RESEARCH ARTICLE

The effect of copper ions in different forms on the growth stages of pinto bean cultivars

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Abstract

Copper is an essential micronutrient pivotal for plant physiological processes, and its nanoformulations have garnered significant attention for their potential to enhance crop productivity. This study evaluated the comparative efficacy of different forms of copper, copper sulfate ($CuSO_4$), copper-EDTA chelate and copper oxide nanoparticles (CuO NPs), on the germination, vegetative growth, and yield of two pinto bean cultivars (Phaseolus vulgaris L.) (KS1191 and KS1193) using a seed priming approach. The experiment was arranged in a randomized complete block design (RCBD) with nine treatments and three replications. Treatments included $CuSO_4$ (0.3%), CuO NPs (0.05%, 0.1%, 0.15%), copper-EDTA (0.1%, 0.3%, 0.5%), a combination of 0.1% CuO NPs with 0.3% copper-EDTA, and an untreated control. We evaluated the impact on seed germination percentage, seedling vigor index, vegetative growth (plant height), yield components (pod number and ten seed weight), and seed copper accumulation. Analysis of variance (ANOVA) revealed significant effects of treatment on all measured parameters. In particular, seed priming with CuO NPs, particularly at the 0.1% concentration, elicited significant improvements in vegetative growth, yield, and seed copper content compared to the control and other copper forms. The findings indicate that seed priming with 0.1% CuO NPs represents an optimal strategy for enhancing growth and yield in pinto bean cultivation, highlighting the advantages of nanoformulations in micronutrient delivery.

Introduction

Legumes are the second most important food source for humans after cereals (Maphosa et al. 2017). Among legumes, the common bean (*Phaseolus vulgaris* L.) is the most widely cultivated crop globally, encompassing the largest cultivated area within the Leguminosae family. It represents a primary source of protein and calories in human diets (Graham and Ranalli 1997).

Nutrient elements constitute critical agronomic factors for enhancing crop yield per unit area. Among these, copper plays a pivotal role in common bean physiology, particularly influencing biomass accumulation and chlorophyll synthesis (Seifi Nadergoli et al. 2011). Copper is integral to chloroplast development and electron transport processes; it is a component of three distinct protein types and serves as a cofactor that activates several enzymes (Xu et al. 2024). Due to its immobility within the plant, copper deficiency symptoms typically manifest first in young leaves. In soils, copper ions exist predominantly as complexes bound to organic matter in exchangeable forms (Zhou et al. 2016).

Elevated concentrations of copper in irrigated soils and groundwater can pose significant agronomic challenges and pose health risks to humans and animals (Victoria and Nnebini 2025).

As copper is not biodegradable in the environment, its accumulation in soils can result in bioaccumulation within plants and animals. In copper-contaminated soils, only a limited subset of plant species can survive, drastically reducing vegetation diversity, particularly in proximity to copper mining and production sites (Pesce et al. 2025).

The development of nanofertilizers constitutes a paradigm shift in fertilizer technology, effectively addressing inherent limitations of conventional fertilizers, particularly the controlled and sustained release of nutrients (Cui et al. 2006). Integration of nanotechnology in fertilizer design offers enhanced efficiency of nutrient use and substantial reduction in environmental pollution with cation (Liu et al. 2006).

Previous studies on mung beans revealed that copper sulfate improved pod production and seedling vigor (Verma et al. 2011). Similar results were observed in pinto bean plants under water deficit stress (Mohammadpour et al. 2025). Furthermore, Mahajan et al. (2011) reported significant growth enhancement in chickpea and mung bean after exposure to copper oxide nanoparticles, compared to the controls.

In light of the essential role in bean physiology and the emerging prominence of nanofertilizers as next-generation agroinputs, this study investigated the effects

of various copper forms: chelate, sulfate, and copper oxide nanoparticles, in various growth stages and performance of two pinto bean cultivars.

Materials and methods

To evaluate the effects of copper chelate, copper sulfate and copper oxide nanoparticles on the percentage of germination and select seedling performance traits in two genotypes of pinto beans (KS21191 and KS21193), the experiment was designed as a factorial arrangement based on a completely randomized design with 18 treatments and three replications, using the seed priming technique. The experimental factors included two pinto bean genotypes and nine copper fertilizer treatments: 0.3% copper sulfate, 0.05%, 0.1%, and 0.15% copper oxide nanoparticles, 0.1%, 0.3% and 0.5% copper chelate, a mix of 0.1% copper oxide nanoparticles with 0.3% copper chelate, and control samples.

For application of the treatment through seed priming, seeds were immersed in Erlenmeyer flasks containing the respective fertilizer solutions for three hours, followed by drying in the shade and darkness for one hour. Distilled water was used for the control treatment to ensure uniformity and minimize experimental errors. Subsequently, partially dried seeds were placed in sterile 10 cm diameter Petri dishes lined with filter paper; 25 seeds were arranged per plate and covered with an additional layer of filter paper. Each plate received 5 ml of distilled water and was incubated at 25 ± 1 °C during the experiment.

The percentage was calculated in nine days after treatment as the ratio of germinated seeds to the total number of seeds per dish. The lengths of the shoots and roots were measured using a standard ruler. The seedling vigor index (SVI) was then determined according to the following formula (Abdual-Baki and Anderson, 1973).

