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## REVIEW

# Copper Nanoparticles: Roles extending beyond antimicrobials to plant defense and growth

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This review explores the emerging role of copper nanoparticles (CuNPs) as a sustainable alternative to conventional agrochemicals in plant disease management. With increasing concerns over global food security, environmental degradation, and the limitations of conventional fungicides, nanotechnology offers innovative solutions with improved efficiency and reduced ecological impact. CuNPs exhibit enhanced antimicrobial activity due to their high surface area, increased reactivity, and ability to release bioactive copper ions, enabling effective control of a wide range of plant pathogens. In addition to their direct antifungal and antibacterial effects, CuNPs function as defense-elicitors, activating plant defense mechanisms through modulation of antioxidant enzymes, phytohormone signaling pathways, and expression of defense-related genes. Advances in characterization techniques have facilitated a deeper understanding of CuNP's properties and their interactions with plant systems. Importantly, the physiological and molecular responses of plants to CuNP's exposure are highly concentration- and plant species-dependent. Despite their potential, concerns regarding environmental fate, phytotoxicity, and regulatory challenges persist. Therefore, optimized application strategies and comprehensive risk assessments are essential to ensure the safe and effective integration of CuNPs into sustainable agricultural practices.

**Keywords** : Antimicrobial activity, copper nanoparticles, defense response, phytotoxicity

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## INTRODUCTION

The escalating global population, coupled with the increasingly unpredictable challenges posed by climate change and environmental degradation, exerts immense pressure on agricultural systems to ensure global food security and promote environmental sustainability.

Plant diseases, instigated by a diverse array of pathogens including fungi, bacteria, and viruses, stand as a perpetual and significant threat to crop

yields worldwide. The economic repercussions of these diseases are staggering, resulting in billions of dollars in losses annually and directly impacting the livelihoods of farmers and the stability of food supply chains (Ristaino *et al.* 2021; FAO, 2023; Gai and Wang, 2024). Consequently, the development of efficacious, economically viable and environmentally responsible strategies for plant disease control remains a paramount global research priority (Burdon *et al.* 2021). Traditionally, plant disease management has heavily relied on the widespread application of synthetic agrochemicals. While these conventional chemical control measures have played a crucial

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role in protecting crops, their widespread and often indiscriminate use has inadvertently given rise to a cascade of detrimental issues. For example, the copper-based pesticides (e.g., Bordeaux mixture, copper oxychloride) have been used long-standingly as broad-spectrum antimicrobial agents (Yu *et al.* 2023). Copper ( $\text{Cu}^{2+}$ ) is effective due to its ability to disrupt protein and enzyme functions in pathogens, and it is also an essential micronutrient for plants (Dey *et al.* 2023). However, traditional copper formulations suffer from several critical limitations, including poor dissolution and uptake necessitating high application rates, phytotoxicity, the environmental accumulation of heavy metals in the soil and water bodies, and the induction of pathogen resistance (Ghorbani *et al.* 2024). These inherent limitations of traditional agrochemicals underscore a pressing need for innovative solutions that can deliver targeted efficacy while minimizing environmental impact and combating the growing threat of microbial resistance. Nanoscience and Nanotechnology, involving the manipulation of matter at the nanoscale (typically 1 to 100 nanometers), offer a revolutionary paradigm shift for the agricultural sector. However, in "Guidelines for evaluation of nano-based agri-input and food products" released by Department of Biotechnology, Government of India in 2020, nanomaterials are defined as any material that ranges in size from 1 to 100 nm at least in one dimension or any material that possesses improved properties or phenomenon because of the effect of the dimension even if the dimensions falls outside the nanoscale range up to 1000 nm (Department of Biotechnology, 2020). This interdisciplinary field presents the opportunity to overcome the shortcomings of conventional agriculture by engineering materials with unique physico chemical properties, such as high surface area-to-volume ratios, enhanced reactivity, and quantum effects (Khot *et al.* 2012; Kah and Hofmann, 2014; Wang *et al.* 2016). Nano-technology's contributions span the entire agricultural value chain, from soil health and nutrient delivery to crop protection and post-harvest management. Nano-enabled agricultural innovations include nano-fertilizers for enhanced nutrient uptake, genetic modification and trait improvement (RNA interference), environmental remediation, and climate resilience (Kah *et al.* 2018; Duhan *et al.* 2017).

In crop protection, the development of nano-pesticides and nano-fungicides inarguably one of the most promising pathways for sustainable agriculture (Singh *et al.* 2021; Abd-Elsalam *et al.* 2024; Singh *et al.* 2024). The formulation of Copper Nanoparticles (CuNPs) represents a compelling and rational advancement in plant disease management. The use of copper is grounded in its dual roles as an essential micronutrient for plant physiology (a cofactor for enzymes involved in photosynthesis and respiration) and its established history as a broad-spectrum antimicrobial agent (Fig. 1). Cu is an essential plant micronutrient, and it is a cofactor of various enzymatic activities. It is required in little amounts, and the critical deficiency level may fall in the range of 1-5  $\text{mg kg}^{-1}$  DW, and the threshold limit for toxicity is over 20-30  $\text{mg kg}^{-1}$  DW of the plant (Nguyen *et al.* 2017). In addition, Cu has a pivotal role in the electron transport chain, photophosphorylation, and cell wall metabolism, and also enhances photosynthetic activity (Pradhan *et al.* 2015; Mir *et al.* 2021). And it is also a well-known fact that Cu is indispensable for chlorophyll formation and plant growth (Viera *et al.* 2019). CuNPs exhibit a high surface area, leading to enhanced release of  $\text{Cu}^{2+}$  ions and greater contact with pathogen surfaces, significantly boosting their antimicrobial activity compared to macro-sized particles (Dizaj *et al.* 2014). The increased efficacy allows for the use of substantially lower concentrations of copper, mitigating the risks of phytotoxicity and environmental accumulation associated with traditional high-dose applications. The small size of CuNPs potentially allows for more effective coverage and uptake, providing a more uniform and potent protective layer against pathogens. Nano-agrochemicals represent sophisticated formulations where the active ingredient is either encapsulated within a nanoscale carrier or is itself a nanoparticle. Nano-agrochemicals offer enhanced bioavailability and efficacy, leading to a significant reduction in the required chemical load, enabling targeted and controlled release, and can potentially reduce the rate of resistance development (Nuruzzaman *et al.* 2016; Kah *et al.* 2018). Nanotechnology enables the creation of smart delivery systems that are responsive to specific environmental or biological triggers, such as changes in pH,

temperature, or the presence of a pathogen (de Oliveira *et al.* 2014; Hou *et al.* 2021; Huanget *al.* 2018; Jiang *et al.* 2024). These systems release the active chemical only when and where it is needed, minimizing off-target effects and environmental dispersal. Considering the remarkable efficacy of CuNPs and their utility in plant disease management, this review aims to cover recent advances in CuNPs. While the benefits are substantial, the introduction of any new technology, especially at the nanoscale, necessitates a careful and responsible approach. Responsible and safe use of nano-agrochemicals requires rigorous testing to evaluate their long-term potential environmental and health impacts and the establishment of clear regulatory frameworks.

### **Synthesis and Characterization of Copper Nanoparticles (CuNPs)**

The synthesis of Copper Nanoparticles (CuNPs) is fundamentally guided by two core strategies, the Top-Down and Bottom-Up approaches (Tripathy *et al.* 2023; Palagati *et al.* 2024). The Top-Down approach involves the reduction of bulk material into nanosized particles, analogous to carving, offering good control over gross size and shape, and often being scalable for large-scale production, like Mechanical Milling, Plasma Etching (Tripathy *et al.* 2023; Palagati *et al.* 2024). Conversely, the Bottom-Up approach is building with molecular bricks, involves the assembly of atoms or molecules into nanoparticles, enabling precise control over size, shape, and surface properties, which is crucial for tailored functionalities. CuNPs synthesis methods are broadly categorised into Chemical, Physical, and Green (Biosynthesis) routes, each with distinct advantages in controlling particle morphology (Tripathy *et al.* 2023; Palagati *et al.* 2024).

