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Cobalt in the emergence and initial growth of maize (*Zea mays* L.), bean (*Phaseolus vulgaris* L.) and tomato (*Physalis ixocarpa* Brot. ex Horm.)

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ABSTRACT

Objective: To evaluate the effect of cobalt on the emergence and early growth of maize (*Zea mays* L.), common bean (*Phaseolus vulgaris* L.), and husk tomato (*Physalis ixocarpa* Brot. ex Horm.) seedlings.

Design/Methodology/Approach: The effect of cobalt was independently assessed for each plant species. Seeds of maize hybrid H-52, Pinto Saltillo bean, and husk tomato variety Selecto were sown in 15×15 cm plastic containers filled with a 70:30 (w/w) peat-to-perlite substrate. Cobalt concentrations of 0, 2, 4, and 6 mM were applied via irrigation over a 35-day period. Each container represented one experimental unit, with four replicates per treatment. Beginning six days after sowing (DAS), emergence percentage was recorded. At 35 DAS, the following growth parameters were measured: seedling height, number of leaves, stem diameter, root length, number of roots, root volume, shoot fresh biomass, and root fresh biomass.

Limitations/Implications: Sensitivity to cobalt varied among the evaluated species.

Findings/Conclusions: Cobalt application reduced seedling height and root length in both bean and husk tomato, while these parameters remained unaffected in maize. Overall, cobalt inhibited the growth of all three species, with maize seedlings displaying the lowest sensitivity, followed by beans and husk tomatoes.

Keywords: dose-response, food interest plants, homeostasis, transition metal, tolerance.

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INTRODUCTION

Seedling emergence is one of the most critical phenological stages for annual plants. This phase marks the transition from dependence on seed reserves provided by the maternal plant to autotrophic growth sustained by photosynthesis (Forcella *et al.*, 2000). During this stage, both intrinsic seed factors and biotic or abiotic environmental conditions can negatively impact or even prevent successful emergence (Lamichhane *et al.*, 2018). Heavy

metal contamination in soils can adversely affect seedling emergence and development by disrupting enzyme production, antioxidant activity, and photosynthetic processes (Seneviratne *et al.*, 2019). The impact of heavy metals on emergence varies among species, primarily due to differences in seed structure particularly the seed coat as well as species-specific exclusion mechanisms such as chelation and subcellular compartmentalization (Jan & Parray, 2016). Cobalt (Co) is a transition metal essential for certain organisms, as it forms part of cobalamin (vitamin B12) and plays enzymatic and coenzymatic roles (Hu *et al.*, 2021; Osman *et al.*, 2021). It contributes significantly to nitrogen fixation in legumes and, when applied exogenously, may stimulate plant growth. Its reported benefits include enhanced stem development, coleoptile elongation, bud formation, and increased growth and yield (Ulhassan *et al.*, 2023). However, cobalt's effects are highly dose-dependent and vary according to plant species and application methods. Elevated Co concentrations can inhibit nodule formation in legumes and negatively impact overall plant development, yield, root formation, nutrient translocation, and water uptake (Akeel & Jahan, 2020). Moreover, excessive Co can disrupt physiological and biochemical processes, including chlorophyll content, photosynthesis, and enzyme activity (Salam *et al.*, 2024). Industrial activities, particularly the manufacturing of hard metals, are the primary sources of cobalt pollution in the environment (Klasson *et al.*, 2016). Industries such as cement production, tool grinding, electronic waste processing, and the use of cobalt-containing products including cosmetics and batteries contribute to Co release, resulting in its accumulation within the soil-plant system (Banerjee & Bhattacharya, 2021). Plant species differ in their tolerance to and accumulation of Co (Peng *et al.*, 2021). Hyperaccumulator species absorb and translocate Co to aerial tissues without exhibiting visible signs of toxicity (Hu *et al.*, 2021), whereas non-accumulator species tend to retain most of the metal in their roots, limiting its movement to aboveground organs and reducing the risk of damage to photosynthetic and reproductive structures (Bakkaus *et al.*, 2005). Most food crops lack efficient mechanisms for cobalt translocation to aerial parts, resulting in low mobility and high root retention (Khan *et al.*, 2024). This trait becomes particularly important for root vegetables cultivated in contaminated soils or irrigated with wastewater (Genchi *et al.*, 2023). Given these risks, monitoring Co accumulation in plant tissues using high-sensitivity analytical techniques such as inductively coupled plasma optical emission spectrometry (ICP-OES) is essential (Soh *et al.*, 2024). In addition to the risk of Co accumulation in edible plant parts, the vulnerability of crops during early developmental stages must also be considered. Most cultivated species lack effective Co tolerance mechanisms, making early growth a particularly susceptible phase under metal exposure. This sensitivity varies by species, necessitating comparative evaluations across different taxonomic groups. Within this context, the aim of the present study was to evaluate the effect of Co application on the emergence and early seedling growth of maize (*Zea mays* L.), common bean (*Phaseolus vulgaris* L.), and husk tomato (*Physalis ixocarpa* Brot. ex Horm.).