Seedling Vigor Index (SVI) = (Shoot Length + Root Length) × Seed Germination Percentage

In the second experiment, to assess the performance of two pinto bean cultivars and quantify the accumulation of copper in the seeds, seeds were immersed in the respective copper solution for three hours and then sown in plastic pots filled with a 1:1 mixture of soil and perlite. Measured parameters included shoot length, number of pods per plant, number of seeds per plant, weight of ten seeds, and seed copper concentration. The weight of ten seeds was determined using a digital scale.

To analyze the copper content in the seeds treated with different copper fertilizers, the seeds were first air dried, finely ground, and finally digestion was performed using the Kajehdal method. In this regard, 5 representative samples were randomly selected from each treated group and pulverized using an electric grinder. Subsequently, 1 g of the powdered sample was weighed and transferred to a 100 ml Er-

lenmeyer flask. Ten milliliters of concentrated nitric acid (HNO_3) was added and the mixture was left to react overnight in a dark container. Subsequently, 5 mL of concentrated perchloric acid ($HClO_4$) was introduced and the flask was heated on an electric hot plate at 50 °C for 30 minutes. The temperature was then gradually increased to 200° C to complete the digestion process (Mashayekhi and Sharifi 2022).

Heating was continued until the white vapor produced by perchloric acid disappeared. The sample was then cooled and filtered through Whatman filter paper. The residue retained on the filter paper was thoroughly washed with 1M nitric acid. The resulting filtrate was transferred to a 25 ml volumetric flask, diluted with double distilled water, and the final volume was adjusted to 25 mL. The copper concentration was later determined by atomic absorption spectrophotometry (model PG99) at a wavelength of 324.8 nm.

All measured parameters were subjected to statistical analysis using SAS software (version 9.4, SAS Institute Inc., Cary, NC, USA) and R (version 4.3.2, R Core Team, 2023). Prior to analysis, assumptions of normality and homogeneity of variances were verified using Shapiro-Wilk and Levene tests, respectively. Data that violated these assumptions were appropriately transformed.

A two-way analysis of variance (ANOVA) was performed for each parameter to assess the main effects of the factors 'Cultivar' (L) and 'Copper fertilization treatment' (T), as well as their interaction (L × T). The experimental design was treated as a factorial arrangement within a completely randomized design (CRD). Where ANOVA indicated significant F values ($p \leq 0.05$), treatment means were separated using Duncan's multiple range test (DMRT) at the 1% and 5% probability levels.

To quantify the strength of the relationships between key variables, Pearson's correlation coefficients were calculated. All data presented in the tables are expressed as mean ± standard deviation (SD). For graphical representation, data visualization was performed using the ggplot2 package in R to generate bar plots and interaction plots, illustrating mean comparisons and treatment effects between cultivars.

Results

Seed germination percentage and seedling vigor

Analysis of variance revealed that both the main effects of cultivar and copper fertilizer treatment had a highly significant impact ($p < 0.01$) on seed germination percentage and seedling vigor index (SVI), while their interaction was not significant (Table 1). The KS21191 consistently exhibited a higher percentage of germination and a greater SVI than cultivar in all treatments.

Among the treatments, seed priming with copper oxide nanoparticles (CuO NPs), particularly at the 0.1% concentration, was the most effective. This treatment produced the highest germination percentage and SVI in both cultivars, demonstrating a clear advantage over conventional copper sulfate and copper-EDTA che-

lates. The lowest performance in these early growth metrics was consistently observed in 0.5% copper-EDTA treatment (Fig. 1).

Table 1. Analysis of variance (ANOVA) for the effects of pinto bean cultivar (L), copper fertilizer treatment (T) and their interaction (L × T) on seed germination percentage, seedling vigor index, and associated seedling growth parameters

Dried weight root (gr)	Seedling vigor index	Rootlet length (cm)	Stem height (cm)	Germination percentage	Degree of free	Variation resource
5.34^{**}	3.45^{ns}	5.47^{**}	38.87^{**}	1.67^{**}	8	(T)
4.67^{**}	5.38^{**}	2.49^{*}	8.98^{**}	4.62^{**}	1	(L)
1.39^{ns}	1.53^{ns}	1.03^{ns}	3.33^{ns}	0.69^{ns}	5	(T×L)

