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Original Research

Improving Morphological Attributes in *Petunia hybrida* Through Foliar Application of Copper Nanoparticles under Salinity Stress

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ABSTRACT

Salinity stress poses a major challenge to global agriculture, adversely impacting plant growth, yield and quality. *Petunia hybrida* is a widely grown ornamental plant in urban areas due to its attractive flower colours. Salinity is a major limiting factor for its growth and development as it adversely affects its morphological and physiological attributes. The current study was conducted to assess the mitigation effect of copper nanoparticles (CuNPs) on salinity stress in *Petunia hybrida*. A pot experiment included two levels of salinity (control and 100 mM NaCl) and two levels of CuNPs (10 mg L^{-1} and 20 mg L^{-1}). Morphological parameters such as plant surface area, leaf area, root length, shoot length and biomass were measured. The results depicted that the application of CuNPs not only alleviated salinity stress but also enhanced plant growth and biomass. Plants treated with 20 mg L^{-1} CuNPs under saline conditions exhibited the highest surface area (624.33 cm^2), shoot length (21.67 cm), and fresh biomass (46.97 g). Furthermore, CuNPs improved dry biomass and leaf area under both saline and fit soil conditions. The findings highlight the efficacy of CuNPs as a sustainable approach to enhance salinity stress tolerance in petunia, with promising implications for growing ornamental plants and broader agricultural applications.

Keywords: *Petunia hybrida, salt stress, copper nanoparticles (CuNPs), nanotechnology.*

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INTRODUCTION

Petunia hybrida, a member of the Solanaceae family with around 30 subspecies, is renowned for its annual and perennial herbaceous plants (Geitmann, 2009). Among these, *Petunia hybrida*, a hybrid of *Petunia axillaris* and *Petunia integrifolia*, commonly known as the garden Petunia, is widely recognized (Gerats and Vandenbussche, 2005). *Petunia hybrida* is cultivated annually from seeds for outdoor ornamental purposes. Furthermore, *Petunia hybrida* serves as a model for exploring flavonoid biosynthesis, botanical improvement, and self-incompatibility, contributing significantly to advancements in these areas (Tamari et al., 2013). Petunia plants are found on all continents, often alongside other annual species, boasting a wide array of colours and an extended flowering period, spanning about 6 months. They exhibit profuse blooming, even thriving in hot summers and producing new varieties in seasons with high humidity (Bala, 2012). It is an adaptable annual ornamental that can be used in numerous ways, i.e., including borders, hanging baskets, containers, colour masses and groundcover during specific seasons.

Salinity is a global problem for farmers. Around 2050, the anticipated loss of cultivable land due to increased salinity is projected to reach up to 50% (Wang et al., 2003). Salinity is caused by a variety of ionic species, including NaCl, Na_2SO_4, $MgSO_4$, $MgCl_2$, KCl, and Na_2CO_3. NaCl is the most prevalent and impactful because it dissociates into Na^+ and Cl^- ions (Munns and Tester, 2008). These salts alter osmotic and/or ionic conditions at the cellular level, disrupting plant nutrition and influencing growth and performance (Yang and Guo, 2018). The plant response to salt stress is complex, involving morphological, physiological, and metabolic changes (Neves et al., 2010). Soil salinization reduces plant growth, yields and product quality (Yang and Guo, 2017). Over 950 million hectares of land are affected by salinity stress and the incidence of soil salinization is increasing (Yang and Guo, 2018).

In modern agriculture, various methods are employed to enhance crop quality, yield, and growth, but most crucially, to strengthen a plant's capacity to withstand environmental stressors. Nanotechnology is a burgeoning scientific domain focused on altering substances at the atomic or molecular level and is used in many industries, including manufacturing,

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pharmaceuticals and agriculture (Singh et al., 2014). It encompasses the creation of materials on a nanoscale through the manipulation of atoms and molecules (Khan et al., 2019). Copper-based nanoparticles, including copper oxide and copper nanoparticles, are one of the five most commonly used nanoparticles (Ma et al., 2014). The widespread use of copper nanomaterials is attributed to their abundance, accessibility, and cost-effectiveness compared to gold and silver. Consequently, diverse physical and chemical methods are employed for large-scale production of copper nanoparticles. The "Green Synthesis" method for nanoparticle synthesis is characterized by its simplicity, cost-effectiveness and reproducibility, yielding stable products. Notably, it avoids the need for high energy, pressure, elevated temperatures, or toxic chemicals. In the bottom-up approach of green synthesis, similar to the chemical reduction of nanoparticles, the distinguishing factor is the substitution of chemical-reducing agents with extracts from plants, fruits, flowers and alga (Pal et al., 2019).

