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Copper oxide-based nanoparticles in agro-nanotechnology: advances and applications for sustainable farming

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Abstract

Feeding the world's population sustainably and addressing food insecurity are among the most pressing global challenges. Agriculture, a key sector in addressing these challenges, faces disease outbreaks, inefficient fertilizer use, and adverse environmental impacts. This review explores the green synthesis and sustainable application of copper oxide nanoparticles (CuO NPs) in agriculture, focusing on their role as nanoagrochemicals. The green synthesis of CuO NPs using plant extracts produces nanoparticles (NPs) with tailored properties that enhance their efficacy as nanoagrochemicals. Copper oxide NPs have shown promising applications as nanopesticides, providing potent antimicrobial and antifungal effects, primarily through reactive oxygen species (ROS) generation, which disrupts cellular functions in pathogens. Moreover, CuO NPs improve plant growth by enhancing nutrient uptake and photosynthetic efficiency, making them effective nanofertilizers. Furthermore, nanopriming, an innovative seed treatment using CuO NPs, enhances seed germination and plant growth by activating stress-related genes and improving biochemical responses, especially under drought and salinity. Studies demonstrate the superior efficacy of CuO NPs over conventional agrochemicals in enhancing seedling vigor, nutrient uptake, and crop yield. However, concern regarding phytotoxicity at higher concentrations and potential environmental impacts necessitate careful dosage optimization. This review provides insights into the sustainable application of CuO NPs in agriculture, highlighting their potential to revolutionize crop productivity while emphasizing the need for further research into long-term environmental safety and optimized application methods.

Keywords Nanoagrochemicals, CuO NPs, Green synthesis, Nanopesticide, Nanofertilizer, Nanopriming

Introduction

Safely and sustainably feeding the rapidly growing global population is a formidable challenge of our time. According to the United Nations, the global population is projected to reach approximately 8.5 billion by 2030, necessitating a 50% increase in food production to meet the escalating demand [1]. Agriculture is critical for the economies of many countries, including Afghanistan, where it contributes 23% of the GDP and employs 61.6% of the labor force, given that 70% of the population in rural areas live in poverty, enhancing agricultural productivity and implementing effective agronomic measures are essential for ensuring food security and economic stability globally [2]. Traditional agricultural methods face numerous challenges, such as inefficient fertilizer application, which leads to environmental pollution [3], and diseases caused by bacteria, fungi, pests, and viruses, which reduce crop quality and yield [4]. Nanotechnology

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offers promising solutions to address food security challenges, particularly through its applications in improving nutrient delivery, and crop resilience. These advancements hold significant potential for resource-constrained regions, where ensuring sustainable agriculture is critical to achieving long-term food security [5]. One key advantage of nanotechnology lies in its ability to enhance nutrient uptake and delivery through nanofertilizers (fertilizers that utilize NPs), which improve the solubility and absorption of essential nutrients, reducing environmental impact and enhancing productivity [6]. For instance, CuO NPs improve micronutrients such as P, K, Mg and Ca in *Medicago polymorpha* L. [7]. In addition, nanotechnology provides innovative approaches to pest control, targeting pests more precisely and effectively, mitigating pesticide resistance and reducing chemical load on the environment [5, 7]. Copper oxide NPs were found to be over ten times more efficient than conventional $CuSO_4$ in antimicrobial performance while also reducing environmental impacts such as renewable energy use (64.7%), human health risks (69.6%), and ecosystem quality degradation (53.2%) [9]. Furthermore, nanotechnology addresses water scarcity by using nanofiltration techniques, such as graphene-based filters, to remove contaminants from irrigation water, improving water quality and ensuring efficient crop growth [5, 7]. Despite these advancements, the widespread adoption of agricultural nanotechnology faces several challenges, including market development, regulatory hurdles, and potential health and environmental risks associated with NPs use [10]. Regulatory frameworks are especially crucial for enabling the safe and equitable application of CuO NPs, ensuring their benefits reach smallholder farmers while minimizing environmental risks. Moreover, geographical disparities and regulatory barriers in emerging economies further complicate the deployment of nanoagrochemicals, although the potential for improving food security and climate resilience remains significant [7, 9].

Copper oxide NPs can be engineered to release nutrients in a controlled manner, ensuring plants receive a steady supply of essential elements while minimizing excess fertilizer use and environmental pollution [11]. Their nano-size allows deeper penetration into plant tissues, increasing nutrient uptake and lowering the fertilizer requirement [12]. In pest management, CuO NPs formulated as nanopesticides offer a sustainable solution to growing pest resistance, enhancing the delivery of active ingredients to target organisms and controlling plant diseases caused by bacteria and fungi due to their antimicrobial properties [12, 13]. Nanopriming with CuO NPs enhances plant resilience by triggering stress-response pathways, improving seed germination, root development, and overall plant tolerance to environmental stresses such as drought and salinity [14, 15]. The integration of CuO NPs into agricultural practices has the potential to significantly contribute to global food security efforts. Their applications in nanofertilizers and nanopesticides can reduce resource dependence and environmental impact, while nanopriming enhances crop resilience to climate change [12, 13]. However, addressing potential regulatory and safety challenges is vital to their widespread adoption, particularly in regions facing severe food insecurity. Furthermore, the use of NPs, including CuO NPs as nanofertilizers and nanopesticides, could contribute to reducing greenhouse gas emissions by decreasing the need for large-scale agrochemical applications, addressing both agricultural productivity and climate change challenges [17]. This highlights the importance of CuO NPs in developing sustainable agricultural practices, though more research is needed to fully understand their synthesis, application, and long-term environmental impact.