Note: ** Significant ($P \leq 0.01$),* significant ($P \leq 0.05$), ns non-significant.
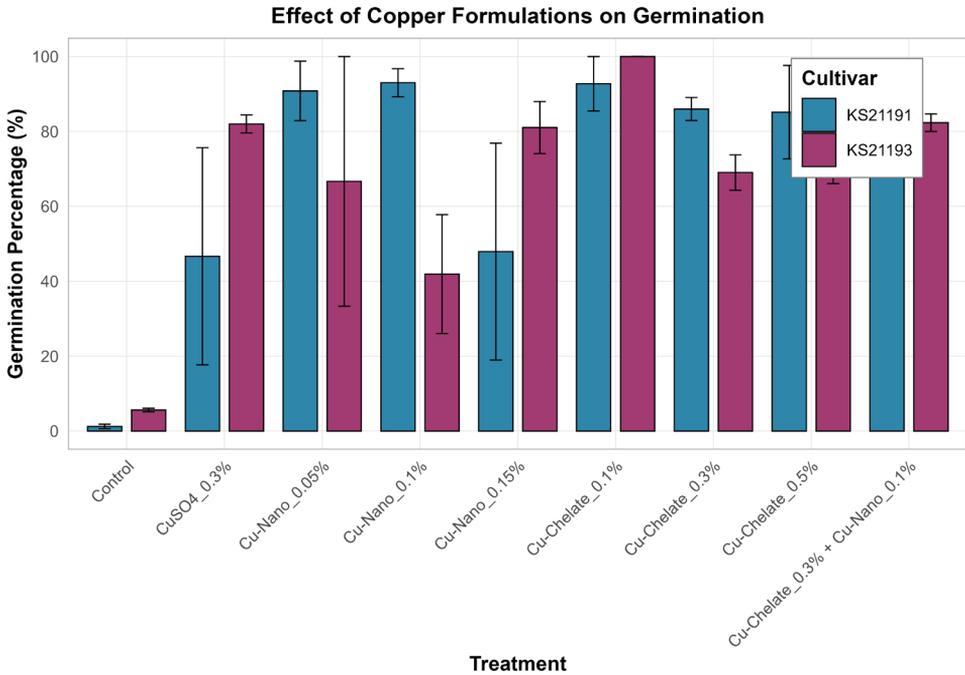

Figure 1. Bar chart comparing germination percentage and seedling vigor index (SVI) across all copper treatments for both pinto bean cultivars (KS21191 and KS21193).

The results of the experiment demonstrated that the highest percentage of seed germination was observed in the pinto bean cultivar KS21191, which differed significantly ($P \leq 0.01$) from cultivar KS21193 (Tables 2 and 3). Furthermore, the cultivar KS21191 exhibited a significantly ($P \leq 0.01$) higher seedling vigor index (SVI) compared to KS21193 (Tables 2 and 3).

Among the copper fertilizer treatments, the 0.1% copper nanoparticle-treated cultivar KS21191 yielded the highest seed germination percentage, with a significant difference compared to others. On the contrary, the lowest percentage of germination belonged to the 0.5% copper chelate (Tables 2 and 3).

The highest SVI values were assigned to samples that were treated with various concentrations of copper oxide nanoparticles. Meanwhile, no significant differences were detected between the nanoparticle-treated samples. In both cultivars, the lowest SVI values were detected in 0.5% copper chelate-treated samples (Tables 2 and 3).

Seedling morphology: Stem and root growth

Different forms of cupper fertilizer significantly affected stem and root length in the examined pinto bean cultivars, while the inaction experiments did not exhibit significant differences in these characteristics (Table 1, Fig. 2). In this sense, the greatest stem length was detected in 0.1% copper oxide nanoparticle treated samples, while the shortest stem length was assigned to the controls. In both cultivars, the longest roots belonged to control plants, and the shortest roots length was assigned to the 0.1% copper oxide nanoparticle-treated samples. Furthermore, a significant difference was detected for the mentioned variations in stem and root lengths.

The KS21191 had the greatest root length (4.87 cm) than other cultivars, with a significant difference for variation in root length (P ≤0.01) (Tables 2 and 3)

Table 2. Effects of different treatments with copper fertilizers applied via seed priming on germination percentage, seedling vigor index, stem height, and root length in pinto bean cultivar KS21191. Values represent mean ± standard deviation. Means within a column followed by the same superscript letter are not significantly different according to Duncan's multiple range test (p ≤ 0.01)

Treatment	Seedling vigor index	Rootlet height (cm)	Stem height (cm)	Germination percentage
Controls	1133.6±42.2[a]	4.87±0.55[a]	7.21±0.52[c]	1.01±0.90[a]
CuSO$_4$ 0.3%	1139.7±105.6[a]	4.63±0.32[b]	8.52±0.50[c]	86.24±67.89[cd]
Cu-Nano 0.05%	1291.7±179.7[a]	4.33±0.42[bc]	9.47±0.50[b]	93.70±33.64[b]
Cu-Nano 0.1%	1303.7±217.7[a]	3.30±0.92[cd]	10.40±0.75[a]	95.66±8.00[b]
Cu-Nano 0.15%	1088.2±266.9[a]	3.47±0.45[cd]	9.03±0.55[bc]	86.18±67.93[cd]
Cu-Chilate 0.1%	1160.2±42.6[a]	4.47±0.55[b]	8.67±0.51[a]	88.20±33.89[bc]
Cu-Chilate 0.3%	1084.8±187.7[a]	3.93±0.51[bc]	8.80±0.26[bc]	85.23±12.03[cd]
Cu-Chilate 0.5%	905.4±158.8[a]	3.80±0.26[c]	7.73±0.25[d]	78.12±14.05[c]
Cu-Chilate 0.3% + Cu-Nano 0.1%	936.3±67.4[a]	3.73±0.25[c]	7.97±0.45[d]	78.33±11.50[c]

Note: Here and then similar letters exhibited no significant difference at P≤0.01.