Chemical synthesis is fundamentally a bottom-up strategy for producing CuNPs that involves the reduction of copper ions ( $\text{Cu}^+$  or  $\text{Cu}^0$ ) in solution, where the central challenge is the strict control over nucleation and growth kinetics to achieve mono-dispersity and the prevention of rapid re-oxidation of the resultant CuNPs (Kharchenko *et al.* 2016; Khan *et al.* 2016). The predominant chemical reduction methods utilize various agents

such as Ascorbic Acid, which is a relatively mild and eco-friendly choice, often requiring elevated temperatures (e.g., 80-95°C), a capping agent like Polyvinyl Pyrrolidone (PVP) for stabilization, and precise pH adjustment (using NaOH or  $\text{NH}_3$ ), which is critical for controlling morphology. The reaction's progression is indicated by a characteristic colour change associated with the CuNPs' Surface Plasmon Resonance (SPR) band (Pariona *et al.* 2019; Sadek *et al.* 2022; Pham *et al.* 2019; Wu, 2007) (Fig. 2).

Alternatively, Hydrazine Hydrate is a more potent reducing agent, frequently paired with natural stabilizers like Pectin or Sodium Alginate and sometimes accelerated by microwave irradiation or catalytic seeds (e.g., AgNPs) (Akturk *et al.* 2020; Venkatakrishnan *et al.* 2014; Yu *et al.* 2015; Grouchko *et al.* 2009) while Sodium borohydride is a very strong reducing agent, necessitating low temperatures (e.g., 0°C) and robust stabilizers (e.g., PVP, SDS) to manage its extremely rapid nucleation rate and inhibit agglomeration (Rajesh *et al.* 2016; Solanki *et al.* 2010). Precise size and shape control is achieved by tuning the relative rates of nucleation versus growth where high reducing agent concentrations and/or high temperatures generally favour rapid nucleation and thus smaller particles and through the use of polymeric stabilizers (like PVP) or surfactants (like CTAB) which adsorb onto the surface, limit Ostwald ripening, and stabilize the colloidal suspension, with the capping agent-to-precursor ratio being a vital parameter (Solanki *et al.* 2010; Hokita *et al.* 2015).

Physical methods primarily rely on top-down approach, including techniques like thermal decomposition (Mott *et al.* 2007) and those involving high-energy input. Microwave irradiation is also utilized to accelerate chemical reactions (Nikam *et al.* 2014). The thermal decomposition involves heating organometallic precursors (e.g., Copper II acetylacetonate) in high-boiling point solvents (e.g., octyl ether) with surfactants (e.g., oleic acid, oleyl amine) to yield CuNPs via controlled pyrolysis (Mott *et al.* 2007). In microwave-assisted synthesis, it provides uniform and rapid heating, which can lead to highly crystalline nanoparticles in a short reaction time (Nikam *et al.* 2014).

Green synthesis is highly favoured due to its eco-friendly nature, eliminating hazardous chemicals, and operating under ambient conditions, often at room temperature or slightly elevated. This method is a crucial application of the Bottom-Up strategy. It relies on biological entities—primarily plant extracts, fungi, or bacteria—to reduce copper ions ( $\text{Cu}^{2+}$  to  $\text{Cu}^0$ ) and stabilize the resultant nanoparticles. Phytochemicals (e.g., flavonoids, phenolic compounds, terpenoids) in plant extracts act as both reducing agents (donating electrons via hydroxyl or carboxyl groups) and capping/stabilizing agents (adsorbing onto the CuNPs surface to prevent aggregation). For example, extracts from *Lawsonia inermis*, *Ficus religiosa* and *Tabernaemontana divaricata* are being used to synthesize CuNPs (Nasrollahzadeh *et al.* 2014; Sankar *et al.* 2014; Sivaraj *et al.* 2014). This approach is generally performed in aqueous media and often at mild temperatures, minimizing energy consumption. Similarly, in fungal and microbial-mediated synthesis, microorganisms utilize secreted enzymes (e.g., reductases) and biomolecules (e.g., proteins, polysaccharides) to achieve the biogenic reduction of copper ions (Dorjee *et al.* 2024a; Truong *et al.* 2023). Moreover, several biomolecules such as primary and secondary amine, amide group, aldehydes and carboxyl groups have been associated with CuNPs synthesised using fungi, thought to act as reducing agent as well as stabilizing agents (Dorjee *et al.* 2022, 2024a,b; Kamil *et al.* 2017). This method offers potential for highly specific and monodispersed synthesis, leveraging the native biochemical pathways of the organism. Methods used for synthesis and characterization of Cu-nanoparticles have been laid out in Tables 1 & 2.

### **Characterization methods of copper nanoparticles**

Characterizing CuNPs is essential for understanding their physicochemical properties, behaviour, and potential applications, as nanoparticles exhibit unique size-dependent electronic and catalytic properties. Among various techniques (Tables 3 & 4), transmission electron microscopy (TEM) provides high-resolution images that enable direct measurement of nanoparticle size and shape; therefore, it serves as an important tool for

morphological analysis (Fig. 3). Moreover, TEM data can be used to determine particle size distribution across different ranges (Rice *et al.* 2013; Kestens *et al.* 2021; Cheng *et al.* 2010; Van den Berget *et al.* 2016). Since size and shape significantly influence nanoparticle properties, their precise determination is critical. However, due to orientation effects, misleading images can be generated and quantification of a large number of particles using TEM is a major challenge (Mourdikoudis *et al.* 2018). With advancements in analytical technologies, high-resolution techniques such as HRTEM, energy-dispersive X-ray spectroscopy (EDS), and scanning tunnelling microscopy have further enhanced characterization accuracy. In addition, scanning electron microscopy (SEM) is widely employed to obtain surface morphology and topographical details along with size estimation (Saranyaadevi *et al.* 2014; Usman *et al.* 2013). LaGrow *et al.* (2017) demonstrated that advanced high-resolution Environmental Scanning Electron Microscopy can even be used to study the redox behaviour of CuNPs, revealing dynamic oxidation and reduction mechanisms. Furthermore, UV-Vis spectroscopy offers a rapid and simple method to analyze optical properties, particularly the surface plasmon resonance (SPR) band, which depends on particle size, shape, and aggregation (Dorjee *et al.* 2023; Moniri *et al.* 2017; Thiruvengadam *et al.* 2019). The position and intensity of the SPR peak provide valuable insights into nanoparticle concentration and uniformity, as shown in Fig. 4 (Pan *et al.* 2020; Paramelle *et al.* 2014). Complementarily, X-ray diffraction (XRD) is used to determine the crystalline structure, phase composition, and lattice parameters, thereby contributing to an understanding of structural stability (Fig. 5) (Thakar *et al.* 2022; Ahamed *et al.* 2014). Fourier-transform infrared spectroscopy (FTIR) helps identify functional groups on the nanoparticle surface, revealing information about capping agents and surface interactions influencing their surface chemistry and stability (Fig. 6) (Dorjee *et al.* 2023, 2024a, 2024b; Harishchandra *et al.* 2020). Energy Dispersive X-ray (EDX) coupled with SEM or TEM allows analysis of the elemental composition of CuNPs confirming their copper content and revealing potential impurities or surface modifications (Luna *et al.* 2015; Powar

*et al.* 2019). Surface area and porosity affect CuNPs reactivity and catalytic activity are analysed using Brunauer-Emmett-Teller analysis (BET) techniques which determine specific surface area and pore size distribution, enabling understanding of the nanoparticles' surface properties by measuring gas adsorption (Phul *et al.* 2018). It basically calculates the amount of gas needed to form a monolayer on a solid surface to evaluate properties like porosity, shelf life, and catalytic activity (Thommes *et al.* 2015). Zeta potential analysis provides information about the surface charge and stability of CuNPs in various solvents, which provide insights into the colloidal stability, aggregation behaviour, and interactions with surrounding media, and provide insight into their size and distribution (Dorjee *et al.* 2023; Manikandan *et al.* 2015). Dynamic light scattering (DLS) measures the fluctuations in scattered light intensity caused by the Brownian motion of nanoparticles in a suspension (Rodriguez-Loya *et al.* 2023). DLS is based on the Stokes-Einstein equation, which correlates the diffusion coefficient of nanoparticles to their hydrodynamic diameter (Fig. 7) (Izzi *et al.* 2023; Qi *et al.* 2017). As nanoparticles undergo Brownian motion, they collide with molecules in the surrounding medium, causing intensity fluctuations in the scattered light. In addition, thermal analysis methods such as thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC) reveal information about the thermal stability, weight loss, and phase transitions of CuNPs and associated coatings or capping agents (Al-thabaiti *et al.* 2015; Vyazovkin *et al.* 2011) (Fig.8).