MATERIALS AND METHODS

The experiment was conducted under controlled greenhouse conditions, with an average temperature of 28 °C. The plant material included seeds of hybrid maize (*Zea* *mays* L.) H-52, common bean (*Phaseolus vulgaris* L.) cultivar Pinto Saltillo, and husk tomato (*Physalis ixocarpa* Brot. ex Horm.) cultivar Selecto. The growth substrate consisted of a 70:30 (v/v) mixture of peat (REKIVA®) and perlite. Each species was evaluated independently. Six seeds were sown in plastic containers measuring 15×15×10 cm, each filled with the substrate. One container represented one replicate, with four replicates per treatment. Treatments were arranged in a completely randomized design. After sowing, 40 mL of cobalt solution was applied at concentrations of 0, 2, 4, and 6 mM. Cobalt was supplied as cobalt (II) chloride hexahydrate [$CoCl_2 \cdot 6H_2O$] (J.T. Baker; Center Valley, PA, USA), dissolved in distilled water. The solution was applied directly to the substrate using a graduated cylinder and was administered every 24 hours for 35 days. No additional fertilization was provided. Daily and cumulative seedling emergence were recorded starting 24 hours after sowing to calculate the emergence percentage for each replicate. At 35 days after the onset of treatments, the seedlings were removed from the containers, and the following variables were measured: seedling height (cm), stem diameter (cm), number of leaves, root length (cm), number of roots, root volume (cm^3), and fresh biomass (g). Root volume was determined using the water displacement method with a graduated cylinder, and fresh shoot and root biomass were measured with an analytical balance (OHAUS Explorer; NJ, USA). The data were subjected to analysis of variance (ANOVA), and means were compared using Tukey's test ($p \leq 0.05$), with the Statistical Analysis System software (SAS Institute, 2011).

RESULTS AND DISCUSSION

In this study, cobalt application had no significant effect on the emergence percentage of maize (Figure 1A) across all tested concentrations. However, emergence was significantly reduced in bean (Figure 1B) and husk tomato (Figure 1C), with the highest Co concentration (6 mM) completely inhibiting emergence in husk tomato. Cobalt is known to impair germination and emergence in higher plants by increasing protease activity, inhibiting amylase enzymes, and disrupting sugar transport (Lassoued *et al.*, 2018). Seedling height in maize was not significantly affected by cobalt at 2, 4, or 6 mM (Figure 1D). In contrast, 6 mM Co reduced seedling height in bean by 29% (Figure 1E), and a reduction in tomato seedlings was evident starting at the lowest concentration tested (2 mM) (Figure 1F). Species-specific responses to Co are closely related to their morphological traits and the homeostatic mechanisms each plant employs to mitigate the adverse effects of the metal (Jan & Parray, 2016; Lwalaba *et al.*, 2020). Previous studies have shown that maize can tolerate high concentrations of heavy metals by compartmentalizing them in vacuoles and accumulating them in the cell wall and intercellular spaces (Sun & Luo, 2018). Furthermore, the reduction in plant height is associated with oxidative stress induced by cobalt, leading to damage in lipids, proteins, and membranes, which disrupts photosynthetic processes, water uptake, and ultimately inhibits growth (Ali *et al.*, 2018).