Petunia hybrida, a popular winter annual ornamental plant in Pakistan, suffers from abiotic stressors, particularly salinity, which has a negative impact on growth, yield, and vase life. Nanotechnology, specifically nanoparticles (NPs), has emerged as a potential management strategy for these issues (Torabian et al., 2018). Carbon nanotubes, fullerene, SiO_2, TiO_2, CeO_2, Al_2O_3, Ag, ZnO, Fe_3O_4/Fe_2O_3, and CuO are among the NPs that have been extensively studied and shown to modulate reactive oxygen species (ROS) and likely reactive nitrogen species (RNS) (Prasad et al., 2017). Ranjbari et al. (2020) studied the effects of zinc nanoparticles and zinc sulphate on *Petunia hybrida* under various moisture conditions and discovered that zinc nanoparticles were more effective at promoting growth and flowering than zinc sulphate. Similarly, Al-Dulaimy et al. (2022) investigated the effects of spraying regular and nano iron on *Petunia hybrida* L., finding improvements in both vegetative and flowering aspects. Copper nanoparticles (CuNPs) have gained popularity due to their remarkable ability to combat a variety of bacterial and fungal strains. With previous research highlighting the positive effects of nanoparticle use, especially CuNPs, it's reasonable to expect that applying CuNPs to plant foliage could enhance growth, flowering, and resilience to salt stress. Therefore, this study aims to investigate how petunia plants respond to salt stress when treated with CuNPs.

MATERIALS AND METHODS

The experiment was conducted in the Botanical Garden of Govt. Sadiq College Women University in Bahawalpur during the winter. Petunia seedlings of uniform size and health were purchased from a local ornamental plant nursery in Bahawalpur. The selected cultivar was "Ultra Red Star," known for its striking red blooms. These seedlings were transplanted into individual pots filled with nutrient-rich potting soil. The pots were chosen to provide adequate space for root development, only one seedling was transplanted in each pot (Fig. 1).

Petunia seedlings were acclimatized for one week to minimize transplant shock and ensure uniform conditions. The experiment was divided into two groups of plants, i.e., without salinity and 100 mM NaCl salinity; after transplanting two concentrations of Cu Nanoparticles (10 mg L^{-1} and 20 mg L^{-1}) were applied to both groups of petunia plants grown in pot. Plants were carefully, harvested and were placed inside labelled paper bags and brought to the laboratory for further measurements of growth parameters. In the laboratory, the following parameters were recorded i.e.

Plant surface area

Plant surface area was measured in cm^2 at the time of harvesting with the help of a measuring scale.

Leaf surface area

Leaf area was measured in cm^2 by multiplying the length and width of the leaf. Leaf area was measured from the leaf of each replication of different treatments randomly following the method of Zhang and Brandle (1997) and applying the following formula:

$$\text{Leaf area} = \text{leaf length} \times \text{leaf width} \times 0.66$$

Root length and shoot length

The length of the roots was measured in cm after the plants were harvested from pots. The measurements were made using a measuring scale.

Figure 1: Transplanting of *Petunia hybrida* (a). *Petunia hybrida* plants in pots (b).

Fresh biomass and dry biomass

When the plants were harvested, their fresh biomass, including their roots, was extracted. For the calculation of dry biomass, plants were dried by putting them on Petri dishes and placing them inside the electrothermal incubator at 60°C for one hour. Fresh and dry biomass was calculated in g with the help of an electronic analytical balance.

RESULTS

Numerous morphological parameters showed different outcomes when *Petunia hybrida* seedlings were treated with varying concentrations of copper nanoparticles in saline and non-saline environments. Measurements of all the morphological parameters were taken at the time of harvesting when *Petunia hybrida* plants were at the flowering stage.

