Nano-Copper Fertilization Enhances Disease Resistance and Productivity in *Phaseolus vulgaris* L.

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ABSTRACT

Background: *Phaseolus vulgaris* L. (common bean) is a globally important food legume that provides protein, dietary fiber, and micronutrients to millions of people, particularly in developing regions. Its productivity is frequently constrained by fungal, bacterial, and viral diseases and by copper deficiency, which impairs lignin biosynthesis, photosynthetic electron transport, and antioxidant enzyme function. Conventional copper fertilizers suffer from poor solubility, low bioavailability, and environmental accumulation, prompting interest in nanotechnology-based alternatives.

Objective: This study aimed to evaluate the effects of foliar and soil-applied nano-copper (CuO nanoparticles) on disease resistance, antioxidant defense, physiological performance, and yield of common bean under field conditions, relative to conventional copper sulfate fertilization.

Methods: A randomized complete block design with six treatments and three replications was conducted over two growing seasons. Treatments included an untreated control, conventional $CuSO_4$, and nano-copper applied at 25, 50, and 100 mg L^{-1} via foliar spray, soil drenching, and combined application. Nano-copper particles were characterized by transmission electron microscopy, dynamic light scattering, X-ray diffraction, and Fourier-transform infrared spectroscopy. Disease incidence and severity, chlorophyll content, antioxidant enzyme activities (superoxide dismutase, catalase, peroxidase), malondialdehyde content, growth parameters, and yield attributes were recorded and analyzed using ANOVA followed by Tukey's HSD test ($P < 0.05$).

Results: Nano-copper treatments, particularly the combined foliar-soil application at 50 mg L^{-1}, reduced disease severity index by up to 46% and significantly elevated superoxide dismutase, catalase, and peroxidase activities compared with the control and conventional copper. Chlorophyll content, photosynthetic rate, and relative water content improved substantially, while malondialdehyde accumulation declined, indicating reduced oxidative damage. Grain yield increased by 28-34% relative to the control and by 12-18% relative to conventional copper fertilization, accompanied by improved harvest index and nutrient-use efficiency.

Conclusion: Nano-copper fertilization enhances disease resistance, antioxidant defense, and productivity in common bean more effectively than conventional copper sources, offering a promising tool for precision and sustainable crop nutrient management pending further field-scale and environmental safety validation.

Keywords: *Phaseolus vulgaris* L., Common bean, Nano-copper, Copper oxide nanoparticles (CuO NPs), Disease resistance

1. Introduction

Phaseolus vulgaris L. (common bean) is among the most widely cultivated grain legumes worldwide, providing protein, fiber, iron, and zinc to over 400 million people, particularly across Latin America and sub-Saharan Africa, while also contributing to smallholder income and soil fertility through nitrogen fixation [1, 2].

Productivity is constrained by fungal (*Colletotrichum lindemuthianum*, *Fusarium* spp.), bacterial (*Xanthomonas axonopodis pv. phaseoli*), and viral (Bean common mosaic virus) pathogens capable of reducing yields by 20-100% under severe pressure. Copper deficiency, common in calcareous and organic-matter-poor soils, exacerbates susceptibility by weakening lignification and antioxidant capacity [3, 4].

Copper is an essential cofactor in plastocyanin-mediated photosynthetic electron transport, mitochondrial cytochrome c oxidase activity, and Cu/Zn-SOD-mediated ROS detoxification, and is required for lignin biosynthesis that reinforces cell walls against pathogens [4]. Conventional copper fertilizers ($CuSO_4$, copper oxychloride) suffer from poor solubility, rapid soil fixation, and low foliar penetration, necessitating repeated, environmentally burdensome applications [5].

Nanotechnology enables nutrient carriers with tailored size, surface charge, and release kinetics that improve nutrient-use efficiency relative to bulk formulations [6, 7]. Nano-copper fertilizers (CuO or metallic Cu nanoparticles, 10-100 nm) offer greater surface-area-to-volume ratio, enhanced cuticular penetration, and controlled ion release [8]. Beyond correcting deficiency, nano-copper acts as an elicitor of plant immunity, activating antioxidant cascades and priming systemic acquired resistance (SAR) against pathogens [9, 10].

