Biological and Clinical Sciences Research Journal
eISSN: 2708-2261; pISSN: 2958-4728
www.bcsrj.com
DOI: https://doi.org/10.54112/bcsrj.v2024i1.1444
*Biol. Clin. Sci. Res. J., Volume, **2024**: 1444*
Original Research Article

EFFECT OF ASCORBIC ACID IN MITIGATING COPPER STRESS IN PEA (*PISUM SATIVUM* L.)

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(Received, 17th September 2024, Revised 9th December 2024, Published 18th December 2024)

***Abstract** This study investigated the impact of excessive copper concentrations and varying ascorbic acid levels on the growth and yield of pea seedlings (Pisum sativum L.). Pea plants were subjected to 50 µM copper stress and subsequent foliar spray of control (distilled water), 30 mM, and 60 mM ascorbic acid were applied. 'Orion' pea seeds were sown in pots filled with sand. Hoagland's solution was provided as a nutrient source. After 35 days, 50 µM copper sulfate was applied to the soil as a basal dose. A week later, ascorbic acid at 0, 30, and 60 mM concentrations was applied as a foliar spray. The experiment was conducted in a completely randomized design with six treatments and three replications. Results showed that ascorbic acid significantly improved plant growth as evidenced by increased fresh and dry weight, shoot length, leaf area, and chlorophyll content. The combination of 60 mM ascorbic acid with 50 µM copper resulted in the highest values for most growth parameters, including yield components such as the number of pods and seeds. These findings suggested that ascorbic acid can effectively mitigate the negative effects of copper stress on pea plants, promoting growth, enhancing photosynthetic efficiency, and ultimately leading to increased yield. Copper, an essential micronutrient, can become toxic at high concentrations, while ascorbic acid plays a crucial role in modulating plant growth and stress tolerance.*

Keywords: *Nutrient toxicity; Essential Micronutrient; Legume; Abiotic stress; Vitamin C*

Introduction

Pea (*Psium sativum*) is an annual and cool-season crop that belongs to the family Fabaceae/ Leguminoseae. The plant is a short-lived herbaceous annual with a climbing nature using leaflet modification as tendrils. Sowing time starts in plains of Pakistan from mid-October to November and its flowering normally starts at 45 days after sowing. Copper plays an important role in plant growth, yield, and development (Sresty and Madhava, 1999). It is a micronutrient, that facilitates the plant's respiration, photosynthesis and is important for its metabolism (Wolukau, 2004). Excess of copper has a detrimental effect on plant growth and morphology (Tuna, 2002). Copper from natural resources is one of the major heavy metals. Copper ion toxicity in plants influences cell division. It negatively affects electron transport in the respiration process and indirectly affects plant growth by reducing enzyme activity (Larcher, 1995). Copper tends to accumulate in the root tissue with little translocated to the shoot. Copper toxicity damaged plant roots firstly with symptoms ranging from disruption of the root cuticle and reduced root hair. Copper toxicity is most intensive on mature leaves rather than on younger leaves and is not accompanied by leaf blades. Plants uptake copper by roots and then move it toward the leaf area through the stem. Copper serves to intensify the flavor of vegetables and the color of flowers (Tuna, 2002). Regarding the mobility of different metals in plants, the root system accumulates them more efficiently than the other parts of plants. Most roots are affected by heavy metal toxicity (Abbas et al., 2024ab; Arshad et al., 2024; Ramask et al., 2002).

Ascorbic acid is a major source of vitamin C. It plays a role in photosynthesis as an enzyme cofactor including the synthesis of ethylene and gibberellins and in control of cell growth (Misako, 2014). Ascorbic acid is also an important co-factor in the synthesis of many plant hormones, including ethylene and abscisic acid (ABA) in plants (Mohamed et al., 2011; Haroon et al., 2024; Junaid and Gokce, 2024; Rehman et al.,2024; Sami et al., 2023;). It also activates the enzymatic reactions

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(Kefeli, 1981) and overcomes the effect of salt stress, and protection of photosynthetic pigment from oxidative damage (Choudhary et al., 1993). It enhances the nutritional value and stress tolerance in plants (Wilson et al., 2012). It regulates the redox state of photosynthetic electron carriers and as a cofactor violaxanthin. Thus enhancing their nutritional value and stress tolerance. It occurs in plants and plays a vital role in plant growth. It also activates enzymatic reactions (Kefeli, 1981) to overcome the effect of salt stress and protect photosynthetic pigment from oxidative damage (Choudhary et al., 1993). Ascorbic acid plays a key role in the metabolic process and regulates the biochemical process in plants (Neubauer and Yamamota, 1992) and acts as an antioxidant compound to associate with stress tolerance in plants (Lopez et al., 2001; Al-Mashhad et al., 2011). It protects plants against environmental stresses such as heavy metals and temperature etc (Shalata and Neumann, 2001). The ascorbic acid content of shoots and roots of pigeon pea showed a positive correlation with dry matter accumulation (Madhava et al., 2000). Keeping in view the importance of copper and ascorbic acid, a study was planned to investigate the impact of excessive concentrations of copper and various ascorbic acid concentrations on pea seedlings.