Effect of CuO NPs on plant surface area and leaf area

Figure 2a shows the mean comparison of the plant surface area of *Petunia hybrida* under different levels of salinity and copper nanoparticles. According to statistical analysis of plant surface area of *Petunia hybrida*, plants showed significant results when treated with different levels of copper nanoparticles in salinized and non-salinized environments. Maximum plant surface area (624.33 cm^2) was noticed in the plants treated with 20 mg L^{-1} copper nanoparticles under 100 mM concentration of NaCl stress with the mean value (433.67 cm^2) followed by plants treated with 20 mg L^{-1} copper nanoparticles under 0 mM concentration of NaCl stress with the mean value (413.33 cm^2) than in plants treated with 10 mg L^{-1} copper nanoparticles under 0 mM and 100 mM of NaCl stress with the mean value (397 cm^2) than control group with untreated plants (354.33 cm^2). The lowest results (173.33 cm^2) were shown in the plants treated without copper nanoparticles under 100 mM NaCl stress. Figure 2b shows the mean comparison of the leaf surface area of *Petunia hybrida* under different levels of salinity and CuNPs. According to the statistics, the leaf surface area of *Petunia hybrida* showed significant results when treated with different levels of copper nanoparticles under salinized and non-salinized conditions. Maximum leaf surface area (14.5 cm^2) was noted in the plants treated with 20 mg L^{-1} copper nanoparticles under 100 mM concentration of NaCl stress with the mean value (433.67 cm^2) followed by plants treated with 20 mg L^{-1} copper nanoparticles solely with the mean value (413.33 cm^2) than in plants treated with 10 mg L^{-1} copper nanoparticles solely and 100 mM of NaCl stress with the mean value (397 cm^2) than in control group with untreated plants with the mean value (354.33 cm^2). The lowest results (173.33 cm^2) were shown in the plants treated without copper nanoparticles under 100 mM NaCl stress.

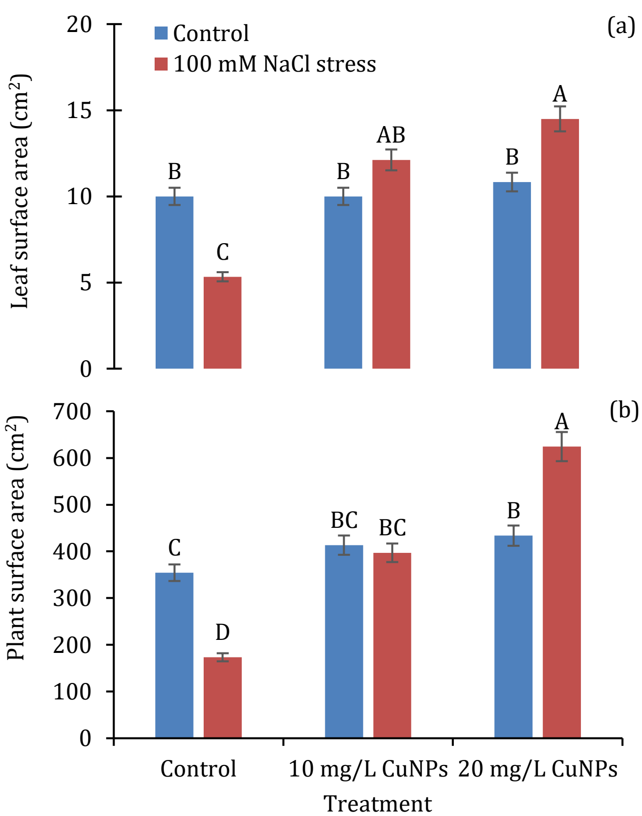

Figure 2: Impact of CuO NPs on the plant surface area of *Petunia hybrida* (a). Impact of CuO NPs on the leaf surface area of *Petunia hybrida* (b).

