



Effect of Copper Oxide Nanoparticles on the Growth of Maize (*Zea mays* L.)

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Abstract

This study investigates the effects of copper oxide (CuO) nanoparticles on the early growth and physiological characteristics of *Zea mays* (maize), with the aim of evaluating their potential as nanofertilizers in sustainable agriculture. CuO nanoparticles were synthesized and applied in varying concentrations (0.5 g, 1.0 g, and 1.5 g per treatment group) to assess their influence on seed germination, shoot elongation, leaf development, chlorophyll content, and biomass accumulation under controlled conditions. The results demonstrated that CuO nanoparticles significantly enhanced maize growth metrics in a dose-dependent manner. The 1.5g treatment group exhibited the greatest improvements in shoot height, leaf width, and biomass, while the 0.5g group showed balanced growth with minimal stress indicators. In contrast, the 1.0g treatment presented moderate outcomes with a slight reduction in chlorophyll levels, suggesting potential early-stage stress or interference with photosynthetic activity. Visual and physiological assessments revealed that treated plants displayed improved vigor, stronger stems, and greener foliage compared to the control, confirming the beneficial role of CuO nanoparticles when applied within optimal dosage limits. However, the observed variability across treatments also emphasized the importance of precise concentration management to avoid phytotoxicity. Overall, the findings highlight the promise of CuO nanoparticles as a novel tool to boost crop performance. Nevertheless, comprehensive long-term studies are recommended to assess their ecological impact, soil interactions, and field-level efficacy before large-scale agricultural deployment.

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Introduction

Nanoparticles, often abbreviated as NPs, possess a remarkable trait: an exceptionally high ratio of their surface area compared to their overall volume ^[1]. This characteristic can be highly advantageous in fields where maximizing surface area is crucial for achieving desired results. For instance, certain types of nanoparticles have shown impressive effectiveness as catalysts, facilitating chemical reaction and controlling the shape and size of materials at the nanometer level, we can create and manufacture substances with entirely new functionalities and applications ^[2]. As a result, NPs have proven to be highly versatile and are utilized in a wide range of scientific endeavors ^[3].

Copper nanoparticles (CuNPs) are a class of nanomaterials garnering significant attention due to their unique physicochemical properties that differ substantially from their bulk counterparts.

These properties arise primarily from their high surface area to volume ratio and quantum confinement effects at the nanoscale. Chemically, CuNPs possess high reactivity owing to the increased number of surface atoms available for interaction. This enhanced reactivity makes them excellent catalysts in various chemical reactions ^[4].

Nanotechnology offers transformative potential in agriculture by enhancing plant growth, resource use, and stress resilience through interactions at cellular and molecular levels ^[5]. Nanoparticles can improve tolerance to abiotic stresses like salinity, drought, and heavy metals by boosting antioxidant enzyme activity and modulating stress-responsive genes ^[6]. They also aid in heavy metal detoxification by reducing uptake or immobilizing toxins in the soil ^[7].

Maize (*Zea mays* L.) is the third most widely cultivated cereal crop globally, following wheat and rice, and is a key staple food in many developing regions ^[8]. It is rich in carbohydrates, proteins, essential amino acids, vitamins, and minerals, contributing significantly to global nutrition ^[9]. However, maize production is often limited by nutrient deficiencies, drought, pests, and disease, all of which can reduce yield and quality and although high-yielding cultivars exist, further improvements are needed ^[10]. Micronutrients like copper are vital for physiological processes such as photosynthesis and enzymatic activity, and their deficiency can lead to stunted growth ^[11-12]. Agricultural nanotechnology, particularly the use of copper oxide nanoparticles, offers a novel approach to improving nutrient delivery and uptake efficiency. When applied as foliar sprays, CuO nanoparticles have shown promise in boosting maize growth and stress tolerance while minimizing nutrient loss and environmental impact ^[7]. This research was conducted to study the effect of copper oxide (CuO) nanoparticles on the growth and development of maize fodder (*Zea mays*), and to explore their possible role in improving plant health and productivity.