Application of 4 mM Co significantly reduced the number of leaves in maize seedlings (Figure 2A). In contrast, the application of 2 and 4 mM Co significantly increased this variable in bean plants (Figure 2B), while it decreased in husk tomato seedlings (Figure 2C). The increased number of leaves in bean seedlings in response to cobalt may enhance

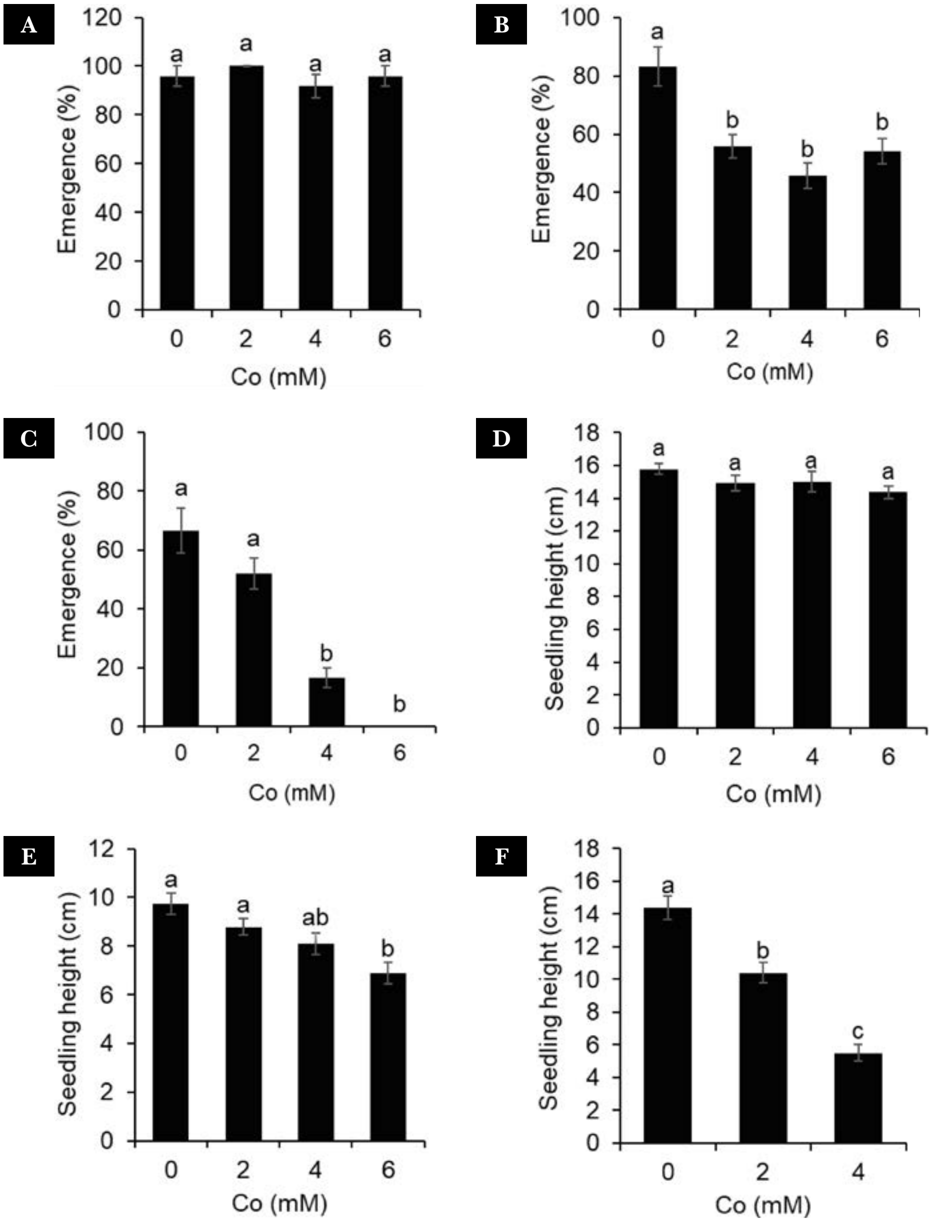

Figure 1. Emergence percentage and seedling height of maize (*Zea mays*) (A, D), bean (*Phaseolus vulgaris*) (B, E), and husk tomato (*Physalis ixocarpa*) (C, F) treated with 0, 2, 4, and 6 mM Co. Values are means ± SE. Different letters within each subfigure indicate statistically significant differences between treatments (Tukey, $p \leq 0.05$).