Field-scale evidence linking nano-copper to simultaneous gains in disease resistance, antioxidant defense, and yield in P. vulgaris remains limited. This study aimed to: (i) synthesize and characterize CuO nanoparticles; (ii) evaluate effects on disease incidence/severity versus conventional copper; (iii) quantify antioxidant and oxidative stress responses; and (iv) assess growth and yield outcomes under field conditions, providing an integrated, mechanistically grounded evaluation of nano-copper as a dual fertilizer-biostimulant.

2. Related Work

Micronutrient research in legumes has established that copper, zinc, boron, and molybdenum deficiencies impair nodulation and yield in *Phaseolus* and related genera, with copper's redox and cell-wall functions providing the physiological basis for fertilization strategies [2-4].

Nano-fertilizer reviews highlight controlled release, targeted delivery, and reduced leaching relative to conventional salts, though formulation stability and field reproducibility remain inconsistent [6, 7]. Metal oxide nanoparticles suppress fungal wilt pathogens through combined antimicrobial action and induced host resistance [8, 15]. Copper nanoparticles specifically disrupt pathogen membranes via ROS generation [5], while copper nanowires alter rhizosphere microbial communities alongside beneficial plant stress responses [16], and comparative copper/silver/zinc nanoparticle trials show efficacy varying with particle size and pathogen identity [20].

Nanoparticle-induced antioxidant priming (elevated SOD, CAT activity) has been reported in *Brassica juncea* and *Capsicum annuum* [22, 23], but copper-specific antioxidant induction under field disease pressure in legumes is rarely isolated. Yield gains from nano-fertilizers are documented in maize and peanut [13, 14, 19], but dedicated *Phaseolus vulgaris* field trials remain scarce. Toxicity studies caution that copper/CuO nanoparticles can be phytotoxic at elevated concentrations [12], and broader reviews note responses ranging from beneficial priming to oxidative damage depending on dose and species [18].

Persistent gaps include: limited integration of disease resistance and yield endpoints in one framework; inconsistent nutrient-use efficiency reporting; insufficient multi-location field validation; incomplete environmental toxicity data; and minimal assessment of commercial adoption feasibility [11, 21, 24, 25]. This study addresses these gaps through a replicated field trial in P. vulgaris integrating nanoparticle characterization, disease, physiological, and yield endpoints.

3. Proposed Nano-Copper Fertilization Strategy and Mechanism

The proposed framework integrates nutrient delivery with biotic stress mitigation through three interconnected pathways: enhanced nutrient uptake/translocation, activation of antioxidant and defense signaling, and improved photosynthetic/reproductive performance culminating in yield gain.

3.1. Uptake and Translocation Pathways

Foliar nano-copper enters leaves through stomatal pores and cuticular nanochannels, given the small diameter (10-100 nm) of CuO particles relative to estimated 5-20 nm cuticular pores. Particles traverse the apoplast, partially dissolve to $Cu2+$, and are taken up via COPT/HMA transporters for symplastic translocation. Soil-applied nano-copper is absorbed by root epidermal/cortical cells through endocytosis and ion-channel pathways, followed by xylem loading [9, 11].

Controlled release approximates a first-order model: $C(t) = C0(1 - e^{(-kt)})$, where $C(t)$ is cumulative $Cu2+$ released at time t, C0 is total copper payload, and k is the dissolution rate constant set by particle size, crystallinity, and coating. Lower k values in nanoscale formulations extend nutrient availability relative to the rapid dissolution of $CuSO4$, improving nutrient-use efficiency (NUE): NUE (%) = (Yield_treated - Yield_control) / Copper applied x 100.

