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Dual Role of Copper Nanoparticles in plants against Abiotic Stress: A Comprehensive Review

***Tabrez Zaki Abbas*[1], *Bilal Akhtar*[2] *and Amit Kumar Singh*[*]**

[1,2,*]Department of Botany, University of Lucknow, Lucknow-226007

Email: amitunpg@gmail.com*, tabrezzaki@gmail.com[1]

Abstract

Abiotic stresses such as drought, salinity, heavy metal stress and extreme temperature stress cause significant damage to crop yield due to their interference in the physiological process of the plant like photosynthesis, nutrient acquisition and oxidative equilibrium. The use of copper nanoparticles (CuNPs) is an innovative as well as effective means to deal with abiotic stresses in nanotechnology-based agriculture because of the role of copper as a necessary micromineral, functioning as an enzyme co-factor of enzymes such as Cu/Zn superoxide dismutase (Cu/Zn-SOD), plastocyanin, and cytochrome c oxidase. Under optimal conditions, CuNPs increase stress tolerance by increasing antioxidant enzyme activity (SOD, CAT, APX), osmoregulation (proline and glycine betaine), regulation of ion homeostasis (low Na+ acquisition, high K+/Na+ ratio) and modulation of phytohormones (ABA, salicylic acid). Through the application of foliar spray and seed priming, Chlorophyll stabilization, photosystem II activity and productivity can be increased without inducing any oxidative damages. On the other side, high concentrations trigger phytotoxicity due to the Fenton-like reactions which produce excessive ROS; results in triggering lipid peroxidation, chlorophyll destruction, nutrient antagonism (deficiencies in nutrients like Fe, Zn, and Mn), DNA breaks, protein oxidation and growth inhibition, which depends on particle size and shapes as well as the types and the duration of exposure to plants. This study reviews various modes of actions, concentration–response relationships and differential impacts on different plants of CuNPs with an emphasis on their applications for mitigating stresses while considering toxicity and environmental hazards.

Keywords: Copper nanoparticles (CuNPs), Abiotic stress, Antioxidant defense, Phytotoxicity, Stress tolerance.

1. Introduction

Factors related to abiotic stress like drought, heat, salinity, heavy metal toxicity and oxidative stress have emerged as the major problems to agricultural production in many geographical regions and can cause high losses in the production of crops, thus causing a risk for global food security (Zhu, 2016). Physiological processes of plant including photosynthesis, respiration, nutrient absorption and hormone balance are affected by the abiotic stress, resulting in poor growth and reduced

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production of plants (Hasanuzzaman et al., 2013). So, by considering the increasing intensity of abiotic stress, it becomes important to look for new strategies that can help in mitigating abiotic stress of plants.

Nanotechnology is emerging as an effective approach in modern agriculture, which facilitates the specific delivery of nutrients, agrochemicals, and stress protectants (Kah et al., 2019). Among various nano based materials, copper nanoparticles (CuNPs) have earned much interest due to its ability to act as micronutrients and as redox agents (Rico et al., 2011). Copper is essential in terms of serving as a co-factor for various important enzymes, including cytochrome c oxidase, polyphenol oxidase, ascorbate oxidase and Cu/Zn superoxide dismutase (Marschner, 2012). The enzymes listed above are critical for lignin biosynthesis, reactive oxygen species detoxification, and electron transport during photosynthesis and respiration processes (Yruela, 2009). In the ionic state as Cu^{2+}, copper is characterized by a rather narrow optimal range where deficiencies and excesses of the mentioned micronutrient adversely affect plant development (Broadley et al., 2012).

In its nanoscale, copper has been found to exhibit specific physicochemical properties, such as a higher surface area/volume ratio, higher solubility, and higher reactivity, which enables copper to efficiently interact with plant cells/tissues (Dimkpa et al., 2018).

The physicochemical properties of copper allow for penetration through the root epidermal tissue or leaf cuticle, translocation through vascular tissues, and release of micronutrients, all of which enhance efficiency in nutrient uptake (Tripathi et al. 2017). Research has demonstrated that at optimal concentrations, copper nanoparticles (CuNPs) can increase antioxidant capacity, photosynthetic pigment stability, osmolytes accumulation (proline, glycine betaine), and phytohormones such as abscisic acid (ABA) and salicylic acid, thereby conferring tolerance to water stress, salinity, and heavy metals (Ahmed et al., 2021). For instance, copper NP treatment under saline conditions has been correlated with reduced lipid peroxidation, increases in water content, and an increase in photosystem II efficiency (Perreault et al. 2013).