photosynthetic capacity, helping the plant cope with cobalt-induced stress. Similarly, various morphological changes in leaves under heavy metal stress have been reported, such as increased leaf thickness and enlargement of epidermal cells (Krzesłowska *et al.*, 2024). Stem diameter increased significantly in maize seedlings treated with 6 mM Co (Figure 2D). In bean plants, this variable remained unchanged (Figure 2E), whereas a significant reduction was observed in husk tomato seedlings (Figure 2F). The increase in stem diameter under Co exposure may be associated with the accumulation of assimilates as reserves to tolerate stress (Akeel & Jahan, 2020).

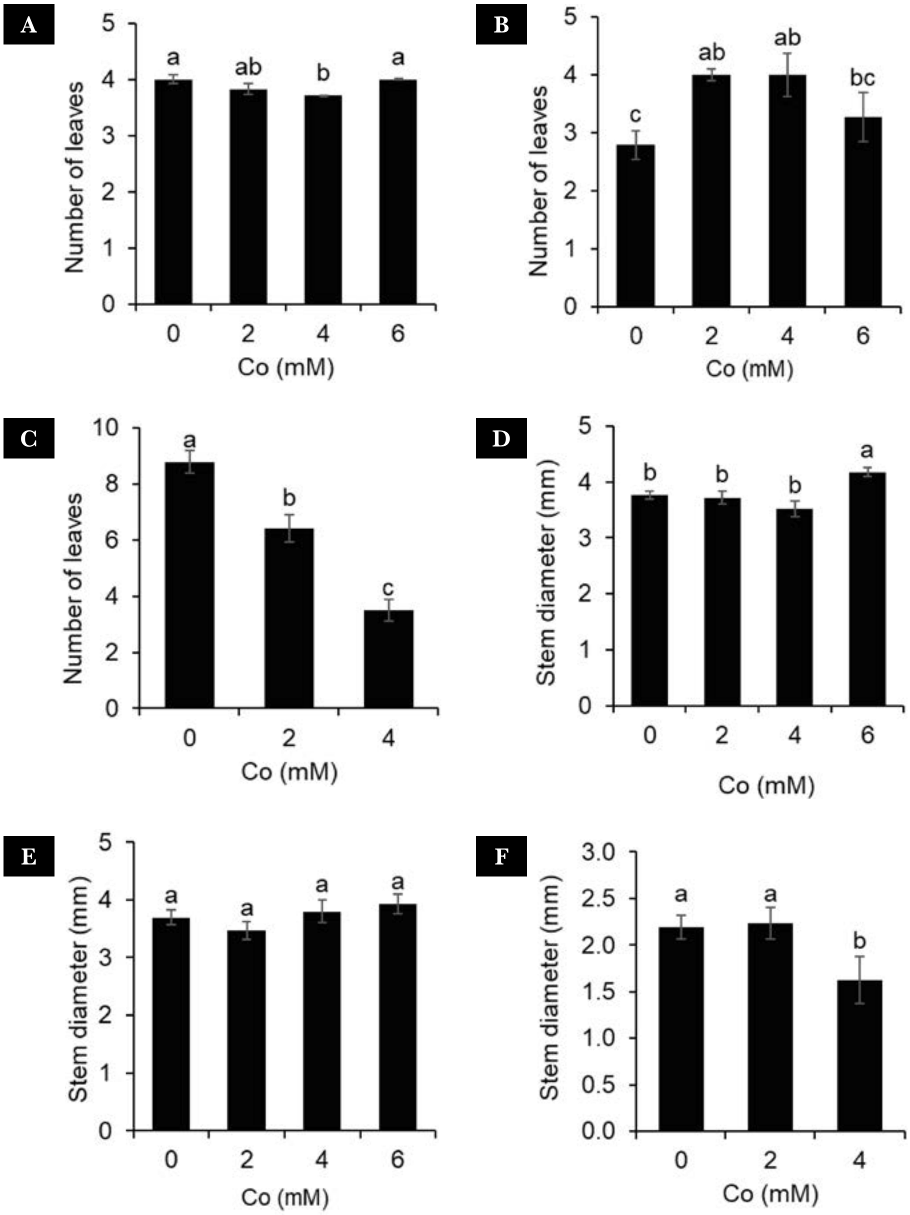

Figure 2. Number of leaves and stem diameter in seedlings of maize (*Zea mays*) (A, D), bean (*Phaseolus vulgaris*) (B, E), and husk tomato (*Physalis ixocarpa*) (C, F) treated with 0, 2, 4, and 6 mM Co. Values are means ± SE. Different letters within each subfigure indicate statistically significant differences between treatments (Tukey, $p \leq 0.05$).