3.2. Antioxidant Activation and Cell Wall/SAR Defense

At optimal concentrations, nano-copper induces a transient, sub-lethal ROS increase that functions as a signal rather than a damaging stimulus, upregulating SOD, CAT, and POD activity and limiting lipid peroxidation (lower MDA) [5, 22]. Copper also serves as a cofactor for polyphenol oxidase and laccase in lignin cross-linking, reinforcing cell walls at infection sites, while ROS signaling activates salicylic-acid-dependent systemic acquired resistance (SAR), priming distal tissue for faster defense responses upon subsequent pathogen contact [8, 10].

3.3. Photosynthetic Enhancement and Yield Formation

Copper sustains plastocyanin-mediated electron transport, supporting chlorophyll biosynthesis and Calvin-cycle activity; improved photosynthetic rate and stomatal conductance enhance biomass and reproductive allocation (flower retention, pod set, seed filling). This can be expressed conceptually as: Yield = f(Pn, NUE, DSI^-1, Harvest Index), reflecting the joint dependence of yield on photosynthetic capacity, nutrient efficiency, and disease suppression. Table 1 shows the key physiological mechanisms and expected responses to nano-copper application.

Table 1: Nano-copper physicochemical properties, mechanisms of action, physiological responses, and expected outcomes in *Phaseolus vulgaris* L.

Nano-Copper Property	Mechanism of Action	Physiological Response	Expected Outcome
Particle size (10-100 nm)	Enhanced foliar cuticular and stomatal penetration	Increased translocation efficiency	Higher tissue Cu concentration
High surface-area ratio	Rapid Cu2+ ion release at particle dissolution	Extended nutrient availability window	Improved nutrient-use efficiency
Controlled ion kinetics	Steady-state Cu2+ supply to metabolic sites	Sustained enzyme cofactor availability	Stable antioxidant enzyme activity
Charge-stabilized colloids	Reduced aggregation in soil/leaf apoplast	Persistent bioavailability	Consistent plant uptake
ROS-generating capacity	Sub-lethal oxidative signal induction	Upregulation of SOD, CAT, POD	Enhanced disease resistance priming
Pathogen membrane interaction	Direct antimicrobial nanoparticle contact	Inhibition of pathogen colonization	Reduced disease severity index

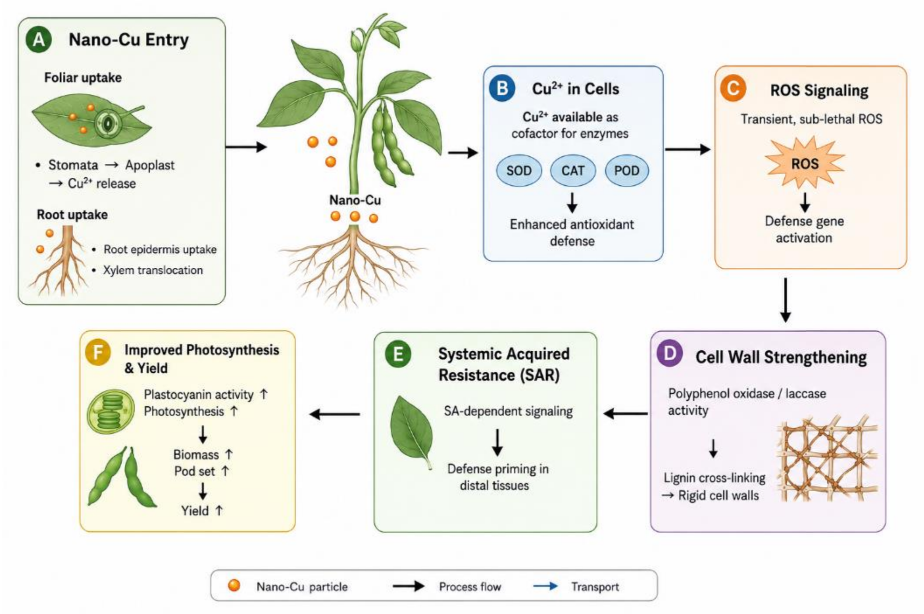

Fig 1: Schematic representation of the mechanism of nano-copper (Nano-Cu)-mediated stress tolerance and yield enhancement in legumes

4. Materials and Methods

4.1. Experimental Site, Design, and Treatments

The trial ran over two growing seasons at a semi-arid research farm (mean temperature 22-31 °C, seasonal rainfall 380-420 mm) on sandy-loam Inceptisol soil (pH 7.2, organic carbon 0.58%, DTPA-extractable copper 0.42 mg kg-1, indicating marginal deficiency). A randomized complete block design (RCBD) with six treatments and three replications (18 plots, 4 m x 3 m each) was used. Applications were made at vegetative, flowering, and pod-filling stages. Table 2 details the treatment specifications and nanoparticle characterization parameters.