The focus of this review encompasses two critical areas related to CuO NPs in agriculture: green synthesis and application. Green synthesis emphasizes the importance of developing environmentally friendly methods for producing CuO NPs, moving away from conventional chemical and physical synthesis that poses significant environmental risks. The application aspect evaluates the effectiveness of CuO NPs as nanofertilizers, nanopesticides and nanopriming agents, aiming to optimize nutrient delivery and enhance pest control while minimizing environmental contamination. Together, these focus areas aim to provide a comprehensive understanding of CuO NPs and their role in promoting sustainable agriculture.

Method

In this review, a comprehensive literature search was conducted to gather and analyze relevant studies on the green synthesis and applications of CuO NPs in agriculture, specifically as nanoagrochemicals. The primary focus was on three key areas: the use of CuO NPs as nanopesticides, nanofertilizers, and their role as nanopriming agents. To identify the most relevant studies, a systematic search was performed across multiple scientific databases, including Web of Science, Scopus, PubMed, and Google Scholar. The search was limited to peer-reviewed articles published in English, and no specific time restriction was applied to capture both early foundational work and recent advancements in the field. The following keywords and their combinations were used during the search: *nanoagrochemicals, CuO nanoparticles, green synthesis, nanopesticide, nanofertilizer, and nanopriming*. The search results were screened based on titles and abstracts to ensure relevance to the subject of CuO NPs in agriculture. Studies that focused on other types of NPs or non-agricultural applications were excluded. Full texts of the selected articles were reviewed, and relevant data on the synthesis, mechanisms of action, and impacts of CuO NPs as nanopesticides, nanofertilizers, and nano-priming agents were extracted. Key information on the efficacy, toxicity, and potential for sustainable agriculture was also considered.

Properties and synthesis of copper oxide nanoparticles

Copper oxide NPs possess unique physicochemical properties, making them highly valuable for various applications. Their vast surface area, robust adsorption capabilities, and numerous reactive sites contribute to their high catalytic activity and chemical stability [18]. Additionally, CuO NPs are known for their antimicrobial and fungicidal properties, which enhance their potential in agricultural applications [18, 19]. These properties are mainly due to their surface-free electrons, specific surface area, and distinct surface activity [21], which allow them to interact efficiently with target organisms or environmental factors. These properties directly address agricultural challenges, such as improving crop yields, reducing post-harvest losses, and preventing crop diseases, which are essential to ensuring global food and nutritional security.

Copper oxide NPs are synthesized through various chemical and physical methods, each with advantages and limitations [22]. The sol–gel method is a popular chemical technique synthesizing CuO NPs with tunable specific surface areas. For example, Dörner et al. [23] synthesized CuO NPs with sizes ranging from 20–40 nm, where the size was dependent on pH levels, demonstrating the method's flexibility in controlling NPs characteristics. Other chemical methods, such as precipitation and hydrothermal techniques, have also been used to synthesize CuO NPs [23] efficiently. However, these methods often require precise conditions, such as high temperatures and pressures, limiting their scalability and environmental sustainability [24].

Despite the effectiveness of traditional chemical and physical methods for CuO NP synthesis, they present several drawbacks. Chemical synthesis often involves toxic reducing agents, such as hydrazine and borohydride, which pose significant health and environmental risks [25]. Additionally, physical methods require sophisticated equipment, high temperatures, and energy-intensive processes. The potential contamination of NPs with hazardous materials and the environmental footprint of these methods have led researchers to seek safer, more sustainable alternatives [26]. These limitations not only raise health and environmental concerns, but also undermine the goal of achieving sustainable agricultural systems that align with global food security and environmental preservation goals.

Green synthesis aligns with the principles of sustainable agriculture by minimizing environmental pollution, reducing the use of hazardous chemicals, and ensuring safer NPs applications in food systems, thereby supporting long-term food security goals [27]. This approach utilizes biological resources, such as plant extracts, as reducing and stabilizing agents, eliminating the need for toxic chemicals [28–30]. Plant extracts are rich in phytochemicals, secondary metabolites, and natural compounds that facilitate the reduction and stabilization of CuO NPs [19]. For instance, Sing et al. [31] used *Morus alba* leaf extract to synthesize spherical CuO NPs with diameters ranging from 40 to 50 nm. Other examples include *Vigna radiata* and *Azadirachta indica*, which produced CuO NPs of 24 nm and 50 nm, respectively [14, 25]. The variation in particle sizes based on plant species demonstrates the flexibility of green synthesis in producing NPs with specific properties tailored for agricultural use. The details of the plant species used for synthesizing CuO NPs are further described in Table 1. Green-synthesized CuO NPs have shown promising applications in agriculture, particularly as antimicrobial agents and nanopesticides. These NPs can inhibit microbial growth by disrupting metabolic activities, making them suitable for organic pest control in phytopathology [32]. However, while green synthesis is a safer approach, evaluating the environmental impact and safety of CuO NPs, especially regarding their accumulation in soil and water systems, is crucial. Addressing these concerns ensures the sustainable use of nanotechnology in agriculture while safeguarding food systems against unintended consequences, such as soil degradation or water contamination, which could exacerbate food insecurity in vulnerable regions.