However, such properties could result in phytotoxicity upon excessive exposure to copper nanoparticles (CuNP). The excessive exposure to CuNP/Cu^{2+} will cause an unrestricted generation of reactive oxygen species (ROS) due to a Fenton reaction, resulting in saturation of antioxidant

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system and causing damage in lipids, proteins, DNA, and inhibition of photosynthesis in plants (Shaw et al., 2013). This will manifest through symptoms of chlorosis, necrosis, reduced rate of root elongation, and inhibited shoot development. Furthermore, excessive concentrations of copper can also cause nutrient imbalance because of competition between Cu^{2+} and other essential elements like Fe^{2+}, Zn^{2+}, and Mn^{2+} (Rico et al., 2011).

CuNPs act as growth promoters when present in small amounts and become toxic when present in higher amounts, so there is a need to assess the dose-response effects, modes of uptake, and interaction with plant metabolic pathways. The response of plants to CuNPs also varies depending on shape, chemical nature of its surface, nanoparticle size, duration of exposure, development stage and kind of stress applied (Gogos et al., 2012).

2. Copper in Plant Physiology

Copper is an essential micronutrient which is required in small concentration for optimal growth and development of plant. It primarily acts as catalytic and structural component of some enzymes (Marschner, 2012)**.** Copper plays a fundamental role in respiratory and photosynthetic electron transport chain by its participation in proteins such as plastocyanin in chloroplast and cytochrome c oxidase in mitochondria (Burkhead et al., 2009). Furthermore, Copper play's crucial role in protecting against oxidative stress, serve as obligatory cofactor for Cu/Zn superoxide dismutase (SOD), that detoxifies superoxide radicals (Alscher et al., 2002). Copper dependent enzymes such as ascorbate oxidase, polyphenol oxidase and amine oxidase, contribute to lignin synthesis, hormone metabolism and cell wall strengthening, hence ensuring the structural stability of plant under stress condition (Broadley et al., 2012).

Plant copper metabolism requires a highly regulated system involving copper uptake, transportation, and sequestration. The primary way in which roots take up copper is by using highly specific copper transporters (family COPT) and the secondary ways through ZIP transporters, facilitating the safe passage of copper ions (Cu^{2+} or Cu^{+}) into specific targets (Sanz et al., 2010). After taking up the copper ion, it is transported to aerial tissues through xylem transport and to the meristem through phloem transport (Mira et al., 2001). Transport of copper in plants occurs through chelation processes where nicotianamine or glutathione plays complementary role, while

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any excess copper is stored in vacuoles or cell wall-bound forms so as to avoid copper ion toxicity (Pilon et al., 2006).

Physiological processes get disrupts due to the deficiency of copper resulting in chlorosis, wilting of tender foliage, leaf curling, and impaired reproduction because of low pollen germination (Marschner, 2012). Insufficient amounts of copper lead to disruption in photosynthesis by means of decreased plastocyanin levels, hence limiting the process of CO_2 fixation (Yruela, 2009). On the other hand, an excess of copper, regardless of its sources, causes an oxidative environment by inducing hydroxyl radicals via Fenton-like reactions (Shaw et al., 2013). Oxidative stress results in DNA damage, oxidized proteins, peroxidation of lipids, as well as enzyme inhibition, causing chlorosis, necrosis, and growth inhibition (Rico et al., 2011).

The presence of copper (Cu) can be viewed through two different perspectives against abiotic stress. Copper not only serves as a protectant but also as a source of stress itself. The presence of an optimum amount of Cu may contribute to effective protection against abiotic stress through ROS elimination, stabilization of chloroplast structure, and accumulation of osmolytes, leading to improved stress tolerance under saline, drought, and heavy metal stress (Burman et al., 2013). On the other hand, excess amounts of copper may cause oxidative damage, nutritional problems, and impaired growth in plants (Dimkpa et. Al. 2018). It is necessary to understand the dual nature of copper to evaluate the possible impact of copper delivered as nanoparticles on plant physiology during stress periods.