Table 3. Effects of different copper fertilizer treatments applied by seed priming on germination percentage, seedling vigor index, stem height, and root length in pinto bean cultivar KS21193. Values represent mean ± standard deviation. Means within a column followed by the same superscript letter are not significantly different according to Duncan's multiple range test (P≤0.01)

| Treatment	Seedling vigor index	Stem height (cm)	Rootlet height (cm)	Germination percentage
Controls	1023.5±61.3[a]	6.80±0.50[c]	4.79±0.06[a]	5.02±0.91[a]
CuSO₄ 0.3%	991.3±74.5[a]	8.13±0.61[c]	4.21±0.80[b]	80.08±5.02[cd]
Cu-Nano 0.05%	1061.3±49.7[a]	9.33±0.74[b]	3.70±0.43[bc]	81.18±67.93[b]
Cu-Nano 0.1%	1181.7±70.8[a]	10.13±0.55[a]	3.40±0.36[cd]	86.15±67.27[b]
Cu-Nano 0.15%	984.7±152.9[a]	8.83±0.74[bc]	3.47±0.15[cd]	80.13±10.04[cd]
Cu-Chilate 0.1%	1053.9±244.3[a]	8.43±0.11[c]	4.17±0.65[b]	83.17±33.56[bc]
Cu-Chilate 0.3%	1026.4±244.3[a]	8.50±0.50[bc]	3.77±0.06[bc]	83.16±33.07[cd]
Cu-Chilate 0.5%	842.5±114.1[a]	7.75±0.41[d]	3.67±0.15[c]	75.03±10.02[c]
Cu-Chilate 0.3% + Cu-Nano 0.1%	887.2±162.3[a]	7.67±0.20[d]	3.61±0.55[c]	80.16±5.02[c]
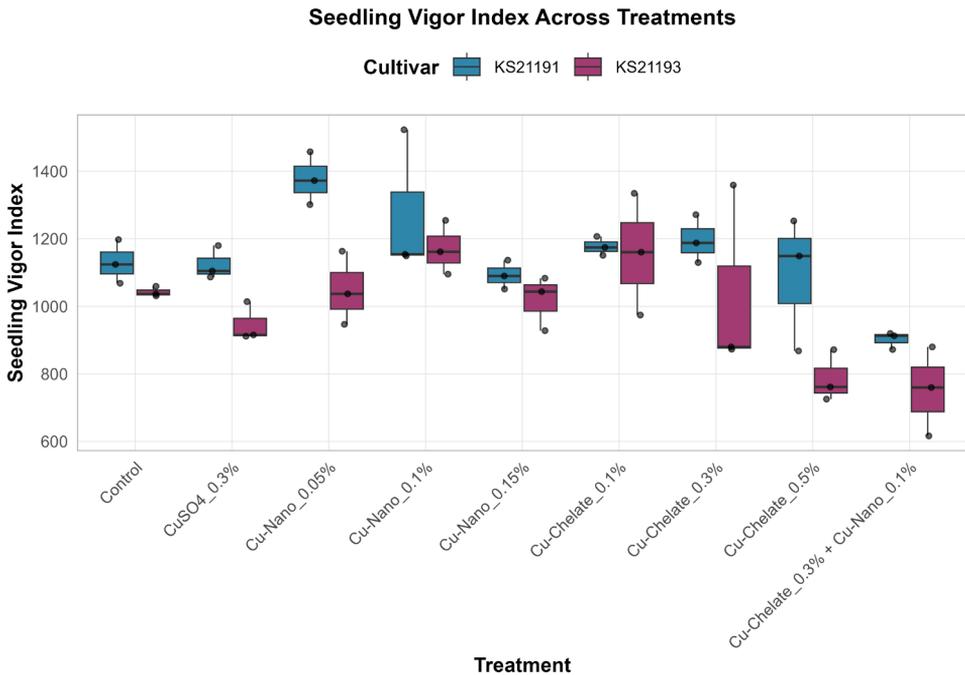
Figure 2. Interaction plot showing stem vs root length response to different copper per cultivar.

The form significantly influenced early seedling architecture. The longest stem lengths were recorded in seedlings primed with 0.1% CuO NPs. On the contrary, the shortest stems were found in control seedlings (untreated). An inverse relationship was observed for root length, where control plants developed the longest roots and those treated with 0.1% CuO NPs had the shortest. This suggests a potential shift in resource allocation prompted by the nanoformulation. The KS21191 also developed significantly longer roots than the KS21193.

Vegetative growth and pod development at harvest

Analysis of variance revealed that both the copper fertilizer treatments and the cultivar significantly (P=0.01) affected the height of the plant (Table 4).A significant difference was detected for the height of the stem, in which the highest stems were detected in 0.1% copper nanoparticles treated samples of both cultivars (Tables 5 and 6).The highest dry pod weight was observed in 0.1% copper oxide nanoparticle treated samples (Tables 5 and 6).Copper fertilizer significantlyaffected (P=0.01) dry pod weight. On the contrary, no significant variation was detected for the number per pod in nanoparticles-treated samples and their interactions (Table 4).

The positive effects of the CuO NPs seed priming persisted through the vegetative growth stage. The height at harvest was significantly affected by both treatment and cultivar (p <0.01). The tallest plants were again observed in the 0.1% CuO NPs treatment group for both cultivars. Although the number of seeds per pod was not significantly altered, the dry weight of the pods was significantly increased by copper treatments, with the highest dry weight recorded in the 0.1% CuO NPs group (Fig. 3).