Effect of CuO NPs on root length and shoot length

Figure 3a shows the mean comparison of plant root length of *Petunia hybrida* under different levels of salinity and CuNPs. According to the statistical analysis, the plant root length of *Petunia hybrida* showed significant results when treated with different levels of copper nanoparticles under saline and non-saline conditions. Average plant root length was higher (5.67 cm) for the plants with 20 mg L^{-1} CuNPs with and without 100 mM salt stress, followed by control followed by the plants treated with 10 mg L^{-1} CuNPs solely with a mean value of 4.67 cm than in control group with untreated plants having a mean value of 4.33 cm than in plants treated with 10 mg L^{-1} CuNPs under 100 mM salt stress having a mean value of 3.83 cm. The average plant root length was the lowest (2.67 cm) for the plants treated with 100 mM salt stress, showing that salinity causes a reduction in plant root length. Figure 3b shows the mean comparison of the shoot length of *Petunia hybrida* under different levels of salinity and CuNPs. According to the statistical analysis, plant shoot length of *Petunia hybrida* showed significant results when treated with different levels of copper nanoparticles under saline and non-saline conditions. The average shoot length of *Petunia hybrida* was higher (21.67 cm) for the plants treated with 20 mg L^{-1} of CuNPs with 100 mM of salt stress, followed by the plants treated with 20 mg L^{-1} of CuNPs solely having a mean value of 17.23 cm than in the plants treated with 10 mg L^{-1} CuNPs with and without 100 mM of salt-stress having mean values of 16.67 cm and 17 cm, respectively. The average plant shoot length was lower for the control group, having a mean value of 15 cm and lowest for the plants treated with 100 mM of salinity stress, having a mean value of 11.33 cm.

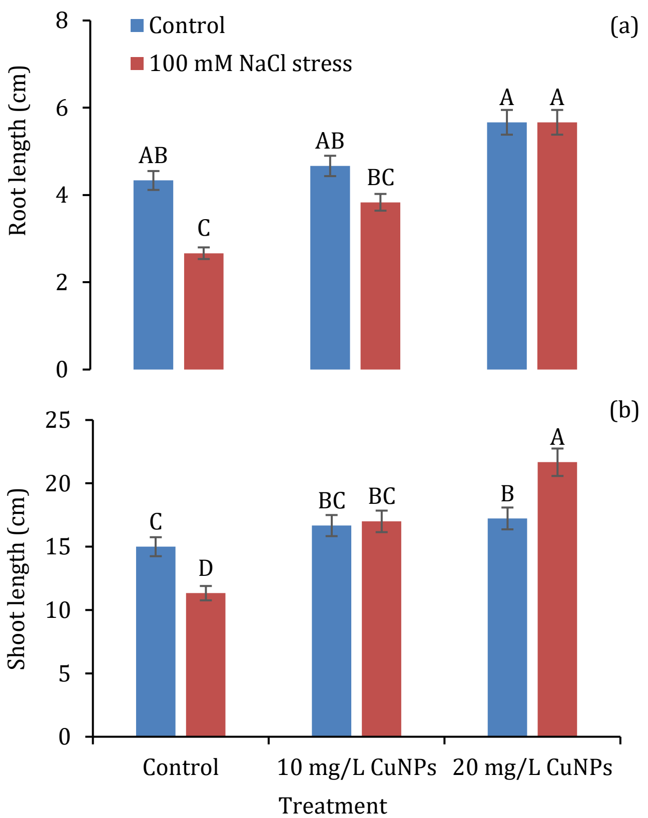

Figure 3: Impact of CuO NPs on the shoot length of *Petunia hybrida* (a). Impact of CuO NPs on root length of *Petunia hybrida* (b).