Since roots are in direct contact with cobalt, they tend to accumulate the highest concentrations of this metal, often resulting in severe damage to this organ (Ulhassan *et al.*, 2023). Consistently, the present study observed complete inhibition of root number and root volume in husk tomato seedlings. In maize seedlings, the application of 6 mM Co significantly reduced both variables (Figures 3A, C). In bean, 2, 4, and 6 mM Co significantly decreased the number of roots (Figure 3B), while 4 and 6 mM Co reduced root volume (Figure 3D). Morphological differences between monocot and dicot plants may lead to differential responses to cobalt toxicity. Root exudation, polysaccharide deposition,

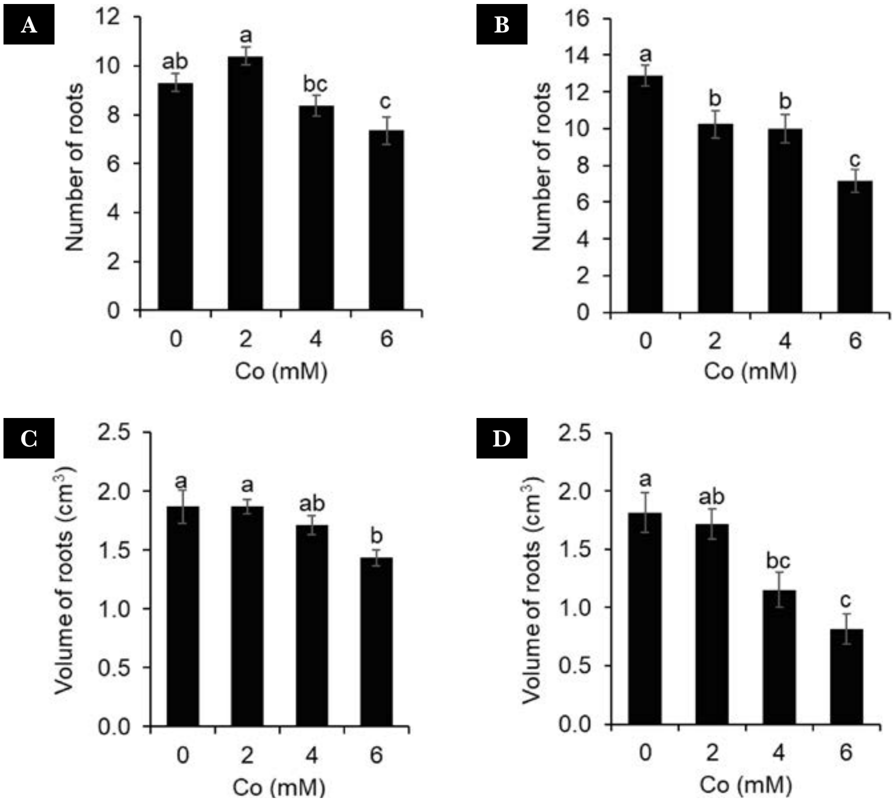

Figure 3. Number and volume of roots in maize (*Zea mays*) (A, C) and bean (*Phaseolus vulgaris*) (B, D) seedlings treated with 0, 2, 4, and 6 mM Co. Values are means ± SE. Different letters within each subfigure indicate statistically significant differences between treatments (Tukey, $p \leq 0.05$).

and thickening of the cell wall are among the tolerance strategies plants may employ under Co-induced stress (Jan & Parray, 2016).