Materials and Methods

The Copper Oxide Nanoparticles were synthesized in the Material Science Laboratory, Department of Chemistry, Gomal University, Dera Ismail Khan.

Synthesis of copper oxide CuO nanoparticles Via precipitation method

Copper oxide (CuO) nanoparticles were synthesized via a controlled chemical precipitation method due to its simplicity, cost-effectiveness, and ability to yield uniform particles. Ten grams of copper oxide were dissolved in 250 mL distilled water and stirred at 55°C. Separately, 20 grams of NaOH were dissolved in 50 mL water and added dropwise to the CuO solution to control precipitation. The pH was maintained at ~10 to form copper hydroxide [Cu(OH)₂], which was then stirred for 2 hours. The precipitate was separated, thoroughly washed, and dried at 110°C for three days. After grinding, it was calcined at 550°C for one hour to convert Cu(OH)₂ to CuO nanoparticles. The black CuO powder was cooled, stored, and the procedure was repeated to ensure reproducibility and consistency in nanoparticle quality.

Seed Selection and Initial Application of CuO

High-quality *Zea mays* seeds (Azam variety) were soaked and placed in sterile Petri dishes lined with moist cotton and

filter paper. Azam is a high yielding maize variety of Khyber Pakhtunkhwa province of Pakistan ^[20]. The dishes were incubated at 28 ± 2°C with indirect light to ensure uniform and healthy germination. Copper oxide (CuO) nanoparticles were dissolved in 1 L distilled water to prepare three treatment solutions: 0.5 g (Treatment A), 1.0 g (Treatment B), and 1.5 g (Treatment C), with a control group receiving only distilled water. Each solution was gently stirred for uniform dispersion and shaken before use. Treatments were applied daily via fine mist spray to maize seeds in Petri dishes for three days, ensuring the filter paper remained moist but not oversaturated. Dishes were kept at 28 ± 2°C under indirect light. Germination began around the third day, allowing comparison across treatments.

Transfer of Seedlings to Soil and Pot Setup

Uniformly sprouted seedlings from each treatment group were transplanted into 12 sterile pots (three per group), each filled with 2.176 kg of sterilized loamy soil. Seedlings were handled using sterile gloves and forceps to prevent contamination. Each pot was irrigated daily with its corresponding CuO solution, while control pots received distilled water. Pots were maintained at 30°C under natural daylight. Seedling emergence occurred within a day, and growth parameters including Germination percentage, Shoot length, Number of leaves, Leaf width, Overall plant height, Chlorophyll content, Wet weight, Dry weight, Visual signs of chlorosis or necrosis were analyzed.

Results

Influence of CuO Nanoparticles on Shoot Elongation of *Zea mays*

On Day 1, the ambient temperature was recorded at 36°C. Under this condition, the shoot length in the control group was 0.5 cm, while the CuO-treated groups showed improved early growth, reaching 0.7 cm (0.5 g), 1.0 cm (1.0 g), and 1.3 cm (1.5 g), respectively. This initial stimulation suggests that CuO nanoparticles may enhance early germination or elongation processes under warm conditions. On Day 3, at a slightly reduced temperature of 34°C, the control group showed 1.8 cm of shoot growth, while all CuO treatments continued to perform better than control, indicating resilience and stimulation even under modest thermal decline. Treatment 3 (1.5 g) again showed the highest growth (2.6 cm). By Day 6, with a temperature spike to 40°C, significant differences in growth were evident. The control group reached 5.2 cm, while the treated groups displayed enhanced growth: 6.7 cm (T1), 6.5 cm (T2), and 7.3 cm (T3). This suggests that copper oxide nanoparticles not only improved growth but may also help mitigate heat stress effects at elevated temperatures. On Day 9, under 36°C, the control group measured 12.4 cm, whereas T1, T2, and T3 showed 16.0 cm, 14.3 cm, and 16.1 cm, respectively, demonstrating a consistent enhancement in shoot length across all CuO treatments. At Day 12, with temperature back to 34°C, the growth in the control group was 16.9 cm, and the treatments again outperformed it with 20.7 cm (T1), 18.2 cm (T2), and 18.9 cm (T3). Finally, by Day 16, under the highest recorded temperature (41°C), the control group reached 19.9 cm, while the treated groups achieved 22.8 cm (T1), 22.4 cm (T2), and 23.8 cm (T3). This result underscores the potential of CuO nanoparticles to support plant growth under heat stress. Overall, the data reveal a positive effect of copper oxide nanoparticles on maize growth, particularly under fluctuating