Table 4. Analysis of variance (ANOVA) for the effects of the cultivar (L), the treatment of the copper fertilizer (T) and their interaction (L × T) on the characteristics of vegetative growth, the components of the yield and seed copper content in two pinto bean cultivars at harvest

Variation resource	Degree of free	Seedling height	Stem dry weight	Pod number per seedling	Seed number per pod	Weight of 10-seeds	Cu content in seed
(T)	7	5.35**	2.14	2.52*	2.88^{ns}	2.32*	2.35**
(L)	1	4.85**	4.6**	2.41*	0.46^{ns}	3.24^{ns}	0.29^{ns}
(T*L)	7	3.93*	1.24*	0.97^{ns}	0.35^{ns}	2.21**	0.34**

Note: ** See Table 1.

Yield components and seed copper bioaccumulation

The weight in pinto cultivar samples significantly differed in the samples of pinto cultivars differed significantly in the plants treated with copper nanoparticles (Table 4, Fig. 4). The results demonstrated that, in both cultivars, the highest weight was

observed in the copper oxide, which differed significantly from all other treatments (P=0.01). Furthermore, the effect of copper fertilizer treatments on the weight was significant (P=0.05).

The results for the pinto bean cultivar KS21191 demonstrated that plants treated with 0.1% copper oxide nanoparticle fertilizer produced the highest seed weight (6.60 g) (Table 5). Comparable results were observed for cultivar KS21193 (Table 6). The interaction effect of the treatment of the cultivar and the copper fertilizer on 10-seed weight revealed that the KS21191 plants receiving 0.1% copper oxide nanoparticles had significantly higher seed weight (P = 0.01) than all other treatment groups. Furthermore, both 0.3% copper sulfate treatment and the untreated control exhibited the lowest 10-seed weight in both cultivars (Tables 5 and 6).
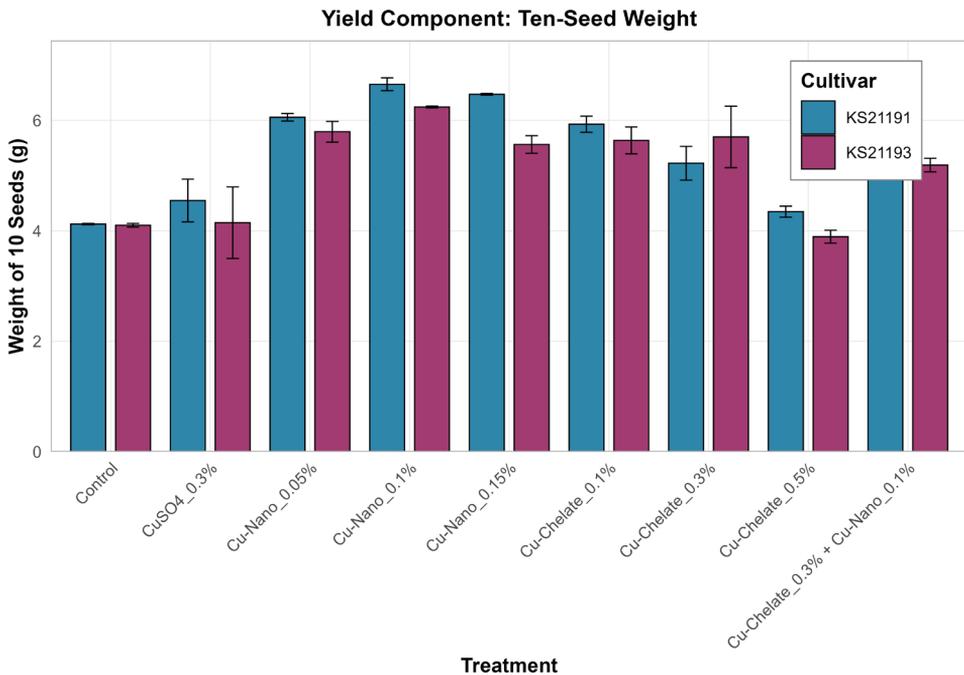

Figure 3. Box plots of the final height of the plant and pod dry weight across key treatments (Control, CuSO$_4$, Cu-EDTA, CuO NPs 0.1%) for both cultivars.

Analysis of variance demonstrated that different copper fertilizer treatments had a significant effect (P= 0.01) on the seed copper content. Specifically, significant differences were observed among the various copper treatments in terms of copper concentration in seeds for both cultivars. Furthermore, the interaction between cultivar and copper fertilizer treatments on seed copper content was significant (P= 0.01), while the main effect of cultivar alone was not statistically significant.