Copper oxide-based nanopesticides

Crop diseases are among the factors that threaten food security worldwide. Plant pathogens reduce global agricultural productivity by 20%, resulting in billions of

Table 1 Green synthesis of CuO NPs from different plant species

Plant species	Size of CuO NPs after synthesis (nm)	References
Morus alba	20–40	[31]
Camellia sinensis	74.6	[14]
Calotropis procera	40–100	[32]
Azadirachta indica	50	[25]
Ocimum americanum L	69.80	[26]
Cannabis sativa L	7.36	[22]
Vigna radiata	24	[15]

annual losses [33]. These productivity losses exacerbate food insecurity, particularly in regions already facing challenges such as limited agricultural resources and growing population demands. Crop diseases caused by pathogens such as insects, viruses, bacteria, fungi, and nematodes decrease yield and compromise quality and shelf life [22]. For instance, to date, fungal diseases account for approximately 70–80% of crop diseases, significantly reducing agricultural production and even causing disastrous reductions in yields and negative economic impacts [34]. Although various alternative control strategies have been applied for protective control, including the use of conventional fungicides and the improvement of cultivation measures [35], the development of pathogenic fungal resistance and adverse effects caused by environmental exposure to fungicides are emerging threats that have become great challenges to agricultural production [36]. Therefore, under these circumstances, more effective and amicable disease management strategies are urgently needed to meet the criteria for sustainable agriculture. To overcome these problems associated with conventional pesticides, recent trends in pest management have moved toward the development of nanotechnology-assisted formulations, which include nanoformulations of pesticides (nanotechnology-enhanced pesticide delivery systems) and nanopesticides, which have the potential to offer increasing benefits and fewer side effects [37]. Nanopesticides align with sustainable agriculture goals by minimizing the environmental footprint of pesticide use, reducing chemical runoff into water systems, and improving resource efficiency, which supports long-term agricultural resilience. Nanopesticides formulations improve the features and behaviour of pesticides, such as their stability, mobility, dispersion, solubility, and targeted delivery [38]. Moreover, it can also improve the safety, efficacy, and cost-effectiveness of traditional pesticides by extending the effect duration, increasing the efficiency, reducing the dose and controlling the release of the active ingredients, and improving the stability of payloads from the environment, which subsequently reduces the amount of environmental residue [39].

Copper oxide NPs have demonstrated fungicidal and insecticidal properties, making them suitable for use as nanopesticides [40]. Studies have shown that compared with conventional pesticides, CuO NPs are more effective at pest control [13]. For instance, the green synthesis of CuO NPs via foliar spraying of green tea in *lettuce* pot experiments has shown promising results. Foliar spray increased antioxidant activity without negatively impacting the *lettuce* defense system, unlike soil irrigation or $CuSO_4$ treatment [14]. This demonstrates the potential of CuO NPs to provide sustainable pest control solutions while maintaining crop health and quality, which is vital for meeting global food security demands. Integrating CuO NPs and ε-PL into a nanogel has enhanced antifungal activity, improving disease control in tobacco plants infected with *Alternaria alternata* [20]. The nanogel outperformed the commercial fungicide $CuCaSO_4$ at lower Cu concentrations. CuONP@ALGNP@PL also effectively inhibited *A. alternata* spores. Additionally, it showed greater safety than $CuCaSO_4$ at the same Cu concentration [20]. The effectiveness of CuO NPs in controlling tobacco black shank (TBS) disease caused by *Phytophthora nicotianae* was investigated by Juan-ni et al. [21]. In this study, CuO NPs effectively suppressed the reproductive growth of the fungus by inhibiting hyphal growth, spore germination, and sporangium production through morphological damage, ROS accumulation, and increased superoxide dismutase (SOD) activity. While in pot experiments, a 100 mg/L concentration of CuO NPs reduced TBS development by 33.69% without harming plants, activated defense enzymes and resistance genes in tobacco, and significantly increased Cu content in both leaves and roots, especially in infected roots. These outcomes not only demonstrate the capacity of CuO NPs to enhance disease resistance, but also underscore their role in boosting crop productivity, which is critical for strengthening food supply chains in a changing climate. Furthermore, cost-effective and environmentally friendly CuO NPs were synthesized and exhibited antifungal activity against *Ziziphus jujuba* in a study by Manzoor et al. [25]. Biologically synthesized CuO NPs were applied as antifungal agents against *Fusarium virguliforme* in soybean plants through foliar treatments, resulting in increased plant growth, improved nutrient content, enhanced photosynthetic indicators, and elevated expression of genes linked to antifungal defense, thereby demonstrating their capacity to boost disease resistance [22].