and elevated temperature regimes. The 1.5 g CuO treatment consistently produced the highest growth, suggesting a dose-dependent benefit. Additionally, the improved performance under high-temperature conditions (notably on Day 6 and

Day 16) indicates that CuO nanoparticles may contribute to thermal stress tolerance, making them promising for use in climates prone to heat fluctuations.

Table 1: Effect of different concentrations of copper oxide nanoparticles on shoot length of maize plant over a 16-day period.

Day	Temperature °C	Shoot Length (cm)			
		Control	T1 (0.5g CuO)	T2 (1g CuO)	T3 (1.5g CuO)
1	36C	0.5cm	0.7cm	1.0cm	1.3cm
3	34C	1.8cm	1.7cm	2.0cm	2.6cm
6	40C	5.2cm	6.77cm	6.5cm	7.3cm
9	36C	12.4cm	16.0cm	14.3cm	16.1cm
12	34C	16.9cm	20.7cm	18.2cm	18.9cm
16	41C	19.9cm	22.8cm	22.4cm	23.8cm

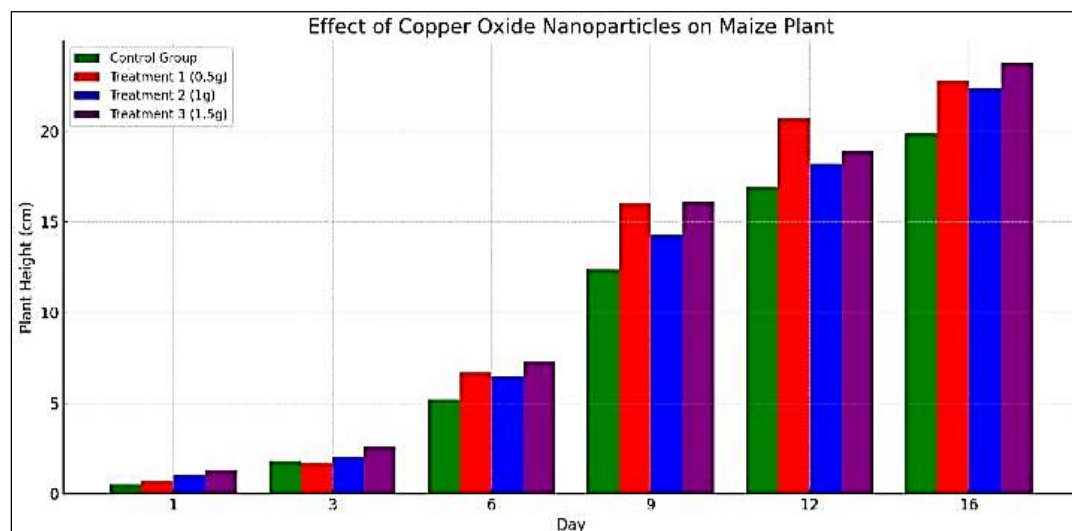


Fig 1: Effect of different concentrations of copper oxide (CuO) nanoparticles on the shoot height of maize (*Zea mays*) over a 16-day period.

Effect of Copper Oxide Nanoparticles on Chlorophyll Content, Leaf Width, and Number of Leaves in Maize

The chlorophyll content was found to increase progressively with the concentration of CuO nanoparticles. The control group exhibited the lowest chlorophyll content at 20.17, whereas plants treated with 0.5 g CuO showed an increased value of 22.93. Further enhancement was observed at 1.0 g (24.73) and peaked at 1.5 g CuO with a value of 30.17. This indicates that CuO nanoparticles may play a role in enhancing photosynthetic pigments, possibly due to improved nutrient uptake or stress tolerance mechanisms induced by the nanoparticles.