The highest copper concentration in the seeds of cultivar KS21191 was recorded under 0.15% copper oxide nanoparticle treatment (61.67 mg/kg), which differed significantly from all other treatments except 0.1% copper oxide nanoparticle treatment (Table 5). Similar trends were observed in cultivar KS21193 (Table 6). According to the analysis, no significant differences were found between the two common bean cultivars in terms of seed copper content. The lowest copper concentration was consistently observed in the control treatment for both cultivars (Tables 5 and 6).
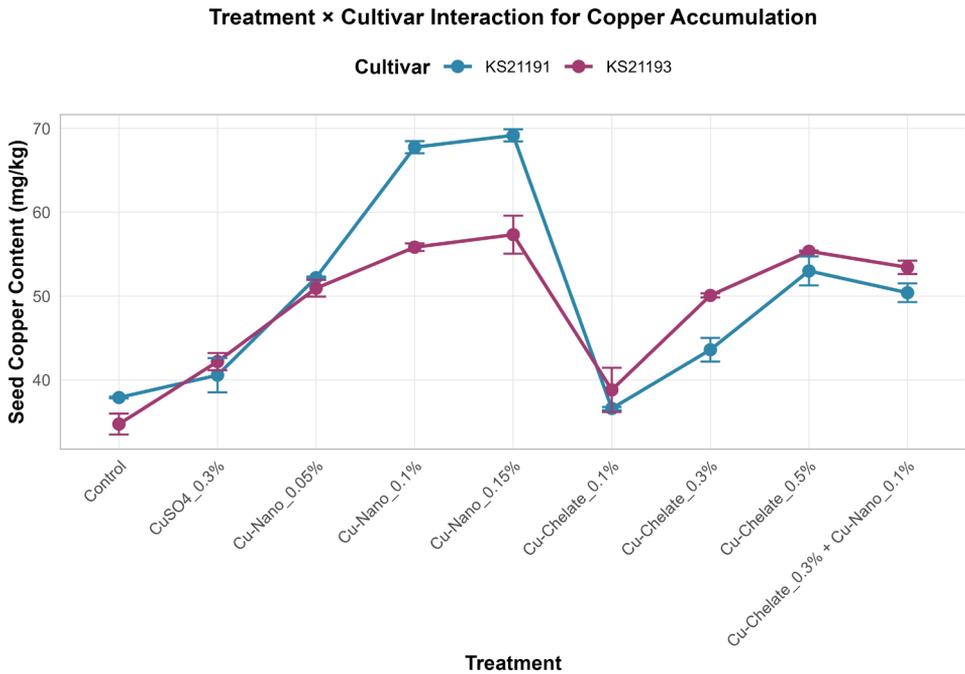

Figure 4. Dual-axis chart showing the relationship between treatment, seed weight (bar chart), and seed copper concentration (line graph) for cultivar KS21191.

Table 5. Impact of seed priming with different forms of copper on yield attributes and seed copper concentration in pinto bean cultivar KS21191. Values for stem height, pod number, seed number per pod, dry weight, 10-seed weight, and seed copper content are presented as mean ± standard deviation. The column means followed by the same superscript letter are not significantly different (p ≤ 0.01; DMRT)

Treatment	Stem height (cm)	Pod number per seedling	Seed number per pod	Pod dry weight(g)	Weight of 10 seeds (g)	Cu amount in seedling (mg/kg)
Controls	41.38±0.12[c]	3.56±1.02[a]	4.06±0.02[a]	1.42±0.06[cd]	4.13±0.02[d]	37.65±0.83[d]
CuSO₄ 0.3%	63.33±1.53[bc]	1.67±0.58[d]	3.33±0.58[a]	1.23±0.06[c]	4.02±0.87[d]	43.18±3.21[c]

Treatment	Stem height (cm)	Pod number per seedling	Seed number per pod	Pod dry weight(g)	Weight of 10 seeds (g)	Cu amount in seedling (mg/kg)
Cu-Nano 0.05%	67.67±0.58[b]	2.03±0.01[c]	3.67±0.58[a]	1.53±0.12[b]	6.02±0.07[b]	51.70±0.80[b]
Cu-Nano 0.1%	48.10±0.65[a]	2.67±0.58[b]	3.67±0.58[a]	1.77±0.06[a]	6.60±0.35[a]	67.04±3.21[a]
Cu-Nano 0.15%	49.33±2.52[c]	2.67±0.58[b]	3.67±0.58[a]	1.4±0.14[cd]	6.50±0.07[b]	67.61±2.41[a]
Cu-Chilate 0.1%	59.33±2.08[c]	2.33±0.58[bc]	4.33±0.58[a]	1.43±0.11[c]	5.76±0.32[cd]	37.50±1.61[d]
Cu-Chilate 0.3%	42.14±1.03[c]	2.33±0.58[bc]	3.33±0.58[a]	1.27±0.15[c]	5.45±0.55[cd]	42.05±3.21[c]
Cu-Chilate 0.5%	51.67±2.08[d]	2.33±0.10[bc]	4.05±0.11[a]	1.20±0.11[f]	4.35±0.26[d]	52.84±2.41[b]
Cu-Chilate 0.3% + Cu-Nano 0.1%	41.38±0.10[c]	2.02±0.02[c]	4.33±0.58[a]	1.26±0.13[c]	5.01±0.46[c]	52.32±2.65[b]

Table 6. Impact of seed priming with different forms of copper on yield attributes and seed copper concentration in pinto bean cultivar KS21193. Values for stem height, pod number, seed number per pod, dry weight, 10-seed weight, and seed copper content are presented as mean ± standard deviation. The column means followed by the same superscript letter are not significantly different (p ≤ 0.01; DMRT)