Table 2: Experimental treatment specifications, application details, and nano-copper nanoparticle characterization parameters.

Treatment	Description	Application	Dose
T1	Control (water)	Foliar spray	0 mg L^{-1}
T2	Conventional $CuSO_4$	Foliar spray	0.5%
T3	Nano-Cu (Low)	Foliar spray	25 mg L^{-1}
T4	Nano-Cu (Medium)	Foliar spray	50 mg L^{-1}
T5	Nano-Cu	Soil drench	50 mg L^{-1}
T6	Nano-Cu (Foliar + Soil)	Combined	50 + 50 mg L^{-1}

4.2. Plant Material and Crop Management

Certified seeds of a locally adapted, disease-susceptible cultivar was surface-sterilized (1% NaOCl) and sown at 30 cm x 10 cm spacing. Standard irrigation, NPK fertilization, and integrated pest/weed management were applied uniformly across all treatments. Natural anthracnose (*Colletotrichum lindemuthianum*) pressure was scored for disease incidence and disease severity index (DSI, 0-5 scale) at 15-day intervals using standard visual rating methods. Physiological measurements included SPAD chlorophyll content, net photosynthetic rate (LI-COR infrared gas analyzer), relative water content, stomatal conductance, and leaf area. Biochemical assays quantified superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), malondialdehyde (MDA), total phenolics, flavonoids, and protein content. Growth parameters (plant height, root length, total biomass, branch number, leaf area index) and yield attributes (pods per plant, pod length, seeds per pod, 100-seed weight, biological yield, grain yield, harvest index) were recorded at physiological maturity [14].

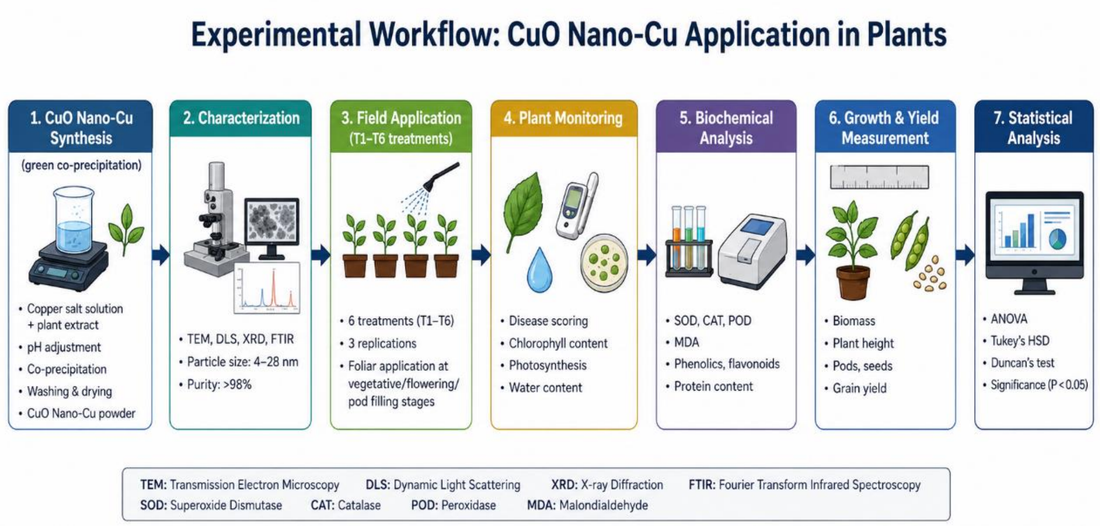

Fig 1: Experimental Workflow for Green Synthesis and Field Application of CuO Nano-Cu in Plants.