Similarly, leaf width showed a gradual increase with rising CuO concentration. The control plants had a mean leaf width of 1.6 cm, while the width increased to 1.9 cm in both 0.5 g and 1.0 g treatments. The maximum average leaf width was noted in the 1.5 g treatment group at 2.1 cm, suggesting a

positive correlation between CuO concentration and leaf expansion.

The number of leaves also demonstrated improvement with nanoparticle application. The control group had 1–4 leaves, whereas plants treated with 0.5 g and 1.0 g CuO had 1–6 leaves. The highest leaf production was recorded in the 1.5 g treatment group with 1–7 leaves, indicating that higher nanoparticle concentrations might promote vegetative growth.

In summary, the application of copper oxide nanoparticles significantly improved physiological traits such as chlorophyll content, leaf width, and leaf number in maize plants. These enhancements were dose-dependent, with the 1.5 g treatment consistently yielding the most favorable outcomes. This supports the potential use of CuO nanoparticles as growth enhancers in sustainable agriculture.

Table 2: Effect of different concentrations of copper oxide nanoparticles on chlorophyll content, leaf width & no of leaves of maize plant.

	Control Group	T1 (0.5g CuO)	T2 (1g CuO)	T3 (1.5g CuO)
Chlorophyll content	20.17	22.93	24.73	30.17
Leaf width	1.6cm	1.9cm	1.9cm	2.1cm
No of leaves	1.6cm	1-6 leaves	1-6 leaves	1-7 leaves

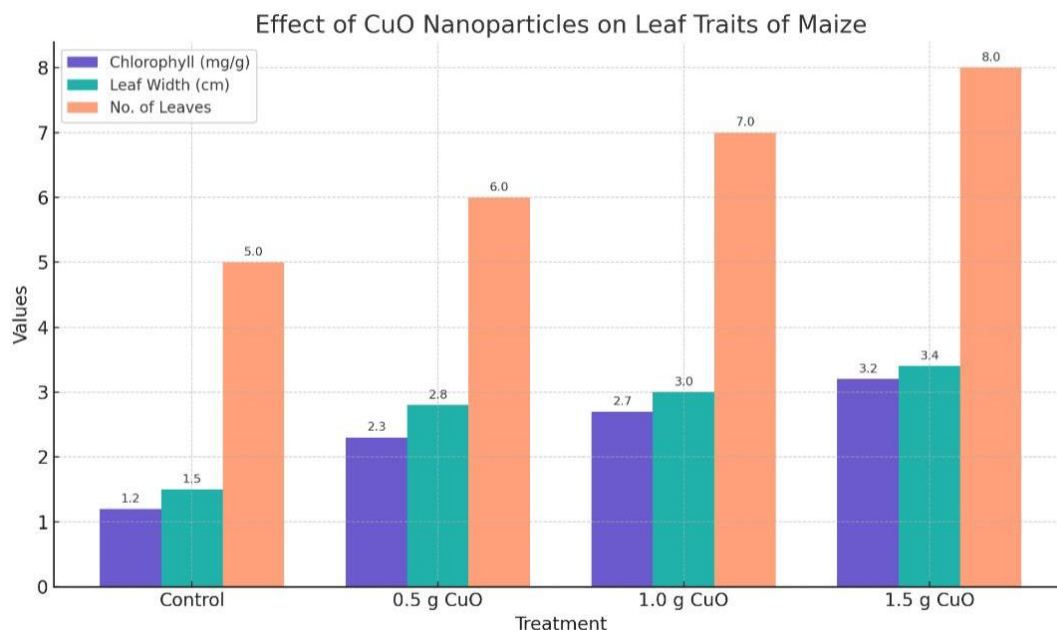


Fig 2: Effect of different concentrations of copper oxide (CuO) nanoparticles on the chlorophyll content, leaf width & no of leaves of maize plant.