3. Overview of Abiotic Stresses in Plants

The effects of abiotic stresses have been found to be much more harmful than biotic stresses due to restriction in plant growth, development, and productivity worldwide (Zhu et al., 2016). Abiotic stress includes salinity, drought, toxic metal ions, temperature, and nutrient deficiency. It affects the physiological, biochemical and molecular functions, which in turn lower photosynthesis efficiency, lower nutrient absorption, and cause cell damage because of high levels of ROS (reactive oxygen species) (Hasanuzzaman et al., 2020).

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Among various abiotic stress, drought stress is widespread that causes limitation of water, which results in the closing of stomata, reduced assimilation of carbon dioxide and changes in hormonal signaling, especially within the abscisic acid (ABA) signaling pathways (Shinozaki et al., 2014). Salt stress occurs due to the presence of excess sodium chloride ions in the soil, thus causing osmotic stress, ionic stress and nutritional imbalance, which all combine to limit the root growth and water absorption (Munns and Tester, 2008).

Heavy metal stress due to excessive accumulation of copper causes change in enzyme behavior, production of free radicals and problems in nutrient metabolism (Sharma et al., 2009). Imbalance in nutrients like nitrogen and phosphorous disrupts photosynthetic process and metabolic processes (Hawkesford et al., 2012).

There are various adaptive responses to these abiotic stresses including increase in accumulation of osmolytes, increased production of antioxidant enzymes and activation of stress response genes. Nanotechnology, especially application of copper nanoparticles (CuNPs) may enhance stress tolerance by regulating the antioxidant response system, efficient uptake of nutrients and affecting signaling pathways associated with stress (Rico et al., 2011).

4. Positive Roles of CuNPs in Mitigating Abiotic Stresses

Copper nanoparticles (CuNPs) have emerged as effective nanotechnological strategies that enhance plant resistance to many forms of abiotic stress. The especial physicochemical characteristics of CuNPs, such as redox potential, high surface-to-volume ratio and ability to regulate biochemical processes, allow them to function at different biological levels, ranging from cellular antioxidative defense mechanisms to ionic balance and gene expression (Rico et al., 2011). The efficiency of CuNPs is mostly regulated by their concentration, particle size and mode of administration.

4.1. Salinity Stress Mitigation

Excessive amounts of Na^+ and Cl^- ions cause ionic toxicity and osmotic stress (Munns and Tester, 2008). Copper nanoparticles (CuNPs) have been identified to have beneficial role against salinity stress by inducing osmotic adjustment and ion homeostasis. Osmotic adjustment occurs through increase in the biosynthesis of compatible solutes like soluble sugars, glycine betaine, and proline,

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which ensure osmotic regulation (Sharma et al., 2009). Ion homeostasis can be achieved through function of membrane transporters, hence keeping a balanced K^+/Na^+ ratio in the cytosolic compartment (Rico et al., 2011). For instance, application of 50 mg L^{-1} of CuNPs on the leaves of tomato plants subjected to NaCl stress enhanced their growth by decreasing Na^+ uptake and retaining K^+ (Fabián Pérez-Labrada et al., 2019).

4.2. Drought Tolerance

Under drought stress conditions, there is limited water availability that causes stomatal closure, photosynthesis inhibition and oxidative stress (Shinozaki et al., 2014). CuNPs facilitate plant adaptation by regulating stomata function, increasing water use efficiency (WUE) and promoting osmolyte accumulation.

CuNPs-mediated stomatal regulation usually leads to partial stomata closure, which reduces water loss through transpiration but retains CO_2 uptake for photosynthesis (Ahmed et al., 2021). In this way, excessive water loss is avoided without inhibiting carbon assimilation significantly. Water use efficiency can be attained by enhancing photosynthesis and maintaining chlorophyll content. For instance, low concentration CuNPs treatment to wheat seedlings showed better RWC and photosystem II efficiency under limited water (Karimi et al., 2022). The osmolyte accumulation observed under conditions similar to salinity stress, such as proline synthesis that contributes to osmotic equilibrium (Hasanuzzaman et al., 2020).