### **Mechanisms of antimicrobial action of copper nanoparticles**

Copper nanoparticles (CuNPs) exhibit exceptional broad-spectrum antifungal activity and possess numerous advantages over traditional agents, primarily due to their multiple mechanisms of action, which contribute to a potentially slower development of microbial resistance. CuNPs interact directly with the fungal cell membrane, inducing structural damage and altering membrane permeability (Fig 9). This disruption impairs essential membrane functions, culminating in cell lysis and mycotoxic activity. In addition, CuNPs catalyze the formation of reactive oxygen species (ROS) (e.g., hydroxyl radicals) within fungal cells. These highly reactive

molecular species cause oxidative stress by damaging critical cellular macromolecules, including proteins, lipids, and DNA, ultimately triggering apoptosis or necrosis. The efficacy of CuNPs is enhanced by their capacity for interchange between the cuprous (Cu[I]) and cupric (Cu[II]) oxidation states, which facilitates this ROS generation (Shobha *et al.* 2014). Similarly, the release of toxic copper ions (Cu<sup>2+</sup>/Cu<sup>+</sup>) from the nanoparticles interferes with vital cellular processes and fungal growth. Furthermore, CuNPs inhibit essential microbial proteins by binding to the sulfhydryl SH and carboxyl COOH groups of amino acids, thereby disrupting cellular functions and membrane integrity (Shobha *et al.* 2014). The generation of ROS, specifically hydroxyl radicals, binds to microbial DNA strands and cause helix structure disruption (Chatterjee *et al.* 2014). They also cause direct membrane disruption and interfere with other vital biochemical processes (Shobha *et al.* 2014). Beyond direct treatment, CuNPs can be incorporated into coatings to create antimicrobial surfaces that prevent fungal proliferation. The feasibility of their application is enhanced by their demonstrated relatively low toxicity to mammalian cells in various contexts (El-Sherbiny *et al.* 2025; Kaningini *et al.* 2025).

### **Antimicrobial activity of copper nanoparticles**

Copper nanoparticles (CuNPs) have gathered significant attention for their potent antimicrobial and antifungal properties. As discussed in the previous section, the antimicrobial action of CuNPs is multifaceted, involving several key mechanisms that lead to cellular damage and death. Experimental evidence strongly supports the efficacy of CuNPs against a wide range of fungi, often showing results that are both concentration and time-dependent. For instance, CuNPs synthesized by Pham *et al.* (2019) achieved complete inhibition of *Fusarium oxysporum* and *Phytophthora capsici* at a concentration of 30 ppm within three days. Viet *et al.* (2016) demonstrated a sequential increase in inhibition against *Fusarium* sp., rising from 67.38% at 450 ppm after 3 days to a near-complete 93.98% inhibition after nine days. Also, chitosan-CuNPs exhibited remarkable efficacy against *Rhizoctonia solani* and *Pythium aphanidermatum*. (Vanti *et al.* 2020). Moreover, soil application of CuNPs was found to be highly

effective against red root rot disease in tea caused by *Poria hypolateritia* (Ponmurugan *et al.* 2016). The preparation method and form also influence effectiveness; sodium alginate-capped CuNPs showed excellent activity against *Candida albicans* and *Candida krusei* with a low Minimum Fungicidal Concentration (MFC) of only 1 ppm (Akturk *et al.* 2020), while ascorbic acid-mediated CuNPs achieved significant *in vitro* inhibition (>92%) against *Botrytis cinerea* and *Sclerotinia sclerotiorum* at 100 ppm, and importantly, reduced disease incidence *in vivo* in cucumber plants at 25 ppm and 50 ppm (Sadek *et al.* 2022). Furthermore, sugar-mediated CuNPs exhibited a high inhibitory effect on *Rhizoctonia solani*, with dextrose-mediated CuNPs yielding 98.31% inhibition at  $1 \times 10^{-14}$  M (Ray *et al.* 2015). Different studies have highlighted variability in pathogen sensitivity. Pariona *et al.* (2019) reported 100% inhibition of *Fusarium solani*, *Neofusicoccum* sp., and *F. oxysporum* at 1 mg mL<sup>-1</sup>, noting that *F. solani* was the most sensitive. Shende *et al.* (2016) assessed biologically synthesized CuNPs against various agriculturally relevant pathogens, finding *Alternaria carthami* to be the most sensitive (inhibition zone 18.5 mm), while *Rhizopus stolonifer* appeared the most tolerant (inhibition zone 10.5 mm). Similarly, Truong *et al.* (2023) established complete inhibition of *F. oxysporum* at 80 ppm, and observed smaller size of CuNPs (26.5 nm) had higher antifungal efficacy than larger one (29.0 nm). Even copper (I) oxide nanoparticles (Cu<sub>2</sub>ONPs) demonstrated efficacy against root rot fungi like *Fusarium solani* both *in vitro* and *in vivo*, significantly reducing disease incidence in cucumber (Kamel *et al.* 2022). Moreover, the evidence consistently confirms the robust antimicrobial activity of CuNPs, including their effectiveness against bacteria like *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli* (Usman *et al.* 2013; Essa *et al.* 2016), solidifying their potential as versatile agents in disease management.

### **Effect of copper nanoparticles on physiological traits of crops**

The influence of nanoparticles (NPs) on crop physiology is highly context-dependent, modulated by factors such as the NPs composition, concentration, size, shape,

application method, and the specific plant species involved. While NPs, including CuNPs, present exciting avenues for enhancing crop production and stress resilience, their unique properties and interactions with plant systems necessitate careful evaluation due to potential unintended negative effects on the environment and human health (Islam, 2025; Okeke *et al.* 2022; Dorjee *et al.* 2023a). Nanoparticles can affect diverse aspects of plant growth and development, often reported to stimulate seed germination, root elongation, and shoot growth, leading to improved overall plant biomass (Rastogi *et al.* 2017). They influence the photosynthetic process by altering chlorophyll content, pigment composition, and photosynthetic rates; positive impacts on photosynthesis and ultimately contribute to enhanced crop productivity. Furthermore, NPs can modulate the accumulation of secondary metabolites like phenolics, flavonoids, and phytochemicals, which are essential for plant defense and crop quality. NPs are also documented to enhance plant tolerance to various environmental stressors, including drought, salinity, and heavy metal toxicity, by activating stress-responsive genes, increasing antioxidant enzyme activity, and mitigating oxidative damage. Mechanistically, NPs influence root morphology, branching, and density, and can also modulate key plant hormone signaling pathways (auxins, cytokinins, abscisic acid), thereby regulating growth, development, and stress responses (Tripathi *et al.* 2022).