Root length in maize seedlings was not affected by Co application (Figure 4A). However, this variable decreased significantly in both bean (Figure 4B) and husk tomato (Figure 4C). This reduction is attributed to cobalt accumulation in the roots, which disrupts cell division and elongation processes (Ali *et al.*, 2018).

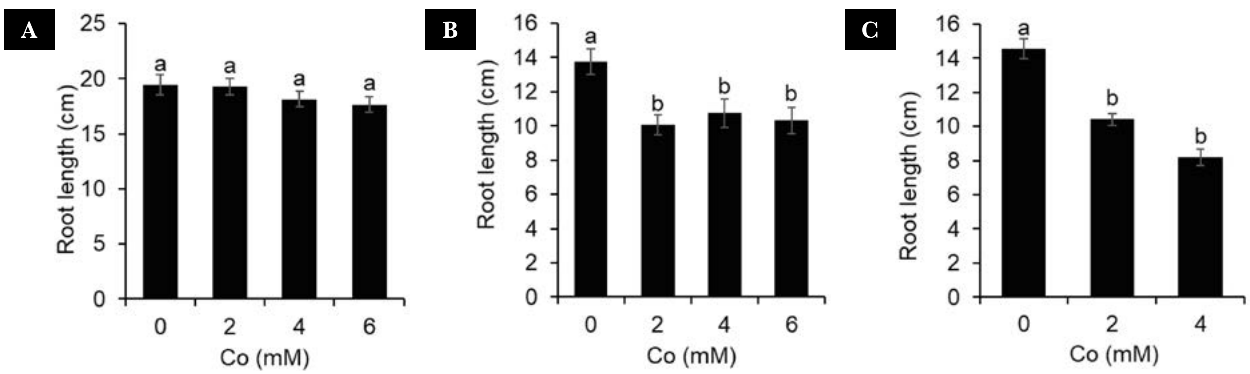

Figure 4. Root length of maize (*Zea mays*) (A), bean (*Phaseolus vulgaris*) (B), and husk tomato (*Physalis ixocarpa*) (C) seedlings treated with 0, 2, 4, and 6 mM Co. Values are means ± SE. Different letters within each subfigure indicate statistically significant differences between treatments (Tukey, $p \leq 0.05$).

Application of 6 mM Co significantly reduced the fresh shoot biomass of maize (Figure 5A) and bean seedlings (Figure 5B). In husk tomato (Figure 5C), reductions were observed starting from the lowest concentration tested, indicating a higher sensitivity of this species to cobalt. A similar pattern was observed for root biomass: in maize, only the 6 mM dose caused a significant reduction (Figure 5D), whereas in bean (Figure 5E) and husk tomato (Figure 5F), all cobalt concentrations had detrimental effects. These biomass losses