Copper oxide NPs exhibit significant potential as nanopesticides due to their diverse modes of action against pathogens and their benefits to plant health. Copper oxide NPs exert potent antimicrobial and antifungal effects primarily through the generation of ROS, which disrupt cellular functions by damaging cell walls, membranes, and DNA, ultimately leading to cell death [41]. For instance, biosynthesized CuO NPs showed a concentration-dependent inhibition of *E. coli*, with a maximum inhibition zone observed at 100 mg/L (11 ± 0.1 mm), and substantial mycelial growth inhibition in *Rhizopus oryzae* inoculated Jujube fruits attributed to ROS production and DNA degradation [25]. Composite nanogels like CuONP@ALGNP@PL further enhance antifungal activity against pathogens such as *Botrytis cinerea* and *Fusarium graminearum*, outperforming conventional fungicides by leveraging Cu^{2+} release and nanogel interactions to destabilize pathogen membranes [20]. Additionally, CuO NPs disrupt cellular functions in fungi by damaging cell walls and membranes, leading to cell death, which is particularly effective against various plant pathogenic fungi, including species of *Fusarium* [20]. Beyond their direct antimicrobial properties, CuO NPs enhance plant resistance to pathogens and improve crop yield by modifying root morphology and promoting beneficial microbial activity in the soil [3]. This dual role of CuO NPs as both nanopesticides and growth enhancers makes them a valuable tool in integrated pest management, offering an environmentally friendly alternative to conventional pesticides with reduced phytotoxicity and improved plant health [20]. However, to maximize their benefits and minimize risks, the adoption of CuO NPs requires robust regulatory frameworks and long-term environmental impact studies to ensure their safe use in diverse agricultural systems. These measures will help balance productivity gains with environmental preservation, particularly in regions highly dependent on agriculture for food security. The use of CuO NPs as nanopesticides against different pathogens is summarized in Table 2.

Copper oxide-based nanofertilizers

Chemical-based fertilizers, while widely used, often result in significant nutrient losses, with studies indicating that 40–70% of nitrogen, 50–70% of potassium, 80–90% of phosphorus, and approximately 95% of micronutrients such as zinc, iron, copper, and molybdenum are lost to the environment through processes such as leaching, runoff, and soil erosion [44]. These inefficiencies are not only unsustainable but also detrimental to ecosystems, as nutrient leaching contributes to eutrophication and soil degradation, severely impacting agricultural landscapes. Despite the potential of chemical-based fertilizers to enhance crop growth, they have not substantially increased plant nutrient uptake or crop productivity in current agricultural practices, raising concerns about their sustainability [45]. This inefficacy is compounded by the fact that overuse of chemical-based fertilizers can lead to diminished soil health and decreased profitability for farmers, ultimately undermining agricultural sustainability and prompting a shift towards more sustainable practices like organic and precision farming. The advent of eco-friendly nanofertilizers offers a promising alternative, addressing traditional fertilizers' environmental and efficacy limitations. Nanofertilizers, often referred to as "smart fertilizers", improve nutrient use efficiency by

Table 2 Application of CuO NPs as nanopesticides

Pathogen	Concentration	Impact	References
Phytophthora nicotianae	0–100 mg/L	• Hyphal colony significantly decreased at 50 and 100 mg/L • Sporangium number decreased by increasing concentrations • Morphological damage, intercellular ROS accumulation, and increased SOD enzyme activity in hyphae	[21]
Spodoptera frugiperda	10–100 mg/L	• 97%, 94%, and 81% larvicidal activity observed at 3rd, 4th, and 5th instar larvae • 98.25%, 98.01%, and 98.42% antifeedant activity on the 3rd, 4th, and 5th instar larvae • After 24-h exposure, the hemocyte levels significantly decreased • Concentration dependent decrease in acetylcholinesterase levels of larval	[44]
Rhizopus oryzae	25–100 mg/L	• The mycelial growth was decreased by increasing concentration • CuO NPs exhibited notable radical scavenging activity • Overall, CuO NPs exhibited remarkable antifungal activity and reduced disease severity against *R. oryzae*	[25]
Spodoptera littoralis	150–600 mg/L	• Mortality of treated larva increased by increasing concentration of CuO NPs • The microflower like CuO NPs exhibited fast entomotoxic effect with LC_{50} = 232.75 mg/L after 3 days • The LT_{50} of CuO NPs at 600 mg/L were 2.69 days • The rectangular CuO also showed fast entomotoxic effect with LC50 = 205.63 mg/L after 3 days and LT50 was 2.13 days	[45]
Alternaria alternata	Cu concentration was 40.09 µg/mL, ε-PL concentration was 11.90 µg/mL	• Higher antifungal activity compared to individual component of nanogel • Affected spore production, spore germination rate and bud tube elongation length • Highest inhibition rate (85.10 ± 1.16%) at 1 mg/mL of the composite nanogel • EC_{50} value were 0.4123 mg/mL	[20]

enhancing the availability of essential nutrients to plants while minimizing environmental impacts [46]. For example, their nano-size increases the surface area, which can significantly improve the absorption efficiency of nutrients compared to conventional fertilizers [44]. This enhanced efficiency is not merely theoretical; practical applications have shown that nanofertilizers can increase plant nutrient uptake through foliar and soil application methods, thus improving plant growth and yield [47, 48].