Materials and methods

This experiment was conducted in the nursery area of the Department of Horticulture, Faculty of Agricultural Sciences & Technology, University of Layyah. Ten seeds of peas (Orion variety) were sown in pots containing sand as a growing medium. Hoagland's solution was used as a nutrient solution, which was applied after fifteen days of seedling emergence. After 35 days of sowing, 50 µM copper (Cu) was applied as basal dose and after a week of basal dose of copper, ascorbic acid (AsA) (30 mM and 60 mM) was applied as foliar spray. Distilled water was applied in control pots as basal dose as well as foliar spray. There were six treatments, replicated thrice, which followed a completely randomized design. The details of the six treatments are outlined in Table 1.

Table: 1. Treatment details of nutrients applied to spinach

Treatment	Concentration
T_0(control)	Distilled water
T_1	30mM (AsA)
T_2	60mM (AsA)
T_3	50µM (Cu)
T_4	50µM (Cu) + 30mM (AsA)
T_5	50µM (Cu) + 60mM (AsA)

Morphological Parameters

Shoot length and root length (cm) were measured using a measuring scale. The fresh and dry weight (g) of pea plant was assessed using a weighing balance (PA-413, Chaus Corporation, USA). The number of leaves per plant was counted and averaged. Leaf area was measured with the help of a measuring scale by the following formula;

Leaf area (cm^2) = length of leaf (cm) × width of leaf (cm)

Nodes of five plants were counted from each pot and an average was taken. The distance between two nodes was measured using a measuring scale, thrice on three selected plants per pot, and the average was noted. The plants used for fresh weight measurement were dried in a dry oven (DGH-9053) at 72°C until the weight became constant. The average dry weight of plants was recorded. Chlorophyll contents were measured with the help of a chlorophyll meter from three leaves of three plants per pot and the average was calculated. The number of seeds per pot was counted from three pods from three plants per pod and averaged.

Results

The application of copper (Cu) and ascorbic acid (AsA) significantly affected the fresh weight of pea plants. Ascorbic acid stimulated the growth with maximum fresh weight observed when applied at the rate of 60 mM in combination with 50 µM Copper as 6.56 g, which is followed by 30 mM AsA applied with 50 µM copper (). AsA also improved fresh weight in the absence of copper. The interactive effect of ascorbic acid and copper was statistically non-significant in terms of dry weight. However, the individual effects of ascorbic acid and copper were significant. Maximum dry weight was observed with 60 mM ascorbic acid as () and 50 µM copper as () treatment also maintained more dry weight of pea seedlings. AsA has a significant impact on the shoot length of pea plants. A maximum shoot length of 26.2 cm was noted in pea plants which received 60mM AsA, followed by 30 mM AsA in the absence of copper. However, in the presence of copper, 60 mM AsA, gave a minimum shoot length as (), followed by 30 mM. The interactive effect of ascorbic acid and copper was statistically non-significant. However, the individual effects of ascorbic acid and copper were significant. Maximum root length was observed with 60 mM ascorbic acid and 50 µM copper treatment also maintained more root length of pea seedling.

AsA and copper had a significant interactive impact on the leaf area of pea plant. Maximum leaf area was 2.66cm noted in pea plant which received 30mM AsA in the presence of copper, followed by 60mM AsA. In the absence of copper the response of 30mM AsA and distilled water, for the leaf area was at par.

The interactive effect of ascorbic acid and copper was statistically non-significant. However, the individual effect of ascorbic acid was significant. And the effect of copper was non-significant.

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Maximum chlorophyll contents were observed with 60 mM ascorbic acid in chlorophyll contents of pea seedlings.