In regard to copper-based nanoparticles, impact on seed germination and subsequent growth parameters is demonstrably species- and concentration-dependent, ranging from stimulatory effects at lower doses to significant toxicity at higher exposures (Dimkpa *et al.* 2012; Dorjee *et al.* 2023a; Yang *et al.* 2020; Gautam *et al.* 2017). For instance, a recent study demonstrated that application of copper nanoparticles enhanced biomass, root and shoot length, germination percentage, chlorophyll a, b and carotenoid content in maize leaves when subjected to copper based nanoformulation at optimum concentration of 250 mg/l; however, reduction of higher concentration caused phytotoxicity and also reduction in concentration of Mn and Zn in leaves of maize (Dorjee *et al.*

**Table 1** : Chemical reduction methods reported for the synthesis of copper nanoparticles and copper-based nanoparticles

Precursor	Reducing agent	Capping agent/Stabilizer	Solvent	Conditions (temperature and duration)	References
CuSO <sub>4</sub>	Ascorbic Acid and NaBH <sub>4</sub>	PEG-8000	Water	100°C and 2 h 40 min	Dorjee <i>et al.</i> (2023a)
CuSO <sub>4</sub> .5H <sub>2</sub> O	Ascorbic acid	PVP	Water	4 h	Sadek <i>et al.</i> (2022)
CuSO <sub>4</sub> .5H <sub>2</sub> O	Hydrazine hydrate	Sodium Alginate	Starch	600 W and 2 min	Akturk <i>et al.</i> (2020)
CuCl <sub>2</sub>	Ascorbic acid	PVP	Water	80°C and 4 h	Pham <i>et al.</i> (2019)
CuSO <sub>4</sub> .5H <sub>2</sub> O	Ascorbic acid	sodium citrate tribasic dihydrate	Water	95°C and 90 min	Pariona <i>et al.</i> (2019)
Cu (CH <sub>3</sub> COO) <sub>2</sub>	Ascorbic acid	Dextrose, dextrin -10, and β - cyclodextrin	Water	---	Ray <i>et al.</i> (2015)
Cu (CH <sub>3</sub> COO) <sub>2</sub>	NaBH <sub>4</sub>	1-Decyne	Water and ethanol	0°C and 3 h	Liu <i>et al.</i> (2014)
Cu (CH <sub>3</sub> COO) <sub>2</sub>	H <sub>2</sub> (3 bar)	PVP	Glycerol	100°C and 180 h	Chahdoura <i>et al.</i> (2014)
Cu (CH <sub>3</sub> COO) <sub>2</sub>	N <sub>2</sub> H <sub>4</sub>	1-Amino-2-propanol	Ethylene glycol	25°C and 24 h	Hokita <i>et al.</i> (2015)
Cu (CH <sub>3</sub> COO) <sub>2</sub>	Glucose		Water	70°C and 2 h	Sabbaghan <i>et al.</i> (2015)
Cu (CH <sub>3</sub> COO) <sub>2</sub> .2H <sub>2</sub> O	Ribose		Water	70°C and 2 h	Sabbaghan <i>et al.</i> (2015)
Cu (NO <sub>3</sub> ) <sub>2</sub>	N <sub>2</sub> H <sub>4</sub> .H <sub>2</sub> O	PAA	Water	Catalyzed by pre -formed AgNPs	Grouchko <i>et al.</i> (2009)
CuCl <sub>2</sub>	NaBH <sub>4</sub>	PVP	Ethanol	RT and 8 h	LaGrow <i>et al.</i> (2014)
Cu (NO <sub>3</sub> ) <sub>2</sub>	NaBH <sub>4</sub>	Tergitol NP-9	Water + ethylene glycol	RT and 2 h	Dharmadasa <i>et al.</i> (2013)
CuCl <sub>2</sub> .2H <sub>2</sub> O	Glucose	Oleic acid+oleylamine	Water + ethanol	50°C and 12 h	Li <i>et al.</i> (2014)
Cu (NO <sub>3</sub> ) <sub>2</sub>	N <sub>2</sub> H <sub>4</sub>	Ethylenediamine	Water	70°C and 30 min	Ye <i>et al.</i> (2014)
Cu (NO <sub>3</sub> ) <sub>2</sub>	-	PEG	Water	75, 100, and 150°C	Ananth <i>et al.</i> (2015)
Cu (NO <sub>3</sub> ) <sub>2</sub>	N <sub>2</sub> H <sub>4</sub>	Amino acids	Alkaline aqueous solution	80°C and 30 min	Yu <i>et al.</i> (2015)
Cu (NO <sub>3</sub> ) <sub>2</sub>	N <sub>2</sub> H <sub>4</sub>	Span-80+Tween-80+Oleic acid	n-butanol+ parafilm	60°C and 6 h	Sun <i>et al.</i> (2011)
Cu <sub>2</sub> ONPs	Ascorbic acid	SiO <sub>2</sub> shel	Water	Acid assisted reduction	Shaygan <i>et al.</i> (2015)
CuCl <sub>2</sub>	N <sub>2</sub> H <sub>4</sub>	Pectin	Water	RT	Venkatakrishnan <i>et al.</i> (2014)
CuCl <sub>2</sub> .2H <sub>2</sub> O	N <sub>2</sub> H <sub>4</sub> .H <sub>2</sub> O	Tris (trimethylsilyl)silane	Oleylamine	120°C and 10 h	Cui <i>et al.</i> (2015)
CuCl <sub>2</sub>	Fructose	Fructose	Water	60°C and 1 h	Sarkar <i>et al.</i> (2014)
CuCl <sub>2</sub>	NaBH <sub>4</sub>	Ammonia	Water+cyclohexane	RT and 5 min	Solanki <i>et al.</i> (2010)
CuCl <sub>2</sub>	Ascorbic acid	Triton-X 100	Castor oil	80°C	Wang <i>et al.</i> (2014)
CuCl <sub>2</sub>	Microwave-assisted		Ethylene glycol	185°C and 30 min	Kawasaki <i>et al.</i> (2011)
CuCl <sub>2</sub>	Na <sub>3</sub> PO <sub>4</sub>		Water	60°C and 20 min	Huang <i>et al.</i> (2015)
CuCl <sub>2</sub>	Ascorbic acid	Branched polyethyleneimine	Water	90°C and 3 h	Tang <i>et al.</i> (2015)
Cu(SO <sub>4</sub> ) <sub>2</sub> .5H <sub>2</sub> O	NaOH	L-Arginine	Water	--	Bhattacharjee <i>et al.</i> (2015)
CuSO <sub>4</sub> .5H <sub>2</sub> O	Glucose	SLS	Water + ethylene glycol	70°C	Shenoy <i>et al.</i> (2013)
CuSO <sub>4</sub> .5H <sub>2</sub> O	Ascorbic acid	Ascorbic acid	Water	80°C and 14 h	Xiong <i>et al.</i> (2011)
CuSO <sub>4</sub>	3-mercaptopropoic acid	Histidine	Water	28°C and 2 days	Sharma <i>et al.</i> (2015)

2023a). Positive effects of CuNPs on the maize were also reported by Van Nyugen *et al.* (2022), where they found that under the drought stress, priming with CuNPs increased plant biomass, water content of leaf, chlorophyll, carotenoid and

anthocyanin content. In consensus, CuO NPs application resulted in an increase in root length, root tip number, total biomass, and photosynthetic rate, but a led to significant decrease in the concentration Mg, Ca, and Mn in *Brassica*