The use of CuO NPs in agriculture has shown promising results in enhancing plant growth, antioxidant activity, and overall plant health. However, the findings across different studies vary, highlighting both the potential benefits and challenges associated with their application. For instance, a study found that the presence of 50 mg/L CuO NPs at 15 °C significantly enhanced the root (by 37%) and shoot (by 13%) growth of *Vigna radiata* [24]. Similarly, the growth metrics of *Triticum aestivum* (wheat) improved markedly due to the enhanced nutrient uptake facilitated by CuO NPs [49]. In another study, the growth of *Lepidium sativum*, *Raphanus sativus*, and *Zea mays* was significantly improved at specific CuO NP concentrations [50]. However, these findings contrast with studies highlighting the potential phytotoxic effects of CuO NPs at higher concentrations. For instance, while lower concentrations of CuO NPs promoted wheat growth, higher concentrations inhibited root and shoot growth, indicating a toxicity threshold [51]. Similarly, the study involving *Lactuca sativa* found that while CuO-Indole-3-acetic acid nanocomposites (CuO-IAA NPs) reduced the toxic effects of CuO NPs, high concentrations of uncapped CuO NPs still led to reduced biomass and shoot length [52]. In contrast, another study on soybeans revealed that while foliar application of CuO NPs and citric acid-coated CuO NPs (CuO-CA NPs) significantly increased soybean yield, ionic Cu showed no impact on yield, suggesting that the method of application and NPs coating play crucial roles in determining efficacy [49]. Moreover, the effect of CuO NPs on different plant species and under various environmental conditions can vary significantly. For example, Nekoukhou et al. [53] reported that *Dracocephalum moldavica L.* treated with CuO NPs exhibited higher bioaccumulation of shoot copper, flavonoids, and anthocyanins compared to chelated-Cu treatments, leading to greater shoot biomass and secondary metabolite yield. These results contrast with findings from another study where excessive CuO NP concentrations were shown to inhibit growth and chlorophyll content in wheat (*Triticum aestivum*) due to toxicity effects [49]. This discrepancy indicates that while CuO NPs can enhance plant growth and secondary metabolite production, their effectiveness is highly dependent on concentration, plant species, NPs surface properties, and application methods, necessitating careful dosage optimization and a tailored approach based on crop and environmental conditions to prevent adverse effects.

The mechanisms through which CuO NPs exert their effects include activating antioxidant defense systems, improving photosynthetic efficiency, and enhancing nutrient uptake. Increased antioxidant activity in plants treated with CuO NPs is a consistent finding in the literature. For example, CuO NPs increased antioxidant concentration by 81% in cowpea cultivars, suggesting a robust activation of the plant's defense mechanisms against oxidative stress [54]. This is consistent with findings in wheat, where CuO NPs enhanced the activity of key antioxidant enzymes, thereby mitigating oxidative damage caused by cadmium stress [49]. Contrastingly, a study involving CuO-IAA NPs found that while CuO NPs alone increased the levels of non-enzymatic antioxidants such as phenolic and flavonoids, the presence of IAA reduced these levels, suggesting a complex interplay between CuO NPs and hormonal regulation [52]. This indicates that while CuO NPs can enhance antioxidant activity, their interaction with other compounds like IAA can modulate these effects, potentially reducing the overall antioxidative response. Nevertheless, increased chlorophyll content and enhanced photosynthetic efficiency are commonly reported benefits of CuO NP application. For instance, using CuO nanofertilizers resulted in a 73% increase in chlorophyll content for soil application, attributed to the NPs' ability to improve light absorption and photosynthetic processes [54]. Similarly, in a study on *Nicotiana tabacum*, applying PGR-ILs combined with CuO NPs improved net photosynthetic rates and biomass yield, suggesting enhanced photosynthetic efficiency [55]. On the other hand, higher concentrations of CuO NPs were found to inhibit photosynthetic activity, as indicated by reduced chlorophyll content and growth inhibition in wheat at concentrations above 0.1 mg/L [51]. The improvement in nutrient uptake is another widely recognized benefit of CuO NP application. Studies have shown that CuO NPs enhance the absorption of essential nutrients like copper and zinc, promoting plant health and growth [25, 46]. For example, biogenic CuO and ZnO NPs increased the total copper and zinc content in both roots and shoots of *Amaranthus hybridus*, without causing toxic accumulation, which contrasts with the harmful effects of traditional copper and zinc salts at equivalent concentrations [57]. However, excessive application of CuO NPs can lead to soil toxicity, as highlighted in a study where high concentrations of CuO NPs resulted in reduced root and shoot growth in wheat due to elevated copper ion release [51]. Table 3 summarizes the application of CuO as a nanofertilizer to different plant species.