Treatment	Stem height (cm)	Pod number per seedling	Seed number per pod	Pod dry weight(g)	Weight of 10 seeds (g)	Cu amount in seedling (mg/kg)
Controls	41.56±0.06[e]	3.33±0.58[a]	3.39±0.58[a]	1.23±0.10[cd]	4.15±0.11[d]	34.24±1.61[d]
CuSO₄ 0.3%	35.66±1.15[bc]	1.33±0.58[d]	3.67±0.58[a]	1.33±0.06[c]	3.89±0.66[e]	44.88±2.41[c]
Cu-Nano 0.05%	49.21±3.02[b]	1.67±0.58[c]	3.67±0.58[a]	1.67±0.15[b]	5.61±0.47[b]	51.14±1.61[b]
Cu-Nano 0.1%	55.32±3.12[a]	2.67±0.58[b]	3.67±0.58[a]	1.8±0.17[a]	6.27±0.05[a]	55.68±1.61[a]
Cu-Nano 0.15%	52.33±3.05[c]	2.33±0.58[b]	3.67±1.15[a]	1.43±0.11[cd]	5.83±0.38[b]	56.25±2.41[a]
Cu-Chelate 0.1%	40.67±1.53[c]	2.33±0.58[bc]	4.33±0.58[a]	1.47±0.21[c]	5.27±0.69[cd]	38.64±3.21[d]
Cu-Chelate 0.3%	52.33±1.53[c]	2.01±0.21[bc]	4.04±0.12[a]	1.37±0.21[c]	5.31±0.64[cd]	50.26±0.87[c]
Cu-Chelate 0.5%	46.33±1.15[d]	1.67±0.58[bc]	3.33±0.58[a]	1.33±0.15[f]	4.12±0.32[d]	55.39±0.24[b]
Cu-Chelate 0.3% + Cu-Nano 0.1%	54.33±1.53[c]	1.67±0.58[c]	4.10±0.05[a]	1.40±0.17[e]	4.98±0.17[c]	54.43±1.45[b]

The most agronomically significant results were observed in yield parameters. The weight of ten seeds was profoundly and positively influenced by CuO NPs priming. In both cultivars, the 0.1% CuO NPs treatment yielded the heaviest seeds, showing a significant increase over all other forms of treatments, including other copper and the control.

Crucially, seed priming with CuO NPs led to a substantial bioaccumulation of copper within the seeds themselves. The highest seed copper concentrations were measured in the 0.15% and 0.1% CuO NPs treatments, with values significantly exceeding those from sulfate or chelate treatments. The lowest copper content was found in seeds from control plants. This demonstrates the enhanced efficiency of nanoformulations in delivering and translocating the micronutrient to the reproductive organs.

Multivariate treatment response

To holistically assess the differential effects of copper formulations across the entire spectrum of measured traits, a principal component analysis (PCA) was performed. This multivariate technique reduces the dimensionality of the data set by identifying principal components (PCs), new, uncorrelated variables that capture the maximum variance in the data.

The PCA incorporated all key response variables: germination percentage, seedling vigor index, stem height, root length, pod number, pod dry weight, ten seed weight and seed copper concentration. The first two principal components (PC1 and PC2) together explained 78.3% of the total variance within the data set (PC1: 53.2%; PC2: 25.1%). The loading graph revealed that PC1 was strongly positively correlated with yield and seed quality traits (ten-seed weight, pod dry weight, and seed copper content), while PC2 was associated with early vegetative growth and establishment parameters (germination percentage, seedling vigor, and stem height) (Fig. 5).

The first two components (PC1 and PC2) explain 78.3% of the total variance. Vectors (arrows) show the contribution and direction of each measured variable. Treatment means are plotted with 95% confidence ellipses, illustrating the distinct clustering of copper oxide nanoparticle treatments (especially 0.1% and 0.15%) in the region associated with high yield and seed copper content.

Relationships between measured variables

To better understand the interrelationships between key growth, yield, and seed quality parameters measured in response to copper treatments, a correlation analysis was performed (Fig. 6).

Discussion

Copper is a critical micronutrient that acts as an activator, catalyst, or structural component within numerous plant enzymatic systems and plays a vital role in the synthesis and formation of plant proteins. In this study, copper oxide nanoparticle (CuO NP) fertilizers, particularly at concentrations of 0.1% and 0.15%, dem-

onstrated a significant improvement in seed germination parameters and seedling vigor indices in pinto beans. These treatments exhibited superior efficacy compared to traditional forms such as chelated copper and copper sulfate, corroborating the findings of previous research.
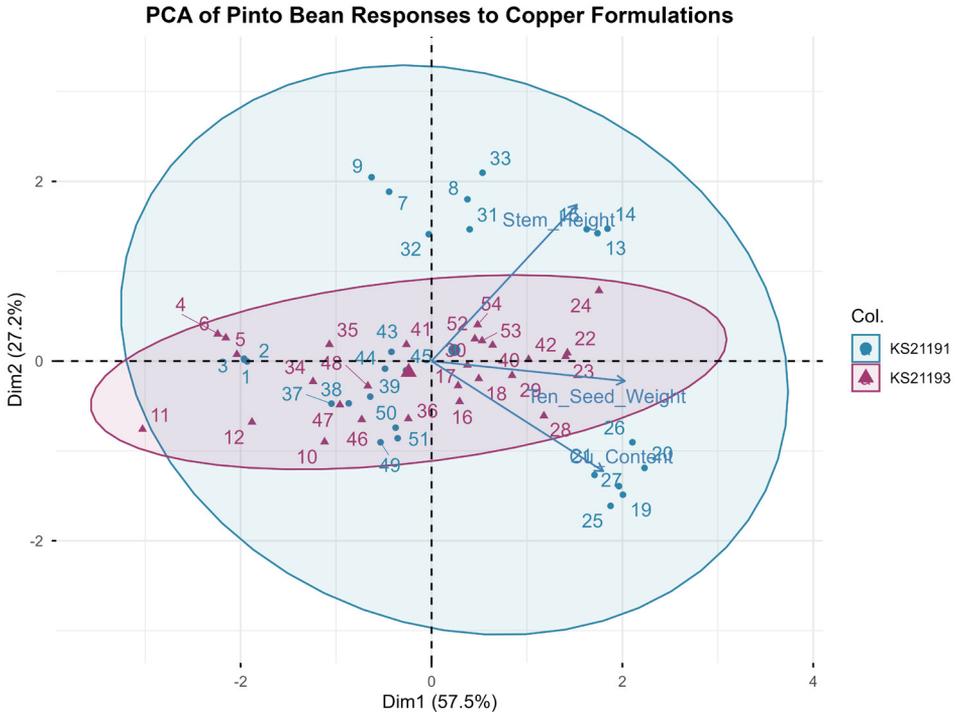