4.3. Heavy Metal Toxicity Reduction

Heavy metals including lead (Pb), cadmium (Cd), and arsenic (As) caused high oxidative stress and interrupt nutrient metabolism (Sharma et al., 2009). The effects of heavy metals could be mitigated by CuNPs through chelation and transporter regulation. In the case of chelation, there will be complexing of heavy metals by phytochelatins and metallothioneins, thus reducing their availability within specific cellular compartments. CuNPs stimulate genes encoding chelators (Rico et al., 2011). Transporter regulation is another protective strategy shown to influence the expression of heavy-metal ATPases (HMAs) and natural resistance-associated macrophage proteins (NRAMPs), thereby reducing translocation of toxic metals from roots to shoots (El-Saadony et al., 2022).

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4.4. Temperature Stress

Temperature extremes such as heat and cold, may cause membrane destabilization, protein denaturation and metabolic imbalance (Bita and Gerats, 2013). CuNPs confer tolerance through heat shock protein (HSP) induction and membrane stabilization.

Induction of HSPs is an important phenomenon that prevent protein aggregation and help in protein folding under heat stress. It has been found that CuNPs induce HSP gene expression in plants under stress mediated by ROS-dependent signaling pathways (Karimi et al., 2022). Membrane stability depends on proper membrane composition and oxidation prevention. During cold stress, CuNPs enhanced unsaturated fatty acid content in membranes of plants, thus providing greater fluidity.

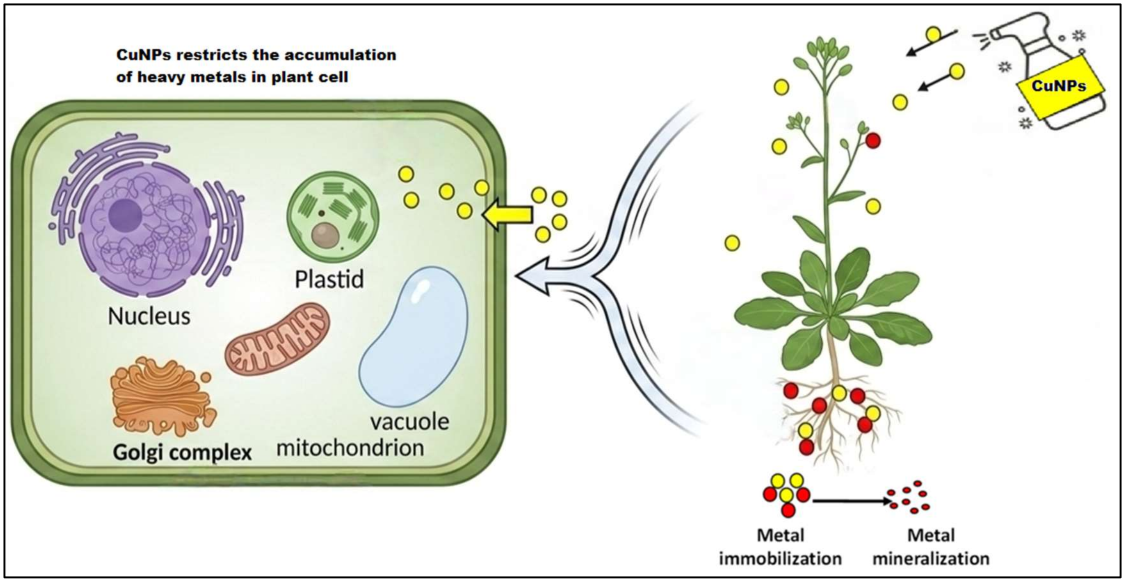

Figure 1. CuNPs initiate antioxidative defense mechanism, maintain photosynthetic attributes, enhance nutrient uptake and reduce heavy metal toxicity

4.5. Table of Beneficial Effects of CuNPs in Abiotic Stress Mitigation in some plants.

Plant species	Stress type	CuNP / Cu-based treatment & application	Observed outcomes	Reference
Tomato (*Solanum lycopersicum*)	Salinity (50 mM NaCl)	Foliar spray of CuNPs at 250 mg L^{-1} on salt-stressed plants.	Improved growth and yield, better Na^+ /K^+ balance, higher phenols,	Fabián Pérez-Labrada et al., 2019 "Responses of Tomato Plants under Saline Stress