Table 3 Application of copper oxide NPs as a nanofertilizer

Plant species	Concentration	Application	Impact	References
Zea mays L	(0.01–0.02 mg/L) for solution culture and 8 mg/L for foliar spray	Solution culture and foliar spray	• The bioaccumulation rate increased by increasing concentration of CuO NPs • CuO NPs enter into the plant cell through roots and leaves • Both types of application increased growth of plants by 51% • The enzymatic activity of plant was highly influenced by CuO NPs	[58]
Medicago polymorpha L	10–100 mg/kg	Solution culture	• The fresh weight of *M. polymorpha* L. increased between 3.7 and 8.1% by CuO NPs exposure • The micronutrient such as P, K, Mg, Ca improved in treatment group in contrast to control • CuO NPs concentration between 10 and 25 mg/kg had best impacts	[7]
Lettuce (*var. ramose Hort.*)	0–400 mg/kg	Solution culture	• Root Cu increased by 67.7–128.0% in all treatment compared to control • Shoot biomass increased by 16.3–19.1% in all treatment group • 200 and 400 mg/kg CuO NPs increased transpiration rate and caused higher stomatal conductance compared to control	[59]

Nanopriming

Nanopriming, an innovative seed treatment approach, leverages the unique properties of NPs to improve crop productivity and resilience under stress conditions, offering significant advantages over traditional priming techniques. Conventional methods, such as hydropriming and osmopriming, focus on osmotic regulation and hydration, helping seeds initiate metabolic processes for early seedling development [48, 54]. However, these techniques often fail to fully address modern agricultural challenges, particularly under environmental stress conditions like drought and salinity [54]. In contrast, nanopriming uses NPs with small size, high surface area, and enhanced reactivity to penetrate the seed coat and interact with cellular components, thereby accelerating metabolic processes, modulating physiological responses, and activating stress-related genes [14, 55, 56]. Nanopriming directly contributes to addressing global food security challenges by enabling crops to withstand environmental stresses and improving productivity. This makes it a valuable tool for achieving sustainable agricultural practices that align with the principles of resource efficiency and climate resilience. Recent nanotechnological advancements have further enhanced the scalability and cost-effectiveness of nanopriming in agriculture. Room-temperature, energy-efficient assembly-based approaches significantly reduce costs by minimizing reliance on expensive equipment and reducing waste through selective material addition [63]. In developing countries, where food insecurity remains a pressing issue, the economic viability of nanopriming can play a transformative role by increasing crop yields without requiring extensive input costs. These innovations make nanopriming a more economically viable option for large-scale agricultural applications [64]. This novel approach holds significant potential for modern agriculture, as NPs can penetrate seed coats and interact with cellular components, triggering accelerated metabolic processes [15]. Nanopriming has shown promise in improving germination rates, seedling vigor, and stress tolerance by activating stress-related genes and enhancing biochemical responses within seeds [16]. Compared to traditional methods, nanopriming reduces germination time and modulates physiological and biochemical processes, offering a more robust solution for addressing the growing challenges of crop productivity under environmental stresses [62]. While conventional priming focuses on osmotic regulation and hydration, nanopriming offers a more dynamic and tailored approach by incorporating advanced materials like metal-based or biopolymer NPs, which can activate plant defense mechanisms more effectively [44]. Consequently, the growing interest in nanopriming reflects its potential to revolutionize seed treatment by increasing agricultural sustainability and productivity.

In nanopriming, various NPs such as copper, iron, gold-based, silver, carbon, zinc, titanium dioxide, and fullerenes are used as seed priming agents, with the nanoformulations being retained by the seed coat; these NPs are categorized into two groups: active NPs (active NPs are engineered with specific properties that affect biological processes [16] and sustained release nanocarrier systems (sustained release nanocarrier systems use