The maximum number of pods per plant was noticed when the plant was sprayed with the 60mM AsA in the presence of copper. The response of 60mM AsA and 30mM AsA was at par. The response of 0, 30, and 60mM AsA was found to be minimal in the absence of copper regarding the number of pods per plant. The maximum number of seeds per pod per plant was 9.83 noticed when pea plants were sprayed with 60mM AsA in the presence of copper. The response of 30mM AsA and distilled water was at par in the absence of copper regarding the number of seeds per pod per plant. The interactive effect of ascorbic acid and copper was statistically non-significant. However, the individual effect of ascorbic acid and copper were significant. Maximum inter nodal distance was observed with 60 mM ascorbic acid and 50 µM copper treatment also maintained more inter nodal distance of pea seedling.

Discussion

The present study investigated the interactive effects of copper and ascorbic acid on the growth and yield attributes of pea seedlings. Results demonstrated that both copper and ascorbic acid significantly influenced various growth parameters Ascorbic acid, applied at 60 mM, consistently promoted growth, leading to increased fresh and dry weight, shoot length, and leaf area. This aligns with previous findings highlighting ascorbic acid's role in cell expansion and division (Smirnoff, 2000). Its antioxidant properties likely protect plant cells from oxidative stress, enhancing overall growth and development. Higher ascorbic acid levels (60 mM) significantly increased the number of pods and seeds per plant, indicating its positive influence on reproductive growth. This could be attributed to its role in nutrient absorption and efficient utilization within plant cells. Its application generally stimulated growth, likely due to its involvement in vital metabolic processes such as photosynthesis and respiration (Wolukau, 2004). Copper application, in combination with ascorbic acid, significantly increased the number of pods and seeds per plant, suggesting a synergistic effect on reproductive yield. While some interactions between copper and ascorbic acid were observed, their overall interactive effects were generally not significant. This suggests that the primary effects of copper and ascorbic acid on pea growth were largely independent. as observed in reduced shoot length and leaf area in some treatments, which aligns with previous studies. Copper can interfere with essential physiological processes, such as electron transport in respiration (Larcher, 1995) and nutrient movement within cells (Meharg, 1994), leading to growth inhibition. Both ascorbic acid and copper significantly increased fresh and dry weight. The combination of 60 mM AsA with 50 µM Cu resulted in the highest values. Ascorbic acid alone significantly increased shoot length. However, in the presence of copper, shoot length was reduced, possibly due to copper toxicity. Ascorbic acid significantly increased chlorophyll content, indicating improved photosynthetic efficiency. This could be due to ascorbic acid's antioxidant properties, which may help to scavenge reactive oxygen species generated by excess copper. Copper, as an essential micronutrient, stimulated plant growth at the applied concentration. However, excessive copper can lead to toxicity, disrupting various physiological processes such as photosynthesis and nutrient uptake. The interaction between copper and ascorbic acid was significant for several parameters, indicating that ascorbic acid can mitigate the negative effects of copper toxicity. This could be due to ascorbic acid's antioxidant properties, which may help to scavenge reactive oxygen species generated by excess copper.

Copper is a micronutrient in plants, which facilitates respiration, photosynthesis and is important for plant metabolism (Wolukau, 2004). Its availability to the pea plants stimulated the growth, which may be attributed to its essential role in plant metabolism. Ascorbic acid has an important role in plant antioxidant system (Foyer and Kunret, 1996), photosynthesis, electron transport (Horemans et al., 1994), and possibly cell expansion (Smirnof, 2000). Application of 30 and 60 mM AsA improved the growth rate of peas when applied with copper.

Conclusion

The study demonstrates that ascorbic acid can effectively mitigate the negative impacts of copper toxicity on pea growth and yield. Furthermore, the combined application of copper and ascorbic acid can significantly enhance various growth parameters and yield attributes. These findings provide valuable insights into optimizing pea production by manipulating nutrient application strategies. Ascorbic acid application, particularly at 60 mM, significantly improved plant growth, yield, and yield components. The combination of 60 mM AsA with 50 µM Cu resulted in the most favorable growth and yield parameters. These findings suggest that foliar application of ascorbic acid can be a promising strategy to enhance pea productivity under conditions of moderate copper stress.