**Table 2:** Biological methods reported for the synthesis of copper nanoparticles and copper-based nanoparticles

Precursor	Reducing agent	Capping agent/Stabilizer	Solvent	Conditions (temperature and duration)	References
CuSO <sub>4</sub>	<i>Bifurcaria bifurcate</i> extract	<i>Bifurcaria bifurcate</i> extract	Water	100-120°C and 24h	Abboud <i>et al.</i> (2014)
CuSO <sub>4</sub>	<i>Macrophomina phaseolina</i>	Primary and secondary amines	Water	28°C and 5-6 days	Dorjee <i>et al.</i> (2022)
CuSO <sub>4</sub>	<i>Aspergillus niger</i> strain STA9		Water	30°C and 24 h	Noor <i>et al.</i> (2020)
CuCl <sub>2</sub> ·2H <sub>2</sub> O	<i>Shizophyllum commune</i>		Water	28° C and 48 h	Fatima <i>et al.</i> (2022)
Cu(OAc) <sub>2</sub>	<i>Kigelia africana</i> fruit extract	<i>Kigelia africana</i> fruit extract	Water	24 h	Alao <i>et al.</i> (2022)
Cu(OAc) <sub>2</sub>	<i>Curcuma longa</i> extract	<i>Curcuma longa</i> extract	Water	200W power and 180 s	Jayarambabu <i>et al.</i> (2020)
CuSO <sub>4</sub>	<i>Trichoderma virens</i> and <i>Chaetomium globosum</i>	Primary and secondary amines	Water	28°C and 5-6 days	Dorjee <i>et al.</i> (2024a)
CuSO <sub>4</sub>	<i>Fusarium verticillioides</i> and <i>Bipolaris maydis</i>	Amines and amides	Water	28°C and 5-6 days	Dorjee <i>et al.</i> (2024b)
CuSO <sub>4</sub> ·5H <sub>2</sub> O	<i>Lawsonia inermis</i> leaf extract	<i>Lawsonia inermis</i> leaf extract	Water		Nasrollahzadeh <i>et al.</i> (2014)
CuSO <sub>4</sub>	<i>Albizia lebbbeck</i>		Water	37°C and 24-72 h	Jayakumarai <i>et al.</i> (2015)
CuSO <sub>4</sub> ·5H <sub>2</sub> O	Extract of <i>Tabernaemontana divaricata</i>	Extract of <i>T. divaricata</i>	Water	37°C and 24-72 h	Sivaraj <i>et al.</i> (2014)
CuSO <sub>4</sub> ·5H <sub>2</sub> O	Hydranium hydroxide	Extract of <i>Kappaphycus alvarezii</i>	Water	100°C and 7 h	Khanehzaei <i>et al.</i> (2014)
CuSO <sub>4</sub> ·5H <sub>2</sub> O	Leaf extract of <i>Ficus religiosa</i>	Leaf extract of <i>Ficus religiosa</i>	Water	Incubation at RT	Sankar <i>et al.</i> (2014)
Cu (NO <sub>3</sub> ) <sub>2</sub> ·3H <sub>2</sub> O	Leaves extract of <i>Calotropis gigantea</i>	Leaves extract of <i>C. gigantea</i>	Water	400°C and 2 h	Sharma <i>et al.</i> (2015)

*chinensis* L. (Di *et al.* 2023). Possibly due to higher oxidative activity of copper oxide nanoparticles (CuO NPs), phytotoxicity to rice was reported at a low concentration of 62.5 mg/l; roots and shoots length, biomass, chlorophyll and carotenoid content were drastically reduced (Yang *et al.* 2020). CuONPs have also been reported to reduce the chlorophyll content in *Hordeum vulgare* L. (Shaw *et al.* 2013). In another study, CuNPs alone were reported to enhance the seedling characteristics of maize, viz., root and shoot biomass, root and shoot length, germination, and vigor (Dorjee *et al.* 2023b). A

recent interesting study showed that CuNPs at 300 mg/l, mitigated drought stress by improving physiological traits viz., chlorophyll content, spike length, plant height, water use efficiency, leaf turgor potential, 1000 grain weight, stomatal conductance of wheat, improving drought resilience and overall grain yield (Raza *et al.* 2024). A similar study on wheat provided clear evidence of improvement in yield and drought tolerance due to CuNPs by rendering better water retention and nutrient uptake (Ahmed *et al.* 2021). However, Zhang and his co-workers recorded CuNPs inhibiting the elongation of primary root

**Table 3** : Different characterization methods for copper nanoparticles

Characterization methods	Primary property determined	Specific insight provided
TEM/HRTEM/ SEM	Morphology/topography	Size, shape, spatial distribution, surface details
X-ray diffraction analysis	Crystalline structure	Crystal phase, lattice parameters, crystal size
UV-Vis spectroscopy	Optical properties	Surface plasmon resonance (SPR), concentration, uniformity
Fourier Transform Infrared Spectroscopy	Surface chemistry	Determination of Functional groups, identification of capping agents
Energy Dispersive X-ray Spectroscopy	Elemental composition	Confirmation of copper content, purity, surface modifications
Dynamic light scattering	Colloidal stability/size	Hydrodynamic diameter, size distribution, aggregation. behaviour
Brunauer-Emmett-Teller Analysis	Surface area/porosity	Colloidal stability, tendency for aggregation
Thermogravimetric Analysis/ Differential Scanning Calorimetry	Thermal properties	Thermal stability, weight loss, phase transitions

but enhancing the lateral root emergence and reduction of relative growth rate of roots by 60% in wheat, which they attributed to an increase in uptake of nitrogen and an increase in concentration of auxin in lateral roots resulting toxicity (Zhang *et al.* 2018). Growth parameters, photosynthetic pigments and fruit quality of strawberry were also reported to enhance significantly upon the application of CuNPs under drought stress (Liu *et al.* 2024). However, CuO-NPs at 200 ppm acted as a potent stimulant in soybean, resulting in a 15% improvement in seed germination and a substantial 50.08% increase in the Seed Vigor Index relative to the control, although application of a higher concentration proved detrimental, reversing these beneficial metrics (Gautam *et al.* 2017). Similarly, studies utilizing increased concentrations consistently highlight CuO-NPs' phytotoxicity. Zafar *et al.* (2016) reported marked negative effects on *Brassica nigra* seed germination and growth, where the maximum concentration of 1500 ppm drastically reduced germination percentage and

led to severe growth inhibition, with plant height shrinking to 0.85 cm compared to the 3.68 cm control, alongside a considerable reduction in root length. Furthermore, Zuverza-Mena *et al.* (2015) observed 50% reduction in the germination of *Coriandrum sativum* when exposed to nano-CuO at 80 mg/kg, accompanied by 12.4% reduction in shoot elongation, although CuO-NPs' effect on rice was less severe, reducing germination by only 7% at 1000 ppm (Shaw and Hossain, 2013). A high toxicity with changes in phenotype, decrease in photosynthetic pigments, reduction in overall biomass, damage to root plasma membrane leading to leakage of electrolyte have been documented when cucumber was exposed to CuNPs (Mosa *et al.* 2018). In a comparative study, Yasmeen *et al.* (2015) found that while CuNPs enhanced wheat seed germination, excessive exposure time, specifically, prolonged soaking (over 2 h) in the CuNPs suspension, led to a measurable decrease in seed germination.