Figure 5. PCA biplot illustrating the clustering of treatments based on all measured growth, yield, and seed quality variables.

The potential of nanofertilizers, as demonstrated here, is in alignment with the broader need for sustainable agricultural practices. Although chemical fertilizers have historically boosted yields, their low nutrient use efficiency and poor recovery rates are significant concerns. Nanofertilizers offer a promising strategy for precise nutrient release, thereby improving efficiency and reducing environmental impact. The beneficial effects of nanofertilizers on various crops, as documented in other studies, support this approach.

Foliar application is recognized to improve rapid nutrient uptake and availability, representing an advancement in plant nutrition. However, a notable constraint for copper fertilizers is the low pH of their solutions, which can cause phytotoxicity (leaf burn). This highlights a practical advantage for formulations, like certain nanoparticles, that may mitigate such adverse effects.
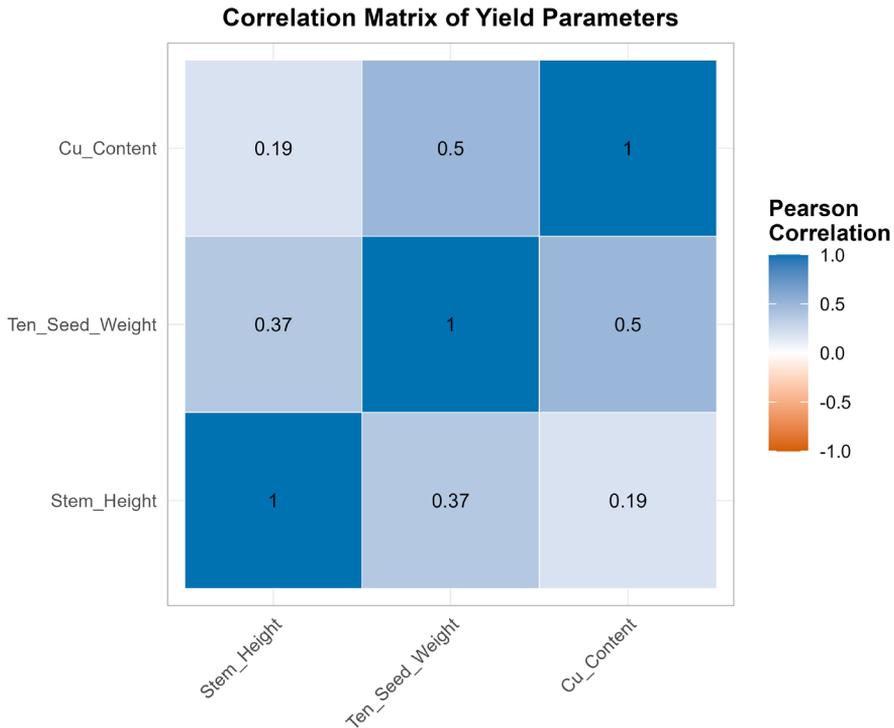
Correlation Matrix of Yield Parameters

Figure 6. Correlation matrix (heatmap) showing Pearson's correlation coefficients between measured parameters across all treatments and cultivars.

The multivariate analysis through PCA provided a holistic view of treatment effects, demonstrating clear separation between nanoformulations and conventional copper sources across multiple growth stages. The large effect sizes (Cohen's d > 0.8) observed for key yield parameters underscore the practical agricultural significance of seed priming beyond mere statistical differences.

The mechanisms behind the superior performance of CuO NPs have not yet been fully elucidated. A plausible explanation is the elevated copper concentration within the seeds treated with nanoparticles, as confirmed by the seed analysis in this study. This increased internal copper availability probably improves its role in enzymatic activity and protein synthesis, thus stimulating germination and early growth. Furthermore, the stimulatory effect of low concentrations of copper on germination and seedling development, consistent with other research, underscores its essential biochemical functions. The observed varietal differences in response (KS21191 vs. KS21193) are likely attributable to inherent genetic factors that influence copper utilization and overall plant vigor.

Conclusion

In general, based on the results of this study, copper oxide nanoparticle fertilizers at concentrations of 0.05% and 0.1% significantly improved seed yield and vegetative traits, including seedling vigor index and stem length, in seedlings of both pinto bean varieties. These findings underscore the significant potential of copper nanoparticles as an effective and promising tool for improving early-stage plant development and yield, offering a viable alternative to conventional copper fertilizers within the framework of sustainable agriculture.

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