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			vitamin C, glutathione, and fruit quality traits under salinity.	to Foliar Application of Copper Nanoparticles.”
Wheat (*Triticum aestivum*)	Drought / water deficit	CuNPs supplied in nutrient solution and soil; optimal effects around 3 mg L^{-1} in hydroponics.	Enhanced drought tolerance with higher chlorophyll stability index, leaf succulence, K content, antioxidant activity, and improved yield components.	Farooq Ahmed et al., 2021 “Applications of copper and silver nanoparticles on wheat plants to induce drought tolerance and increase yield”
Maize (*Zea mays*)	Salinity	CuO-NPs foliar-applied at multiple concentrations to salt-stressed plants.	Increased plant height, biomass, chlorophyll, and antioxidant enzyme activities; reduced oxidative damage and improved salt tolerance.	Shafiq et al., 2024, “Copper Oxide Nanoparticles Induced Growth and Physio-Biochemical Attributes of Maize under Salt Stress.”
Rice (*Oryza sativa*)	Cd–As co-toxicity	Nano-CuO (nCuO) at 10–100 mg L^{-1} added to contaminated soils with Cd and As.	Reduced Cd and As uptake in seedlings, alleviated oxidative stress, and promoted growth by down-regulating metal-transporter genes.	Zhang et al., 2020, “Effects of Copper Oxide Nanoparticles on the Growth of Rice (*Oryza Sativa* L.) Seedlings and the Relevant Physiological Responses.”
Fenugreek (*Trigonella foenum-graecum*)	Salinity	Green-synthesized CuNPs applied at several doses to salt-stressed plants.	Improved shoot and root growth, chlorophyll, osmolyte accumulation,	Fouda et al., 2024, “Ameliorative role of copper nanoparticle in alleviating salt

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			and antioxidant enzymes; decreased Na^+ and oxidative damage under salt stress	stress in fenugreek."

5. Negative Roles and Phytotoxicity of CuNPs

Although CuNPs have shown potential in enhancing the tolerance of plants against abiotic stress conditions, these nanoparticles may also cause toxicity to plants at high concentrations. An overdose of CuNPs affects cellular equilibrium, resulting in harmful biological and chemical effects on plants. It is important to understand these adverse effects as they play a key role in determining the optimum concentration range for copper nanoparticles.

5.1. ROS Overproduction and Oxidative Stress

The excess production of ROS beyond the capability of antioxidative defense mechanisms of plant is among the most common toxicological effects of CuNPs (Perreault et al., 2013). Copper is an important component of photosynthetic electron transport chains in the chloroplasts and mitochondria, but it is involved in Fenton-type reactions, where hydrogen peroxide gets reduced to highly reactive OH• radicals. This process damages the cells through the oxidation of their lipids, proteins, and nucleic acids.

5.2. Chlorophyll Degradation and Photosynthetic Inhibition

Higher concentrations of CuNPs can affect the photosynthetic process via degradation of chlorophyll pigments, reduction in ability of light absorption and damage to PSII reaction centers (Nair et al., 2014; Tripathi et al., 2017). These are caused by generation of reactive oxygen species (ROS) as well as the removal of cofactors such as magnesium from chlorophyll complexes.

5.3. Nutrient Antagonism and Imbalance

The overdose of CuNPs leads to disruption of nutrient uptake due to competition with other necessary nutrients (Dimkpa et al., 2012). Increased concentrations of copper may interfere with the absorption of Fe, Zn, and Mn, thereby causing deficiencies of these metals. In an experiment

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where lettuces were treated with CuNPs, iron deficiency was observed alongside an imbalance of nutrients (Rico et al., 2013).

5.4. Protein Oxidation and Enzyme Inhibition

Excessive level of CuNPs could generate ROS that could cause oxidation of amino acids, which would lead to improper folding of proteins, causing them to aggregate and lose function (Halliwell & Gutteridge, 2015). In addition, copper ions have the potential to bind directly with the active site of enzymes leading to enzyme inhibition.

5.5. DNA Damage and Cytoskeleton Disruption

On a molecular level, the generation of ROS through CuNPs can oxidize the DNA bases, break strands and induce mutations (Wang et al., 2012). The toxicity of CuNPs can also disrupt the cellular organization by attaching itself to microtubules and actin fibers, thereby disturbing the cytoskeleton structure. This disruption will affect cell division, vesicle movement and placement of organelles within cells (Fahmy B et al., 2009).