**Table 4 :** Characterization of nanoparticles using various instruments

Precursor	Reducing agent	UV-Vis ( $\lambda$ ) nm	XRD (nm)	FTIR	PSA	TEM	DLS	SEM	AFM	References
CuSO <sub>4</sub>	NaBH <sub>4</sub>	Peak at 574 nm	Crystalline plane ( <i>hkl</i> of 111,200 and 200)	Peaks at 619.50 cm <sup>-1</sup> and 791.24 cm <sup>-1</sup>						Truong <i>et al.</i> (2023)
CuSO <sub>4</sub>	NaBH <sub>4</sub> and Ascorbic acid	Peak at 466 nm	Face-cubic structure (FCC) ( <i>hkl</i> ) 111, 200, and 220); size: 29.208 nm			Average size: 38.48 nm	Hydrodynamic size: 91.28nm			Dorjee <i>et al.</i> (2023b)
CuSO <sub>4</sub> .5H <sub>2</sub> O	Ascorbic acid		Crystalline plane ( <i>hkl</i> of 111 and 200)			Size: 2-12 nm				Sadek <i>et al.</i> (2022)
CuSO <sub>4</sub> .5H <sub>2</sub> O	Hydrazine hydrate		Face-centered cubic structure (FCC); Crystalline nature	Peaks at 1418, 1051, 875, and 801 cm <sup>-1</sup>			Hydrodynamic size: 263.8 nm	Size: 67 ± 28 nm		Akturk <i>et al.</i> (2020)
CuSO <sub>4</sub>	<i>Aspergillus niger</i>	Peak at 480 nm		Peaks at 3330.07 <sup>-1</sup> and 1636.91 cm <sup>-1</sup>		Size: 5 to 100 nm		Size: 500 nm		Noor <i>et al.</i> (2020)
CuSO <sub>4</sub>	<i>Shizophyllum commune</i>	Peak at 560 nm		Peak at 3435.89 cm <sup>-1</sup> , 2079.04 cm <sup>-1</sup> and 1633.84 cm <sup>-1</sup>		Size: 40 to 65 nm				Fatima <i>et al.</i> (2022)
CuCl <sub>2</sub>	Ascorbic acid	Final peak at 586 nm at 4h	FCC; Crystalline nature			Shape: Spherical; Size: 120 nm at pH 7				Pham <i>et al.</i> (2019)
CuSO <sub>4</sub> .5H <sub>2</sub> O	Ascorbic acid		Crystal structure			Size: 200-500 nm				Pariona <i>et al.</i> (2019)
Cu(CH <sub>3</sub> COO) <sub>2</sub>			FCC; Crystalline nature			Size: 10 nm	Around 100 nm			Ray <i>et al.</i> (2015)
CuSO <sub>4</sub>	Leaf extract <i>Artocarpus heterophyllus</i>	Peak at 640 nm	FCC	Peaks at 3428, 2925, 1614, 1382, 1103, 655.8 cm <sup>-1</sup>				132 nm		Sharon <i>et al.</i> (2018)
CuCl <sub>2</sub> .Cu(NO <sub>3</sub> ) <sub>2</sub>	Ascorbic acid		FCC	3420 and 1635 cm <sup>-1</sup>				Nanowire of size: 142.14±42.6 μm		Tang <i>et al.</i> (2015)
CuSO <sub>4</sub>	Leaf extract <i>Ageratum houstonianum</i> Mill.	Peak at 326 nm		Peaks at 3236.34, 2918.71,235.93, 1595.13 cm <sup>-1</sup>		Size: 80 nm	Size:100 nm	Size: 200 nm		Chandraker <i>et al.</i> (2020)
CuSO <sub>4</sub> .5H <sub>2</sub> O	<i>Ocimum tenuiflorum</i> leaf extract	Peak at 735 nm		Peaks at 3429, 1645.98, 1394.58,1246.06 and 1072.46 cm <sup>-1</sup>	Peak at 22.09 nm; Size: 1-120 nm	Size: 22.09 nm	Size:100 nm			Ramadhan <i>et al.</i> (2019)
CuCl <sub>2</sub>	Ascorbic acid							Size: 100-250 nm		Weisany <i>et al.</i> (2019)
(CuSO <sub>4</sub> .5H <sub>2</sub> O)	Sodium hypophosphitem onohydrate (NaH <sub>2</sub> PO <sub>2</sub> .H <sub>2</sub> O)	—	Average size: 32 nm; FCC			Shape :spherical Size: 30 and 65 nm		Shape: spherical Size: 30 and 65 nm		Lee <i>et al.</i> (2008)
Copper chloride (CuCl <sub>2</sub> .2H <sub>2</sub> O)	Trisodium citrate, myristic acid and hydrazine hydrate (N <sub>2</sub> H <sub>4</sub> )	Peak at 600 nm	Average crystallite size: 30 nm			Agglomerated particles		Revealed the formation of fluffy agglomerates		Khanna <i>et al.</i> (2007)
CuO	Sodium phosphinate monohydrate (NaH <sub>2</sub> .PO <sub>2</sub> .H <sub>2</sub> O) and diethylene glycol		Copper peak at 932.0 eV together with weak CuO peaks at 934.2eV			Size: 1.5 nm.		Size: 45-63 nm		Park <i>et al.</i> (2007)

CuSO <sub>4</sub> ·5H <sub>2</sub> O	Hydrazine (N <sub>2</sub> H <sub>4</sub> ) and ascorbic acid	Peak: 500-600 nm	Size: 10-15 nm.	bands at 3,358cm <sup>-1</sup> ,	Size: 50 nm to 300 nm	Size: 50-270 nm	Usman <i>et al.</i> (2013)
CuSO <sub>4</sub> ·5H <sub>2</sub> O	Ascorbic acid				Size: 35 nm		Kathadand Gajera, (2014)
CuSO <sub>4</sub>	Dendrimers with a trimesyl core (G3–G6),	G3: 310 nm G4:310 nm G5: 307 nm G6: 311 nm		Two broad peaks centered at 1543cm <sup>-1</sup>  ca 1648 and	G3: 5.6 nm G4: 4.8 nm G5: 3.9 nm G6: 3.4 nm		Jin <i>et al.</i> (2008)
CuNO <sub>3</sub>	Chitosan solution and ascorbic acid	peak at 550 nm			Size: 35.5 mV		Zain <i>et al.</i> (2014)
(CH <sub>3</sub> COO) <sub>2</sub> Cu·H <sub>2</sub> O	<i>Syzygium aromaticum</i>		FCC; Crystallite size: 12 nm		Shape: spherical; Size: 15 nm	Shape: spherical; Size: 20 nm	Rajesh <i>et al.</i> (2018)
CuSO <sub>4</sub>		Peak at 551 nm		Shift of peak from 3398 cm <sup>-1</sup> to 3432 cm <sup>-1</sup> , 1624 cm <sup>-1</sup> to 191364 cm <sup>-1</sup> , 2932 cm <sup>-1</sup> and 2898 cm <sup>-1</sup> are shifted to 1637 cm <sup>-1</sup> , 1374 cm <sup>-1</sup> , 2939 cm <sup>-1</sup> and 2899.63 cm <sup>-1</sup> , respectively, 629.73cm <sup>-1</sup> and 1027.06 cm <sup>-1</sup> is to 614.43 cm <sup>-1</sup> and 1021.11 cm <sup>-1</sup>	Size: 9 nm	Shape: spherical; Size: 5-20 nm	Khani <i>et al.</i> (2018)
CuCl <sub>2</sub> ·5H <sub>2</sub> O	NaBH <sub>4</sub>	Peaks at 597 to 569 nm		1226 cm <sup>-1</sup> band to 1234 cm <sup>-1</sup> in capped Cu NPs.		size: 14 nm	Ali Soomro <i>et al.</i> (2014)
[Cu(CH <sub>3</sub> COO) <sub>4</sub> ] [C <sub>12</sub> H <sub>25</sub> NH <sub>3</sub> ] <sub>2</sub>	N <sub>2</sub> H <sub>4</sub>	Peak at 567 nm		Peak at 1200, 1700 cm <sup>-1</sup> and 3400 cm <sup>-1</sup>	Size: 30-85 nm		Kaur <i>et al.</i> (2014)

### Role of Copper nanoparticles in the expression of defense-related genes in plants

CuNPs and other nanoparticles (NPs) act as elicitors, inducing a complex activation of defense-related genes in plants, which is a core component of their defense mechanisms against various stressors, including potential phytopathogens and oxidative stress (Cheng *et al.* 2022; Elmer *et al.* 2018). This response possibly involves the perception of NPs by cell-surface receptors, triggering intricate signal transduction pathways like the mitogen-activated protein kinases (MAPKs) and phytohormone pathways (jasmonic acid (JA) and salicylic acid (SA), ultimately leading to the transcriptional upregulation of defense genes. In support of the statement, OsCERK1, an established Lysin Motif Receptor-like Kinase (LysM-RLK), known for conferring basal resistance in rice, was found associated with stress rendered by CuO NPs and also CuO NPs recognition by OsCERK1 led to enhanced resistance against *Magnaporthe oryzae* (Chen *et al.* 2022). Specifically focusing on CuNPs and Copper Oxide Nanoparticles