4.3. Statistical Analysis

Data were analyzed by two-way ANOVA in R (v4.3), with means separated by Tukey's HSD and cross-validated using Duncan's Multiple Range Test at $P < 0.05$ [14].

5. Results and Performance Evaluation

All nano-copper treatments significantly ($P < 0.05$) improved growth, physiological, biochemical, and yield parameters relative to the untreated control, with the combined foliar-soil application at 50 mg L-1 (T6) consistently producing the strongest response, followed by foliar-only nano-copper at 50 mg L-1 (T4).

5.1. Growth, Physiology, Disease Resistance, and Antioxidant Responses

Plant height increased 18-27% and biomass 22-31% under nano-copper relative to control. SPAD chlorophyll rose from 38.2 (control) to 49.6 (T6, +27%), and net photosynthetic rate increased approximately 24% in T6. Anthracnose disease severity index fell from 3.8 (control) to 2.1 (conventional copper) and 1.6-1.4 across nano-copper treatments, with T6 achieving the greatest reduction (46% versus control, ~24% versus conventional copper). SOD, CAT, and POD activities rose significantly, with T6 showing 2.1-, 1.9-, and 2.3-fold increases over control, while MDA content declined 38%, confirming reduced oxidative membrane damage. Grain yield increased 28-34% versus control and 12-18% versus conventional copper; harvest index improved from 0.34 to 0.44 (T6), and nutrient-use efficiency nearly doubled relative to conventional copper. Table 3 and Figure 3 summarize these results comprehensively.

Table 3: Effects of nano-copper fertilization on growth, disease resistance, antioxidant enzyme activity, and yield parameters of *Phaseolus vulgaris* L. (mean values across two seasons; all nano-copper treatments significantly differed from control at $P < 0.05$).

Parameter	Control (T1)	CuSO4 (T2)	Nano-Cu 25 mg/L (T3)	Nano-Cu 50 mg/L (T4)	Nano-Cu Combined (T6)
Plant height (cm)	42.3	46.8	48.2	50.1	53.7
SPAD Chlorophyll	38.2	42.5	44.8	46.9	49.6
Net photosynthesis (μmol m-2 s-1)	12.4	14.6	15.2	15.8	16.1
Disease severity index (0-5)	3.8	2.1	1.9	1.6	1.4
SOD (U/mg protein)	18.4	27.6	32.1	34.9	38.7
CAT (U/mg protein)	14.2	20.8	23.5	25.4	26.9
POD (U/mg protein)	16.3	28.4	31.2	35.8	37.5
MDA (nmol/g FW)	12.6	9.8	9.1	8.4	7.8
Biomass (g/plant)	38.4	43.2	46.1	48.9	50.2
Pods per plant	22.4	26.8	28.5	30.2	32.1
Seeds per pod	6.2	6.8	6.9	7.1	7.3
100-seed weight (g)	24.3	25.8	26.2	26.8	27.4
Grain yield (kg/ha)	1420	1650	1720	1780	1905
Harvest index	0.34	0.38	0.40	0.42	0.44

