zea mays	Cu and Au NPs	Increased plant development and higher levels of chlorophyll and carotenoids	(Van Nguyen *et al*., 2021)
Triticum aestivum	CuONPs	Improvements in spike length, number of grains per spike, and grain weight	(Yasmeen *et al*., 2017)
Vigna radiata	CuONPs coated with 3-Aminopropyl triethoxysilane	Increased imbibition potential and germination promotion of seeds	(Sarkar *et al*., 2021)
Glycine max	Cu nanocrystalline powders	Improved biological effects on growth and development	(Ngo *et al*., 2014)
Elodea densa	CuONPs	Enhanced photosynthetic activity	(Nekrasova *et al*., 2011)

Table 3. Summary of the effects of CuNPs as potential elicitors for different plant species.

Plant species	Abiotic elicitor	Preferred Conc.	Potential Effect	Ref.
Stevia rebaudiana	ZnO & CuO NPs	10 mg/L	Total phenolic content, total flavonoid content, total antioxidant capacity, and DPPH activity	(Javed *et al*., 2018)
Gymnema sylvestre	CuO0NPs	03 mg/L	Gymnemic Acid and Phenolic Compounds	(Chung *et al*., 2019)
Stevia rebaudiana	Cu and Au NPs	30 µg/L	DPPH activity, total phenolic content, total flavonoid content	(Ghazal *et al*., 2018)
Withania somnigymfera	CuONPs	1 ppm	Flavonoid content, total antioxidant activity, ascorbic acid, tannin content	(Singh *et al*., 2018)
Brassica rapa	CuONPs	50, 250, and 500 mg/L	Reactive oxygen species, malondialdehyde and hydrogen peroxide production, total anthocyanin content	(Chung *et al*., 2019)
Glycyrrhiza glabra	ZnO and CuO NPs	1 and 10 µM	Glycyrrhizin and phenolic compounds contents	(Oloumi *et al*., 2015)
Bacopa monnieri	CuNPs	5 mg/L	Saponins, alkaloids, phenolics, flavonoids, and radical scavenging capacity	(Lala, 2019)
Lens culinaris	CuONPs	0.025 mg/mL	Defense enzymes, total phenolic content, gallic acid, total phenol, and flavonoid	(Sarkar *et al*., 2020)
Stevia rebaudiana	ZnO-PEG, ZnO-PVP, CuO-PEG and CuO-PVP NPs	10 mg/L	Total phenolic content, total flavonoid content, total antioxidant capacity, rebaudioside A and stevioside contents	(Javed *et al*., 2017)
Trigonella foenum-graecum	PVP/PEG CuONPs	200 mg/L	DPPH activity, total antioxidative potential, total reducing power potential along with total flavonoid and phenolic contents	(ul Ain *et al*., 2017)
Artemisia absinthium	Ag and Cu NPs	20 µg/L	Biomass parameters, secondary metabolites production, and antioxidant activity	(Hayat *et al*., *2021)*

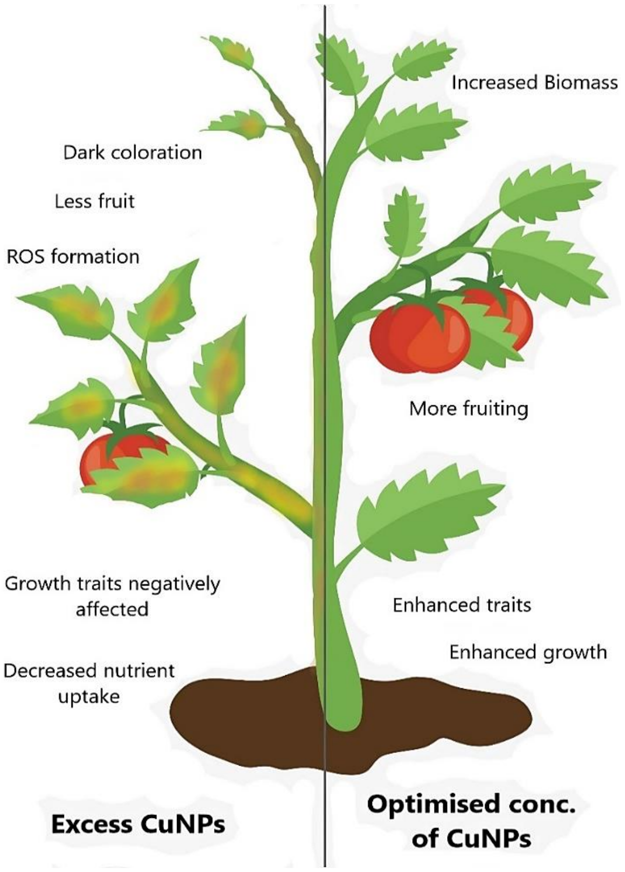

Fig. 4. Beneficial and harmful effects of CuNPs based on concentration level.

Conclusion

The technique of using nanoparticles in green biotechnology is increasing incredibly. Different nanoparticles are used in the agricultural industry because of their useful effects on plants. Likewise, copper nanoparticles have proved to be a potential agent in plant growth and development. CuNPs are employed in the agriculture industry because of easy and cost-effective productions. Plants are a good source of pharmacologically important metabolites. CuNPs are used in cell culture systems, foliar sprays and elicitation strategies to customize metabolite productions in plants in order to meet the excessive industrial needs. Altogether, these strategies are effective if copper nanoparticles are supplied in well-controlled concentrations. In high concentrations, CuNPs are toxic and damaging for plants. Pharmacologically important productions are achieved when copper nanoparticles are utilized in optimized concentrations on plants. Every year, excessive plant yield is lost because of various plant pathogens and that's why antimicrobial properties of copper nanoparticles have been used to maintain crop yield. Nanopriming of seeds with CuNPs has also become common to protect the seeds from microbial attacks. Moreover, copper nanoparticles have been used for phenotypic mutagenesis in plant cells for enhanced traits. These are usually macromutations, but if the studies are more directed then copper nanoparticles could be used for site targeted mutagenesis. The current review highlights many new studies to use copper nanoparticles on plants, but the detailed metabolic pathways triggered by copper nanoparticles in plants are still needed to be explored. For this, research is needed to be directed at the molecular level. Genetic and proteomic studies would be helpful to understand the genes involved and metabolic pathways triggered by copper nanoparticles. In future, these studies would help explore the plants for bioreactor-level productions of secondary metabolites and this in turn would help to reduce the load in natural vegetations.

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