(CuONPs), they are shown to activate genes involved in combating oxidative stress, such as those encoding antioxidant enzymes like superoxide dismutase (SOD), peroxidase (POD), and ascorbate peroxidase (APX), which help to neutralize reactive oxygen species (ROS) that are often generated upon NP exposure (Da Costa *et al.* 2016; Kamel *et al.* 2022; Zhao *et al.* 2017). For instance, CuONPs or their formulations, like Cu-chitosan NPs, have been documented to elevate the activity and expression of SOD, POD, and phenylalanine ammonia-lyase (PAL) in maize and cucumber, conferring resistance against diseases such as *Curvularia lunata* and *Fusarium solani* (Chaudhary *et al.* 2017; Kamel *et al.* 2022). Furthermore, CuONPs have been observed to upregulate pathogenesis-related (PR) genes (e.g., *PR-1* and *LOX-1*) in barley, tobacco, and cucumber, suggesting a defense response similar to that against pathogens (Abbasirad *et al.* 2025; Chen *et al.* 2022; Kamel *et al.* 2022). In addition, antioxidant genes like ascorbate peroxidase and catalase were also found to be upregulated (Abbasirad *et al.* 2025). In tomato plants under salt stress,

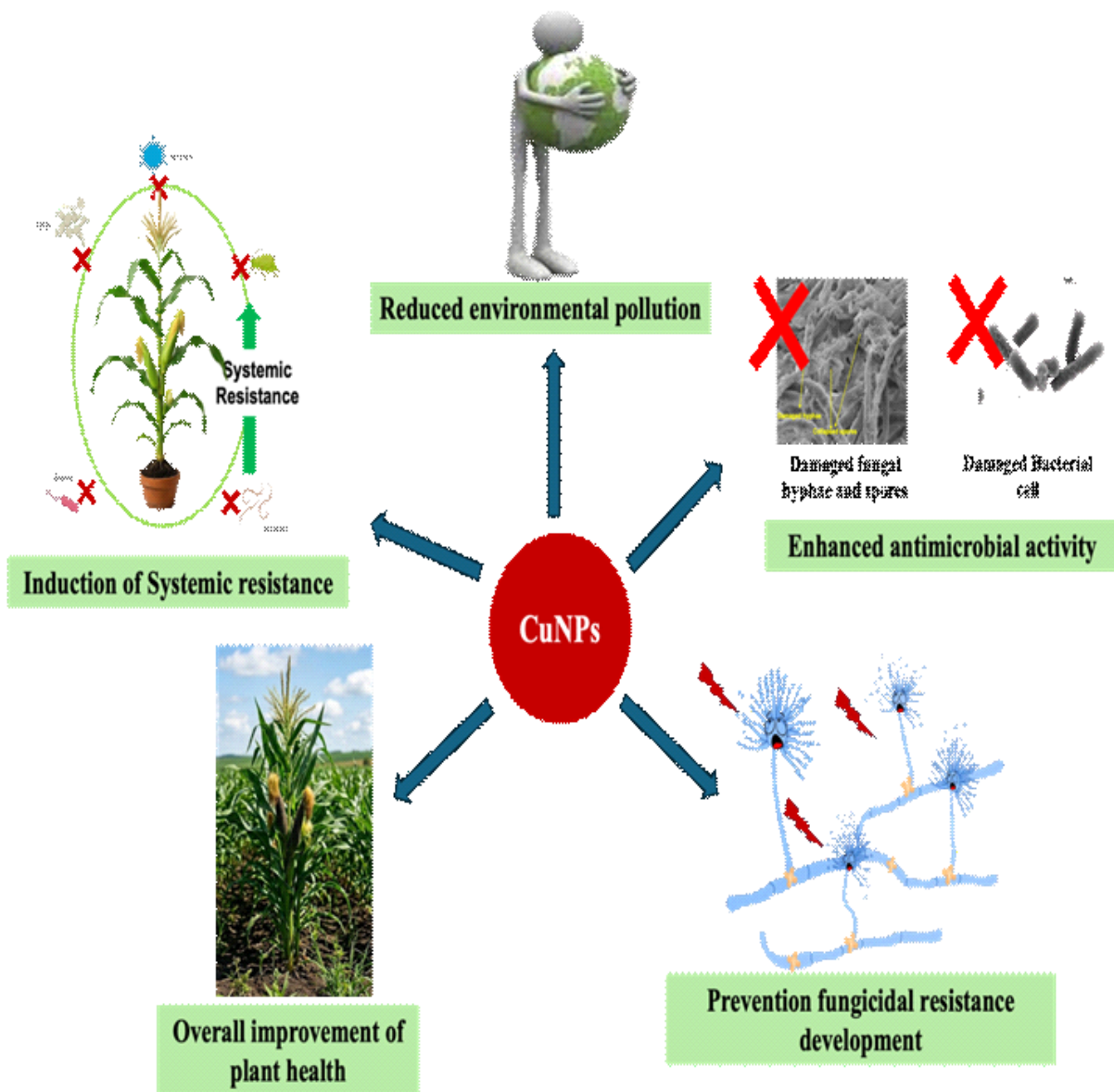


Fig. 1: Multifunctional role of copper nanoparticles (CuNPs) in enhancing plant health and environmental assam.

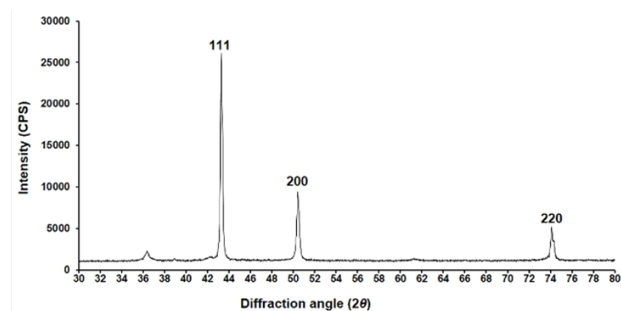
CuNPs combined with chitosan-polyvinyl alcohol hydrogels upregulated *JA* and *SOD* genes, although other genes like *PR-1* and *catalase* (*CAT*) were downregulated, indicating the context-dependent and often concentration-dependent nature of the nano-elicitation effect (Hernández *et al.* 2018). It has been shown that at low concentration CuNPs, normal cell functioning remains unaffected of *Zea mays*, rather defence genes such as ascorbate peroxidase, metallothionein, and catalase get upregulated significantly (Valdes *et al.* 2020). Similarly, important defense genes chitinase 2 (*PR-3*), *PR-1*, and  $\beta$ -1, 3-glucanases (*PR-2*) were expressed

when maize was exposed to an optimum concentration of copper based nanofungicides (Dorjee *et al.* 2025). Also, an increase in the activity of various antioxidant enzymes *viz.*,  $\beta$ -1, 3-glucanase, PAL, POX, and PPO, and non-enzymatic antioxidants *i.e.*, total phenol have been documented, which play an important role in plant defense especially against necrotrophic pathogens (Dorjee *et al.* 2023a). Even in cucumber, an increase in expression of antioxidant and detoxification related genes such as Glutathione peroxidase4 (*GPX4*), Glutathione peroxidase (*GPX*), Monodehydroascorbate reductase (*MDAR*), *POD*, *WRKY6*, *SOD*,

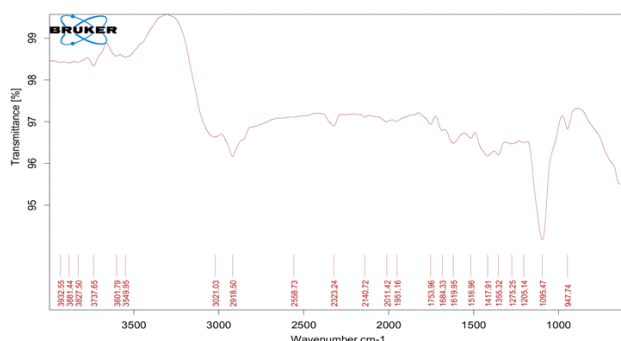


synthesis of protective secondary metabolites, demonstrate the promising role of CuNPs as potent inducers of plant phytoimmunity (Zhao *et al.*, 2017; Kamel *et al.* 2022).