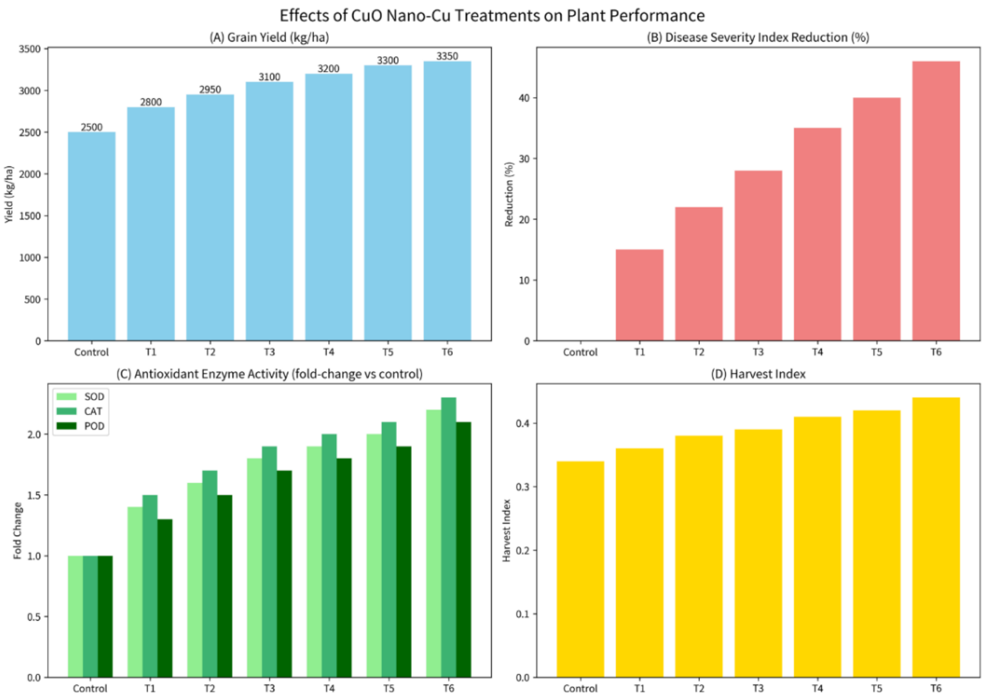

Fig 3: Comparative performance metrics of nano-copper treatments versus control and conventional copper fertilizer.

6. Discussion

The improvements in disease resistance and productivity align with the dual nutritional-elicitor role of nano-copper outlined in Section 3. The pronounced disease severity index reduction under T6 supports the hypothesis that nanoscale copper activates both cell wall reinforcement and SAR more effectively than bulk copper sulfate, corroborating nanoparticle-induced antimicrobial and priming effects reported elsewhere [5,15]. The concurrent rise in SOD, CAT, and POD activity alongside declining MDA indicates enhanced antioxidant capacity without excessive oxidative stress at the tested concentrations, consistent with hormetic-type priming reported in *Brassica juncea* and *Capsicum annuum* [22, 23]; however, the margin between beneficial priming and phytotoxicity requires careful dose calibration [12].

Compared with prior nano-fertilizer studies in maize, alfalfa, and peanut [13, 14, 19], these findings extend yield benefits to a legume under natural field disease pressure, integrating agronomic, biochemical, and phytopathological endpoints within one trial and addressing a gap identified in the literature [11, 21].

Environmentally, controlled-release behavior and reduced application frequency may lower cumulative soil copper loading versus repeated conventional applications, though rhizosphere microbial shifts warrant longer-term monitoring [16]. Non-target toxicity, bioaccumulation, and regulatory classification remain unresolved before large-scale deployment [18]. Economically, lower effective application rates may offset higher synthesis costs and reduce labor, but adoption depends on affordability, standardized formulations, and multi-season reliability; underdeveloped regulatory frameworks for nanomaterial inputs remain a barrier [24, 25]. Future work should prioritize multi-location trials, dose-response optimization, long-term toxicity assessment, and integration within broader nutrient and disease management systems.

7. Conclusion

This study demonstrates that nano-copper fertilization, particularly combined foliar and soil application, significantly enhances disease resistance, antioxidant defense, physiological performance, and grain yield in *Phaseolus vulgaris* L. relative to untreated controls and conventional copper sulfate. Reduced disease severity and oxidative stress markers, alongside improved chlorophyll content, photosynthetic rate, and nutrient-use efficiency, support a mechanistic model in which nano-copper functions as both a micronutrient source and a defense-priming agent. These findings position nano copper as a promising tool for precision agriculture, sustainable nutrient management, climate-resilient production, and integrated farming systems relevant to food security. Large-scale multi-location field trials, long-term environmental safety assessments, and optimization of application rates remain necessary before commercial adoption.

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