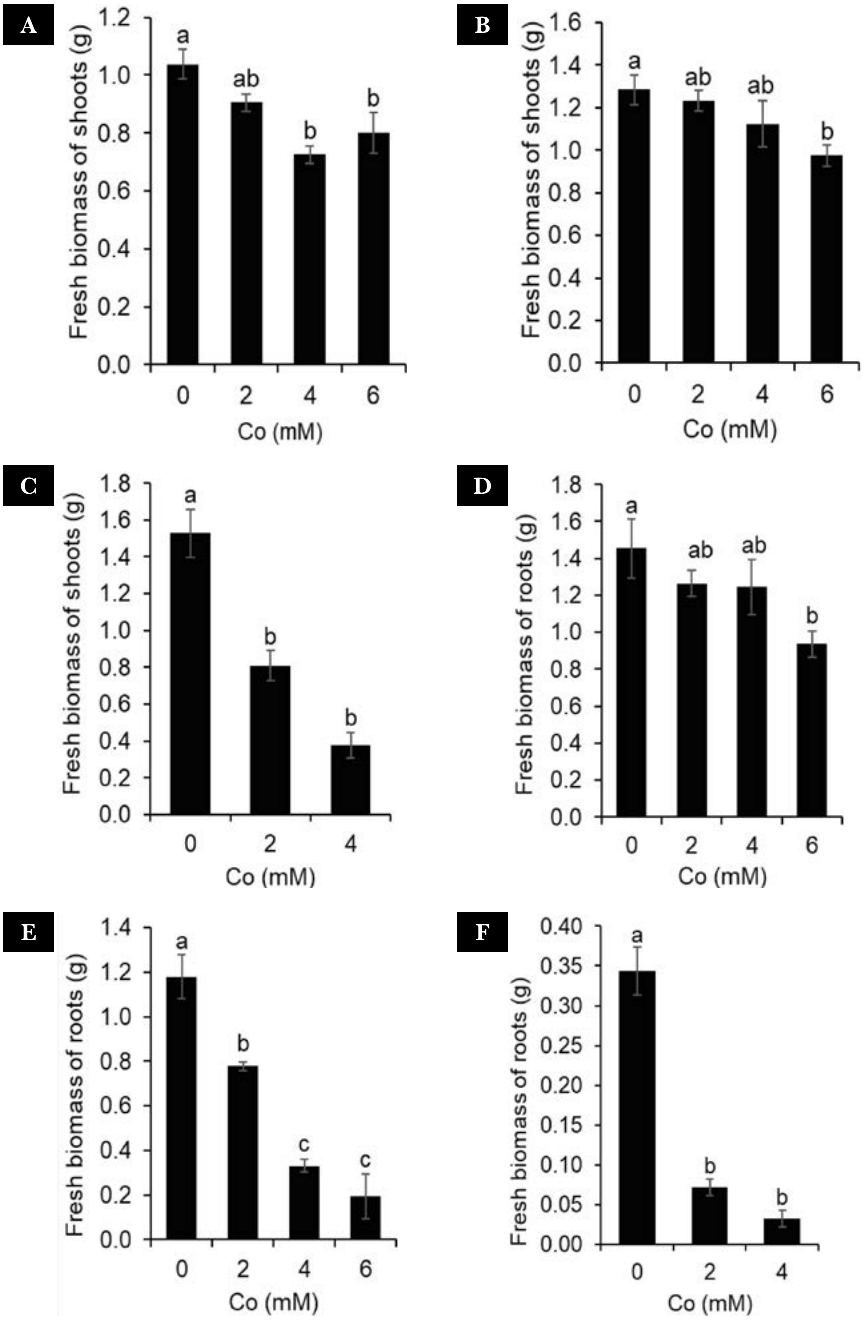

Figure 5. Fresh biomass of shoots and roots of maize (*Zea mays*) (A, D), bean (*Phaseolus vulgaris*) (B, E), and husk tomato (*Physalis ixocarpa*) (C, F) seedlings treated with 0, 2, 4, and 6 mM Co. Values are means ± SE. Different letters within each subfigure indicate statistically significant differences between treatments (Tukey, $p \leq 0.05$).

are attributed to cobalt's interference with water uptake and transport, its inhibition of transpiration, and the reduction in stomatal conductance (Ali *et al*., 2018).

CONCLUSIONS

In this study, cobalt application induced a negative response in the development of all three evaluated plant species, as evidenced by reductions in both shoot and root growth. The magnitude of this effect was dependent on the Co concentration applied. Sensitivity to cobalt varied among species, with maize seedlings exhibiting the highest tolerance, followed by bean and husk tomato. Further research is necessary to investigate the effects of cobalt across different botanical families, focusing on tolerance mechanisms and evaluating the safety of Co exposure in crops intended for human consumption.

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