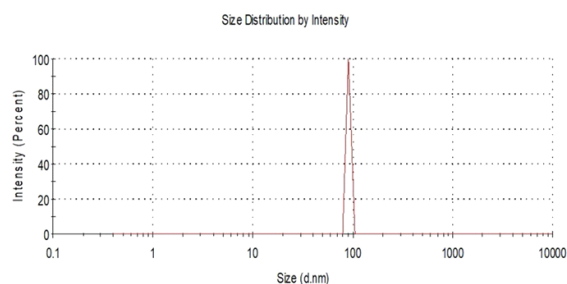
Nevertheless, excessive ROS generation due to CuNPs can cause oxidative damage and disruption of signalling pathways, eventually



**Fig. 5:** X-ray diffraction pattern of copper nanoparticles with diffraction peak at 43.299°, 50.437°, and 74.139° which correspond to miller indices (hkl) 111, 200, and 220, respectively, indicating face-cubic structure (FCC) of EGC (Dorjee *et al.* 2023a)

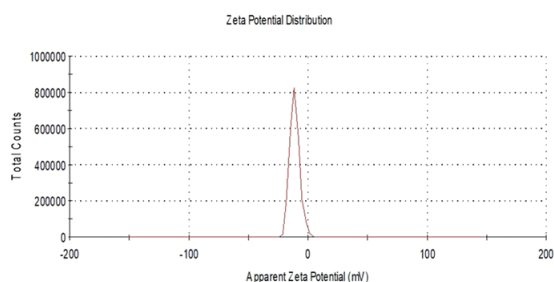


**Fig. 6 :** Fourier Transform Infrared Spectrum (Dorjee *et al.* 2023a)



**Fig. 7:** Dynamic light scattering image indicating hydrodynamic size of around 91 nm (Dorjee *et al.* 2023a)

leading to downregulation of defense-related genes. For instance, birch micro-clones exposed to CuNPs and pathogens (*Alternaria alternata* and *Fusarium spp.*) led to downregulation of Dehydration-Responsive Element-Binding Protein 2 (DREB2), PAL, MYB46, PR-1, and PR-



**Fig. 8:** Zeta potential of copper nanoparticles, which indicated the surface charge, hence the stability (Dorjee *et al.* 2023a)

10 (Grodetskaya *et al.* 2022). CuNPs genotoxicity has been reported by several workers, and it is obvious to see such a result due to the high oxidative properties of copper ions (Mosa *et al.* 2018; AlQuraidi *et al.* 2019; Mehrian *et al.* 2016). Therefore, the impact of CuNPs on plants is concentration-dependent, and the same nanoparticles may elicit varying responses across different plant species. Hence, careful experimentation and precise modulation of CuNPs are essential to optimize their beneficial effects while minimizing potential toxicity.

## CONCLUSION

CuNPs hold significant potential to revolutionize plant disease management by integrating efficiency with sustainability. Their unique physicochemical properties, particularly high surface area and reactivity, enable strong antimicrobial activity against a wide range of plant pathogens with reduced chemical load, and improved environmental compatibility. In addition to direct pathogen suppression, CuNPs also function as plant defence elicitors. These include the upregulation of antioxidant enzymes and pathogenesis-related genes, ultimately enhancing plant resilience against biotic and abiotic stresses. At optimal concentrations, CuNPs improve plant growth, photosynthetic efficiency, and other physiological attributes, thereby contributing to overall crop productivity. However, their effects are highly dependent on factors such as concentration, particle size, method of application, and plant species. While lower concentrations can stimulate beneficial physiological and molecular responses, higher doses may induce oxidative stress, genotoxicity, and phytotoxic effects, leading to impaired growth and suppression of defense-related pathways. Moreover, long-term

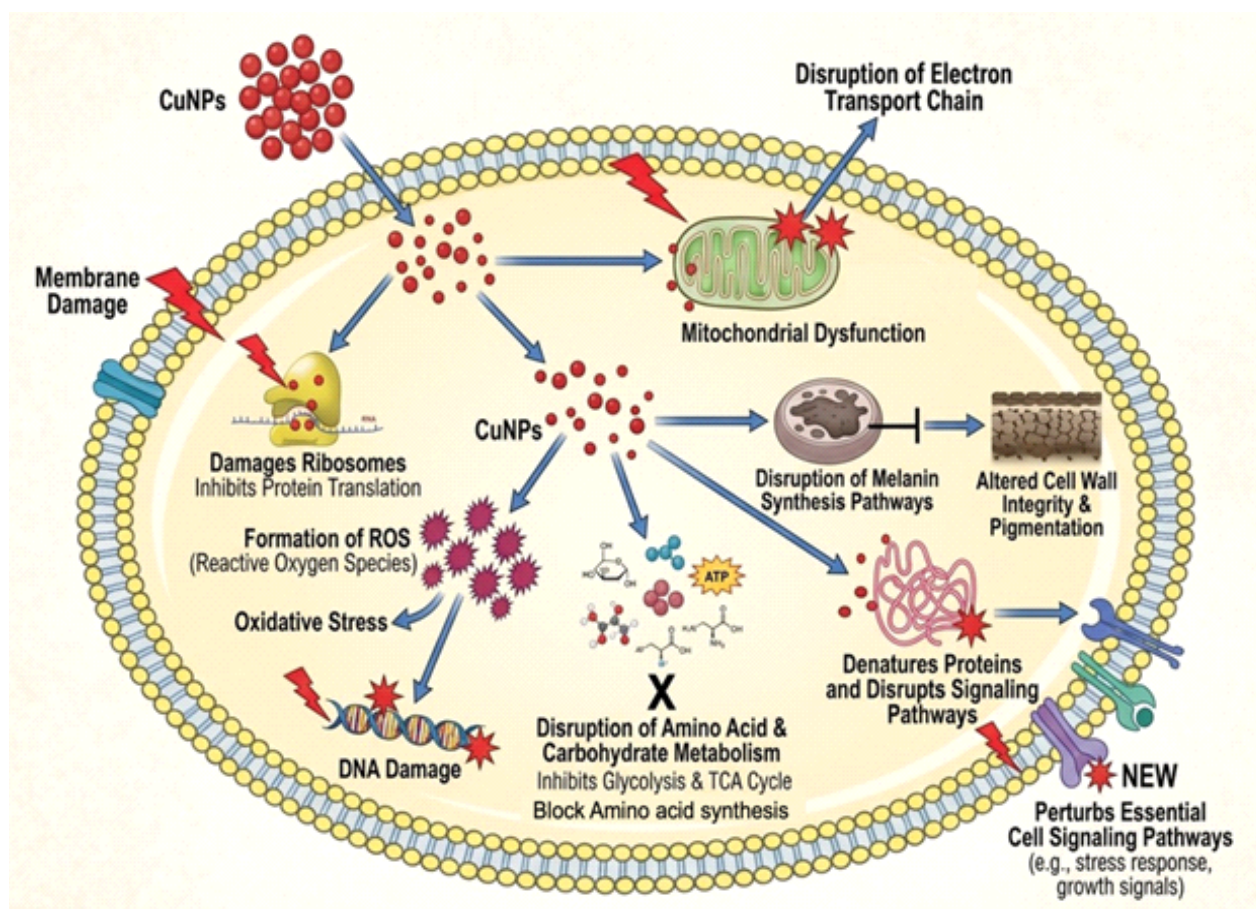


Fig. 9: Putative mechanism of action of copper nanoparticles in a microbial cell

environmental accumulation, potential toxicity to non-target organisms, and regulatory challenges remain.

Looking ahead, future research should prioritize on the development of standardized protocols for synthesis, which can synthesize CuNPs of high monodispersity and field application of CuNPs. Greater emphasis is needed on understanding nano-plant-microbe interactions at the molecular and ecosystem levels. The design of smart, targeted, and stimulus-responsive nano-formulations could further enhance efficacy while minimizing unintended impacts. Additionally, integrating CuNPs into existing integrated disease management (IDM) frameworks and exploring their synergistic interactions with biocontrol agents and other eco-friendly inputs can be fruitful. Establishing clear and standardize regulatory guidelines and comprehensive risk assessment frameworks will also be essential for safe deployment.

## DECLARATION

Conflict of interest. Authors declare no conflicts of interest in regard to publication of this article.

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