NPs to gradually deliver active substances like nutrients, pesticides, or growth regulators over time) [65]. Active NPs typically consist of metal-based NPs, such as zinc, manganese, and iron NPs, with a size of approximately 100 nm. Sustained release nanocarrier systems involve NPs, which can be either active or inactive, loaded with active compounds such as fungicides, herbicides, nutrients, or plant growth regulators that are slowly released into the plant; biopolymer NPs, like chitosan, are commonly used in this system for seed priming, as demonstrated by improved seedling growth in maize seeds primed with chitosan NPs [44]. Nanopriming offers a novel approach to enhance seed germination and seedling vigor, especially under stress conditions, by applying NPs to seeds before sowing [25, 55–57]. This method significantly improves over traditional priming techniques, such as hydropriming, by using the unique properties of NPs, including their small size, high surface area, and reactivity, to penetrate the seed coat and interact with cellular components [35, 55]. Conventional seed priming initiates metabolic processes through hydration, but nanopriming enhances physiological and biochemical responses, particularly under drought and salinity [25, 54, 55]. For example, the application of CuO and silver NPs (AgNPs) as nanopriming agents, has been shown to stimulate early metabolic changes and activate stress-related genes, resulting in better germination rates and increased seedling vigor [56, 63]. Faraz et al. [68] examined using CuO NPs as seed primers. After priming, the *Brassica juncea* seeds were planted in pots and allowed to grow naturally. Priming the seeds with 4 mg/L CuO NPs for 30 min yielded the best results among the different concentrations tested. This treatment significantly increased shoot length (by 30%), root length (by 27%), net photosynthetic rate (by 30%), internal CO_2 concentration (by 28%), and the proline content (by 41%). Additionally, the application of CuO NPs also led to a significant increase in the activity of antioxidant enzymes (such as superoxide dismutase, catalase, and peroxidase) and biochemical parameters (such as nitrate reductase and carbonic anhydrase) in *Brassica juncea* plants. While CuO NPS is widely reported to enhance water uptake and seed germination, leading to a germination rate of 93.33% compared to 76.67% in control groups [15], ZnO NPs have explicitly been noted for their role in enhancing seedling vigor by activating antioxidant enzymes like SOD and CAT, which mitigate oxidative stress [62, 64]. This is a notable advantage over traditional priming methods, which do not directly activate such stress-response pathways at the molecular level [25, 54]. Moreover, nanopriming with biopolymer NPs like chitosan shows promise in sustainable agriculture, as these biodegradable NPs promote seedling growth while minimizing environmental contamination through gradual nutrient release [44]. The potential of nanopriming to enhance crop productivity is further demonstrated by the improved shoot length, dry weight, and photosynthetic activity seen in plants treated with NPs. For example, CuO NPs applied at 4 mg/L resulted in a 30% increase in shoot length and photosynthetic rate, underscoring the capability of nanopriming to boost plant growth metrics under stress conditions [68]. In another study, the seed imbibition and germination coefficients in response to increasing concentrations of CuO and CuO@APTES NPs showed that absorption potential increased with concentration, saturating at 48 ppm for CuO and 80 ppm for CuO@APTES, which likely activated enzymes and accelerated metabolic processes, resulting in improved germination rates of 88.3% and 93.33% compared to 76.67% in the control [15]. These improvements contrast with the limited scope of traditional priming methods, which often do not sustain such benefits beyond early seedling stages [25, 54].

Despite its benefits, nanopriming presents challenges, particularly concerning the concentration of NPs. Higher doses of NPs, such as CuO NPs, can induce phytotoxic effects, inhibiting seed germination and root growth [61, 66]. This concentration-dependent response is crucial, as low concentrations stimulate growth, but excessive NPs accumulation can lead to toxic effects, such as reduced root elongation and delayed flowering [61].In addition, the environmental impact of NPs must be considered. Studies indicate that the accumulation of NPs in soil and water ecosystems can disrupt soil microbiota, affecting soil fertility and plant health [57, 58]. Nanoparticles, especially metal-based, may also persist in the environment due to their slow degradation, posing long-term risks to non-target organisms, including beneficial insects and pollinators [58].

Environmental fate and behavior of copper oxide nanoparticles

The environmental fate and behavior of CuO NPs are important due to their increasing application in agriculture and other fields. Various key factors, including synthesis methods, particle size, morphology, chemical composition, and environmental conditions, influence CuO NPs' environmental behavior.

The synthesis method of CuO NPs plays a critical role in determining their environmental behavior, with green synthesis methods being less toxic and more eco-friendly than traditional chemical approaches [66, 67]. Studies show that plant extracts enhance the stability and environmental compatibility of CuO NPs, with examples like *Bergenia ciliata* rhizome extract demonstrating improved stability [67, 68]. This suggests that green synthesis may yield more favorable environmental profiles. The stability of green-synthesized CuO NPs also highlights their potential in antimicrobial treatments and photocatalysis, contrasting them with traditional organic agents. Still, concerns about long-term accumulation and ecological impacts remain insufficiently explored [76]. Furthermore, the superior colloidal stability and lower toxicity of green-synthesized CuO NPs compared to chemically synthesized ones emphasize the importance of synthesis methods in determining environmental behavior [77]. However, long-term accumulation in soils and the potential for unintended ecological impacts remain concerns that need further study.

The size and shape of CuO NPs play a significant role in their biodegradation rates and environmental behavior, with smaller nanoparticles (4–10 nm in diameter) exhibiting higher surface areas and enhanced reactivity, thereby facilitating their interaction with pollutants and biological systems [71, 72]. For example, hierarchical nanorods (50–200 nm) demonstrate effective adsorption capabilities for hazardous substances like dichromate, illustrating the critical role morphology plays in environmental applications [72, 73]. The porous, sponge-like structure of CuO NPs further enhances their photocatalytic degradation of organic dyes, increasing their efficiency [71, 74]. The higher surface area-to-volume ratio of smaller NPs is correlated with increased reactivity, as confirmed by various studies, although the optimal size range may vary based on environmental conditions [66, 75]. Additionally, rod-like versus spherical morphologies affect dispersion and pollutant interaction, though further empirical comparisons in real-world contexts would help clarify these observations [75].