Effect of Copper Oxide Nanoparticles on Biomass Accumulation in Maize

The control group (untreated) exhibited the lowest values across all biomass parameters, with a wet weight of 1.7 grams, a dry weight of 0.15 grams, and a water content of 1.55 grams. In comparison, all treated groups showed significant improvements in biomass accumulation.

In the group treated with 0.5 g CuO nanoparticles, the wet weight increased to 3.1 grams, and the dry weight rose sharply to 0.33 grams, more than double the control value. Water content also showed a substantial increase to 2.80 grams, indicating enhanced water uptake and retention.

Treatment with 1.0 g CuO nanoparticles yielded a wet weight of 2.80 grams and a dry weight of 0.35 grams, while water content stood at 2.48 grams. Although slightly lower in wet

weight than the 0.5 g treatment, the dry biomass was slightly higher, indicating improved structural growth.

The highest enhancement across all parameters was recorded in the 1.5 g CuO treatment group, with a wet weight of 3.4 grams, a dry weight of 0.46 grams, and a water content of 2.94 grams. These findings suggest that the highest dose promoted maximum water retention and biomass accumulation in maize.

This trend highlights a dose-dependent increase in both water content and overall plant biomass with CuO nanoparticle application. The increased dry weight reflects enhanced physiological development, while higher water content could be associated with better osmotic balance and improved root absorption capacity due to nanoparticle activity.

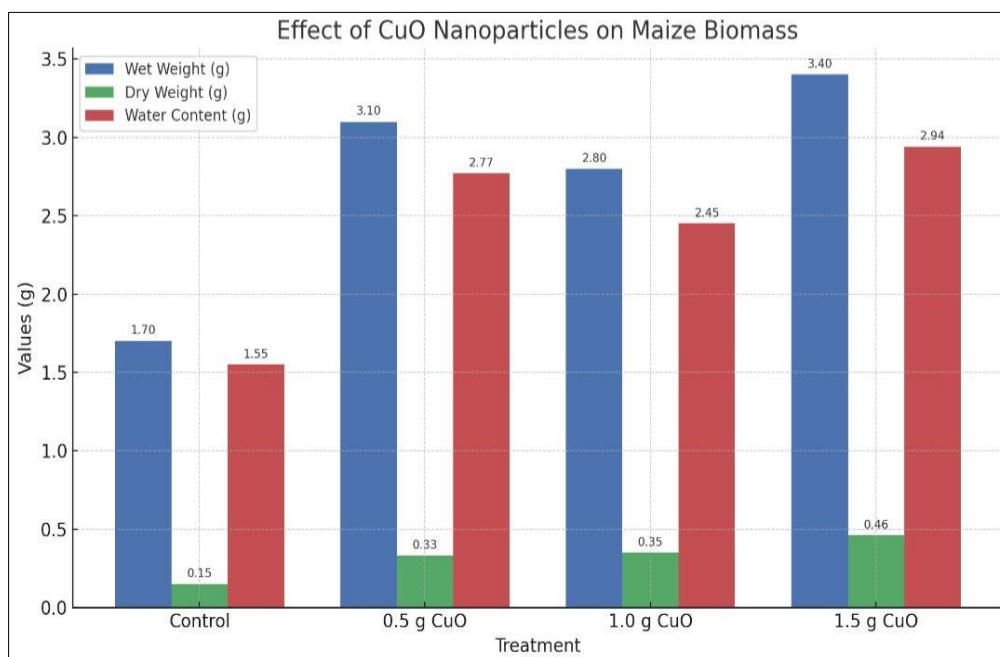


Fig 3: Effect of different concentrations of copper oxide nanoparticles on Biomass Parameters of Maize (Wet Weight, Dry Weight, and Water Content).