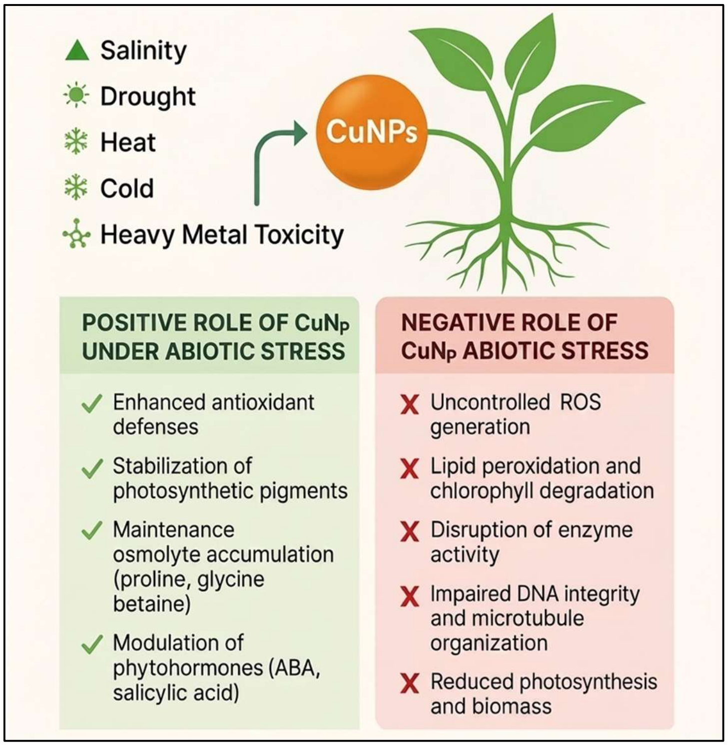

Figure 2. Dual role of CuNPs against abiotic stress

5.6. Table of Reported Toxic Effects of Copper Nanoparticles in some plants.

Plant Species	**Application**	**CuNP Conc.**	**Observed Negative Effects**	**Reference**
Rice (*Oryza sativa*)	Hydroponic	125–250 mg L^{-1}	Increased ROS, high lipid peroxidation (MDA), reduced biomass, reduced chlorophyll	Yang et al., 2020: "Effects of Copper Oxide Nanoparticles on the Growth of Rice Seedlings"
Wheat (*Triticum aestivum*)	Hydroponic/soil	$\geqslant$20 mg L^{-1}	Chlorophyll degradation, decreased photosynthesis, nutrient imbalance	Hong et al., 2015: "Toxic Effects of Copper-based Nanoparticles or Compounds to Higher Plants"
Lettuce (*Lactuca sativa*)	Hydroponic	5–20 mg L^{-1}	Fe deficiency symptoms, reduces root length, nutrient imbalance	Hong et al., 2015: "Toxic Effects of Copper-based Nanoparticles or Compounds to Higher Plants"
Cucumber (*Cucumis sativus*)	Hydroponic	50–100 mg L^{-1}	DNA damage, protein oxidation, increased H_2O_2 and MDA	Mosa et al., 2018: "Copper Nanoparticles Induced Genotoxicty, Oxidative Stress and Redistribution of Essential Metals in Roots of Cucumis sativus"

Conclusion

CuNPs have a dual effect on plants related to abiotic stress depending on their concentrations, where they can act as stress tolerance inducers when present in optimum amounts and become phytotoxicity inducer when present in excess concentration. When present in appropriate amounts,

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CuNPs serve as an essential micronutrient that enhance the efficiency of antioxidant defense systems, regulate osmosis and ensure photosynthesis efficiency under different stresses such as salt, drought, heavy metals, and temperature stresses, as demonstrated in tomatoes, wheat and maize. On the other side, high concentrations affect cell homeostasis by generating ROS, damaging membrane, nutrient imbalance and genotoxicity.

These various outcomes demonstrate the importance of optimizing dosages and modes of applications such as foliar application within specified concentrations. In future studies, priority must be put on eco-friendly techniques, such as green synthesis of nanoparticles, along with engineering of nanoparticles for optimal delivery with reduced toxicity. Furthermore, comprehensive analyses are necessary to gain insight into uptake, transport and regulation of copper nanoparticles by plants.

Field tests conducted to evaluate the impact of copper nanomaterials on soil health and their effect on non-target organisms in the ecosystem are crucial to the development of regulations on their usage. With these issues resolved, the implementation of CuNPs in agriculture becomes feasible to promote sustainability and food security in a changing climate.

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