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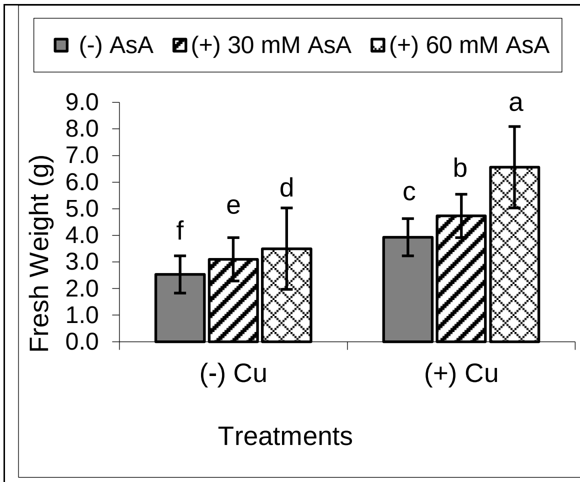

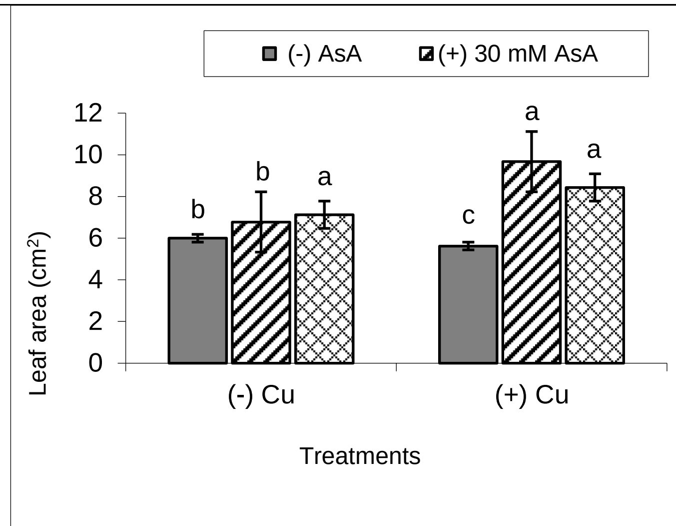

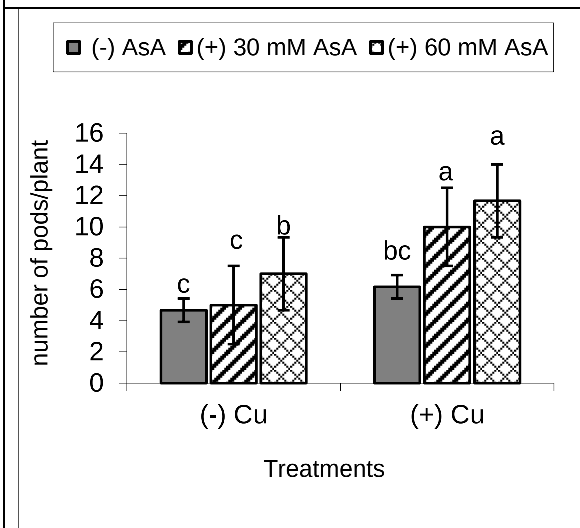

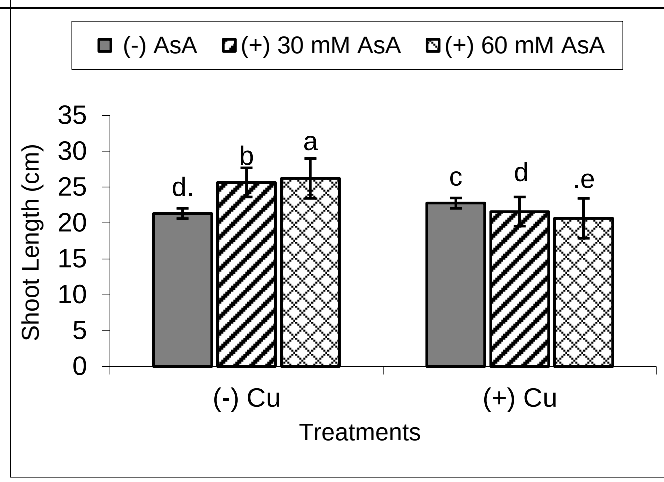

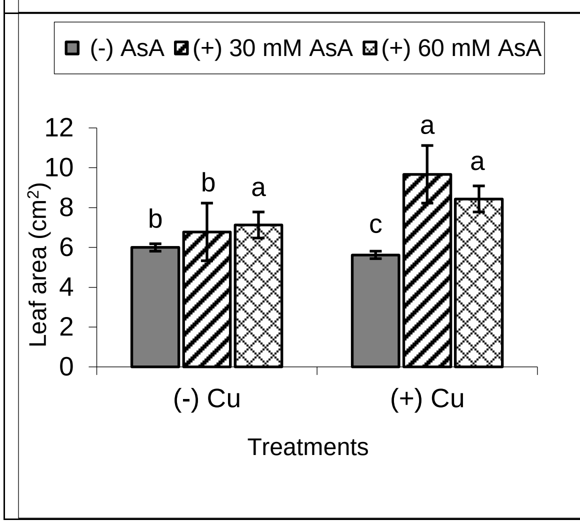

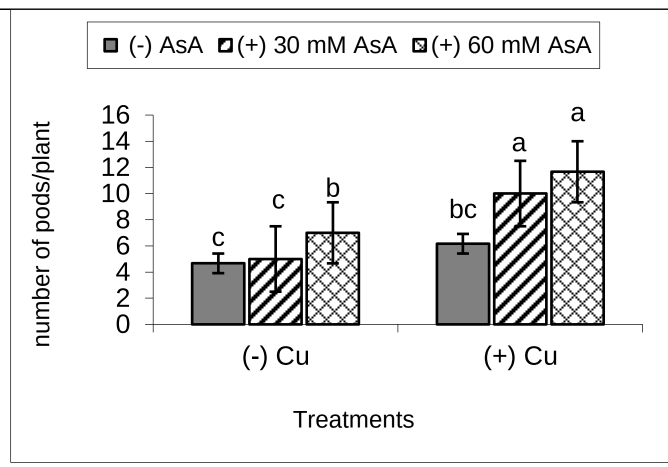

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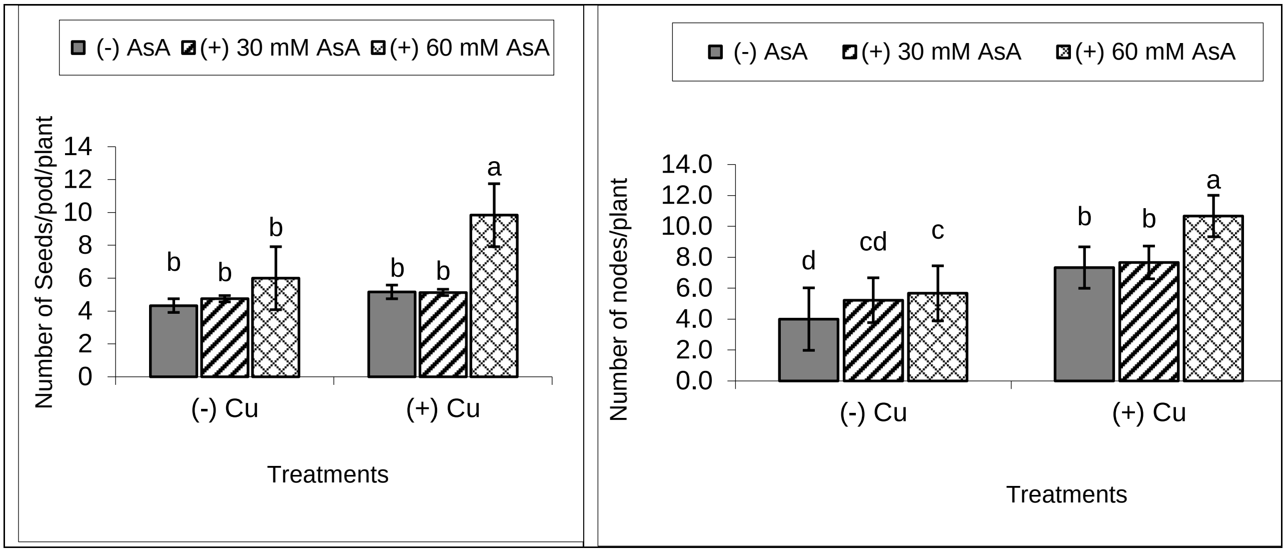

Fig. 1. Effect of ascorbic acid in mitigation of copper stress with respect to fresh weight, leaf area, number of pods/plant, shoot length of pea plants

Declaration

Acknowledgement
Not applicable

Ethics Approval and Consent to Participate
Not applicable.

Consent for Publication
The study was approved by authors.

Funding Statement
Not applicable

Authors' Contribution
All authors contributed equally.

Conflict of interest
There is no conflict of interest among the authors of the manuscript.

[Citation: Abbas, T., Tasadaq, M., Ahmad, W., Younas, H.S., Tariq, U., Irfan, M., Altaf, K. (2024). Effect of Ascorbic Acid in Mitigating Copper Stress in Pea (*Pisum sativum* L.). *Biol. Clin. Sci. Res. J.,* **2024***: 1444*. doi: https://doi.org/10.54112/bcsrj.v2024i1.1444]