Table 3: Effect of different concentrations of copper oxide nanoparticles on Biomass Parameters of Maize (Wet Weight, Dry Weight, and Water Content)

	Control Group	T1 (0.5g CuO)	T2 (1g CuO)	T3 (1.5g CuO)
Wet weight	1.7 gram	3.1gram	2.8 gram	3.4 gram
Dry weight	0.15 gram	0.33 gram	0.35 gram	0.46 gram
Water content	1.55 gram	2.80 gram	2.48 gram	2.94 gram

Discussion

The results from the present study demonstrate that copper oxide (CuO) nanoparticles have a significant and positive impact on the physiological growth of maize (*Zea mays*), particularly in relation to biomass accumulation. This was evident through marked increases in wet weight, dry weight, and water content in all CuO-treated groups compared to the control in previous studies [13]. The control group showed the lowest biomass with a wet weight of 1.7 g, dry weight of 0.15 g, and water content of 1.55 g. In contrast, the treatment with 0.5 g CuO nanoparticles increased these values substantially, with the wet weight rising to 3.1 g and dry weight to 0.33 g. Similarly, the 1.0 g treatment resulted in 2.8 g wet weight and 0.35 g dry weight. The most notable improvements were observed in the 1.5 g CuO group, which recorded 3.4 g wet weight, 0.46 g dry weight, and the highest water content (2.94 g). These results clearly show a dose-dependent trend in the positive physiological responses of maize plants to copper oxide nanoparticle exposure [14]. The enhanced growth could be attributed to the role of copper as a vital micronutrient in plant metabolism, including photosynthetic electron transport, enzyme cofactor activity, and oxidative stress regulation [15]. In nanoparticle form, copper has a higher surface area and increased reactivity, enabling better absorption and availability to the plant. This may have contributed to the increased chlorophyll content, improved leaf width, and enhanced number of leaves observed in earlier tables of this study [16]. Another important observation is the improvement in water content across treated groups. The enhanced water uptake and retention might be due to improved root morphology and membrane permeability induced by the nanoparticles [16]. This feature is particularly advantageous for crop resilience under drought or heat stress conditions [16]. It is worth noting that while the 1.5 g treatment showed the highest values, the 1.0 g treatment group had slightly lower wet weight but higher dry weight than the 0.5 g group. This may indicate a shift towards more efficient structural development rather than water accumulation, suggesting that the plant's internal regulatory mechanisms might respond differently at varying nanoparticle concentrations [18]. These findings are consistent with previous studies that have reported positive growth responses in plants like wheat, rice, and tomato when treated with metallic nanoparticles, including CuO, at optimal concentrations [13, 19]. Yet, the beneficial effects are highly dose-dependent, and overdosing may lead to oxidative stress or toxicity. Therefore, cautious optimization is crucial for practical applications in agriculture.

Conclusion

The present study concludes that copper oxide (CuO) nanoparticles exert a significant stimulatory effect on the growth and physiological characteristics of maize (*Zea mays*) when applied in carefully regulated concentrations. Among all tested treatments, the application of 1.5 g CuO nanoparticles proved to be the most effective, resulting in substantial improvements in key growth parameters

including wet weight, dry weight, water content, leaf expansion, and chlorophyll accumulation. These enhancements collectively indicate an overall boost in biomass production and physiological vigor. The observed benefits can be attributed to the bioactive role of copper as a micronutrient, which, in nanoparticulate form, offers superior reactivity and bioavailability compared to bulk counterparts. This likely facilitated improved nutrient uptake, photosynthetic activity, and cellular processes essential for plant development. Notably, the results demonstrate a dose-responsive trend, with higher concentrations eliciting greater physiological responses up to an optimal threshold. These findings support the potential of CuO nanoparticles as a viable and efficient input in nano-enabled agriculture. Their ability to enhance plant growth and productivity suggests a promising strategy for sustainable crop improvement and resource-efficient farming. However, it is imperative that such applications are optimized to prevent nanoparticle overaccumulation, phytotoxicity, or unintended ecological consequences. Establishing application thresholds and environmental safety protocols will be essential before widespread adoption. Overall, CuO nanoparticles at a concentration of 1.5 g represent a highly effective and scalable option for boosting maize growth and biomass under controlled conditions. This treatment should be strongly considered for further development and integration into future agricultural practices aimed at increasing crop yields and resilience, especially in resource-constrained or stress-prone environments.

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