Environmental conditions such as pH, organic matter, and ionic strength play a significant role in the stability of CuO NPs [78]. Studies indicate that at higher pH levels (around 8–10), CuO NPs exhibit enhanced photocatalytic activity, achieving up to 98.33% degradation of malachite green dye [79]. Conversely, lower pH conditions lead to the formation of CuO complexes and reduced photocatalytic efficiency [80]. Additionally, pH impacts the stability and removal efficiency of CuO NPs during coagulation processes, with optimal removal (up to 90%) occurring at neutral pH [81]. Overall, pH is a critical factor in determining the functionality and environmental fate of CuO NPs in aquatic systems [82]. The presence of natural organic matter (NOM) also modifies the agglomeration and dissolution rates of CuO NPs, with varying effects based on pH [83]. Natural organic matter enhances the stability and retention of CuO NPs in soils by promoting hetero-aggregation with clay colloids, which reduces their aggregation and sedimentation rates [10, 84]. Additionally, the presence of NOM can alter the dissolution rates of CuO NPs, with DHA facilitating increased copper release through complexation, thereby affecting their ecotoxicity [85]. Studies indicate that the interaction between CuO NPs and NOM leads to a more stable colloidal form, which can mitigate the generation of reactive oxygen species and reduce cytotoxicity [86]. Overall, organic matter significantly modulates the environmental dynamics of CuO NPs, influencing their toxicity and accumulation in aquatic and soil ecosystems [87]. Ionic strength (IS) significantly influences the environmental behavior of CuO NPs, affecting their stability, aggregation, and transport in various ecosystems. Studies indicate that higher IS leads to increased aggregation of CuO NPs, which can hinder their mobility in soil and aquatic environments [10, 81]. For instance, in low-IS conditions, CuO NPs form smaller aggregates, enhancing copper bioaccumulation in organisms like zebrafish embryos, while high IS promotes larger aggregates that are less bioavailable [89]. Additionally, the presence of clay colloids and organic matter can alter the critical coagulation concentration, further impacting retention and transport dynamics [10, 83].

Regulatory challenges and future perspective

The regulatory challenges surrounding the application of CuO NPs are diverse and comprehensive, reflecting the need for standardized guidelines and a deeper understanding of their potential risks. Key concerns include the lack of comprehensive biocompatibility assessments and the need for robust toxicity testing to ensure safe use across various fields, including agriculture [84, 85]. Despite their promising antimicrobial, antifungal, and agricultural applications, CuO NPs pose risks due to their potential cytotoxicity and genotoxicity, which are influenced by NPs' shape and concentration. Current regulatory frameworks often fail to adequately address these unique risks, relying on outdated policies that do not account for the distinctive physical properties of NPs like CuO NPs, necessitating updated legislation tailored to nanotechnology [93]. One of the central challenges is the need for standardized testing systems and reference materials, which are critical for accurate risk assessment and ensuring the safe integration of CuO NPs into industrial and agricultural practices. Regulatory oversight is notably lacking in agriculture, where the potential phytotoxic effects of CuO NPs, such as reduced growth and stress induction in plants, raise concerns about their long-term environmental impact and accumulation in ecosystems [87, 88].Studies indicate that exposure to CuO NPs can lead to a reduction in microbial diversity and enzymatic activities essential for soil health, such as dehydrogenase and urease activities, with reductions observed between 20 and 95% depending on the treatment and dosage [96, 97]. Furthermore, the presence of

CuO NPs alters the composition of microbial communities, favoring certain taxa like *Acidobacteria* while suppressing others, which can disrupt biogeochemical cycles crucial for ecosystem stability [96, 98]. Future research must optimize CuO NP application methods and explore the interactions between these NPs and other agrochemicals to fully understand their effects on plant physiology and crop productivity. Additionally, there is a growing need for an authoritative regulatory framework to manage the safe deployment of CuO NPs. This includes adapting current legislation to address the limitations of traditional risk assessment methods, as NPs require a more refined approach due to their complex interactions with biological systems and the environment [99]. A new regulatory paradigm is essential for aligning nanotechnology advancements with safety and sustainability goals, particularly as CuO NPs become more integrated into agricultural applications.

Conclusion

Copper oxide NPs present a transformative opportunity for modern agriculture, offering enhanced productivity through their unique antimicrobial and fungicidal properties and their potential to improve nutrient uptake and resilience under stress conditions. The innovative use of green synthesis methods further underscores the promise of CuO NPs as safer, more sustainable alternatives to conventional agrochemicals. However, adopting CuO NPs in agricultural practices must be cautiously approached, given the potential risks associated with their use. The toxicity of CuO NPs to non-target organisms raises significant ecological concerns, particularly regarding environmental persistence and potential human health hazards. Comprehensive risk assessments are essential to understand the implications of their widespread use and ensure that efficacy and safety are adequately addressed in regulatory frameworks. Current regulations often do not account for the unique characteristics of NPs, highlighting an urgent need for updated legislation emphasizing biocompatibility and toxicity testing. Moreover, further research is crucial to elucidate the long-term ecological impacts of CuO NPs. Investigations should explore their environmental behavior, accumulation in soil and plants, and interactions with other agrochemicals. Addressing practical considerations—scalability, cost-effectiveness, and ease of integration into existing farming systems—will encourage farmer adoption of CuO NP-based products. Overcoming barriers related to education, training, and product accessibility is also necessary for successful implementation. In summary, while CuO NPs offer significant advantages for pest management and plant health, a balanced and collaborative approach involving researchers, regulatory bodies, and agricultural stakeholders is imperative. By prioritizing environmental safety, human health, and regulatory compliance, the agricultural community can effectively navigate the challenges associated with CuO NPs, ensuring their sustainable integration into agricultural practices.

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