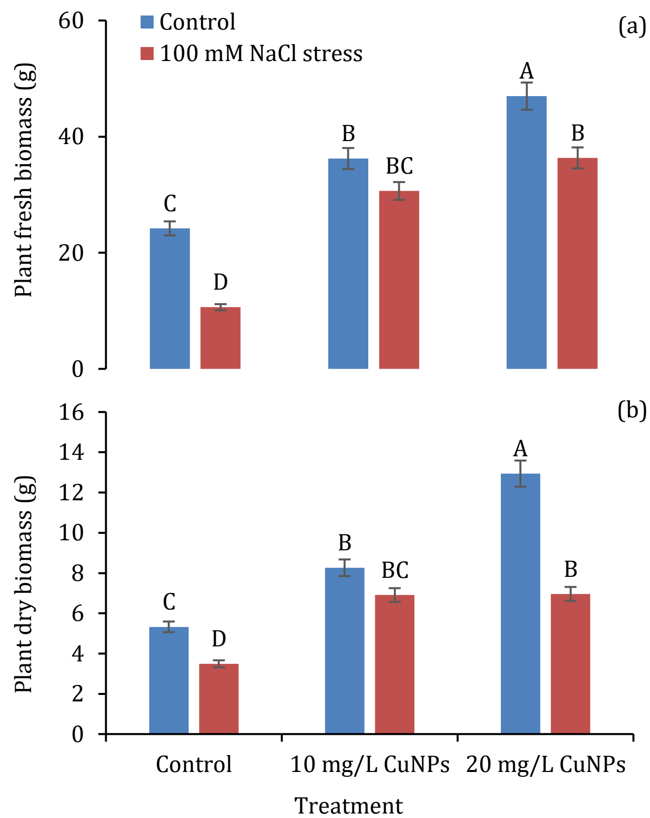

Figure 4: Impact of CuO NPs on plant fresh biomass of *Petunia hybrida* (a). Impact of CuO NPs *Petunia hybrida* on plant dry biomass (b).

Effect of CuO NPs plant fresh biomass and dry biomass

Figure 4a shows the mean comparison of plant fresh biomass of *Petunia hybrida* under different levels of salinity and copper nanoparticles. According to statistics, fresh biomass has a significant effect on plants. Plant fresh biomass was higher (46.97 g) in plants treated with 20 mg L^{-1} CuNPs followed by 10 mg L^{-1} CuNPs with a mean value of 36.328 g, than in the plants treated with 20 mg L^{-1} CuNPs with 100 mM of salt stress with the mean value of 36.328 g than in the plants treated with 10 mg L^{-1} CuNPs with 100 mM of salt stress with the mean value of 30.64 g than in the control group with untreated plants with the mean value (24.19 g). Plant fresh biomass was lowest for the plants with 100 mM salt stress with a mean value of 10.618 g.

Statistics show that there is an eloquent impact of copper nanoparticle treatment on plant dry biomass of plants (Fig. 4b). Average plant dry biomass was highest (12.94 g) in the plants treated with 20 mg L^{-1} CuNPs without salt stress with the mean value of 8.26 cm^2 followed by 20 mg L^{-1} CuNPs with salt stress and 10 mg L^{-1} CuNPs without stress with the mean values of 6.961 cm^2 and 6.905 cm^2, respectively than in plants treated with 10 mg L^{-1} CuNPs with salt stress with the mean value of 5.326 cm^2. Average dry plant biomass was lowest for the plants treated with 100 mM salt stress without applying CuNPs, with a mean value of 3.491 cm^2. This means that salinity reduces dry plant biomass and may change plants' metabolic processes.

DISCUSSION

Production of cut flowers has gained global significance, causing rapid changes in storage and marketing practices (Singh et al., 2010). In Pakistan, floriculture has historically been overlooked, with insufficient efforts from both the public and private sectors to educate farming communities about its importance (Manzoor et al., 2001). The purpose of this pot-based study was to determine how petunia plants respond to salt stress and how copper nanoparticles affect their ability to grow and reproduce.

Salinity poses a significant challenge to crop production, causing more than 50% of agricultural losses (Vijayakumar et al., 2018). This issue is deteriorating over time, threatening food security and sustainable crop yields (Guo et al., 2020). It puts plant survival in danger and severely reduces growth and yield in later stages (Ahenger et al., 2019). The study revealed that salt stress had detrimental effects on various traits of petunia plants. Osmotic stress and inadequate water intake by seedlings because of ion toxicity from sodium and chloride salts can cause reduced germination in salt-stressed circumstances (Hnilickova et al., 2019). Petunia plant growth parameters, including plant height, shoot length, and fresh weight, are drastically reduced by salt stress. But as compared to plants only experiencing salt stress, plants treated with CuNPs (10 mg L^{-1} and 20 mg L^{-1}) showed a notable improvement in growth. Osmotic stress can cause reduced development in salt stress circumstances, which hinders nutrient and water absorption and transport, leading to reduced cell turgidity and expansion, thereby hampering growth (Yadav, 2020).

In the present work, large leaf surface area increased fresh and dry biomass, and more leaves were produced in plants that were treated with 10 mg L^{-1} and 20 mg L^{-1} CuNPs. In contrast, plants that were present under 100 mM of salt stress showed fewer leaves with smaller leaf surface area and reduced fresh and dry biomass. The photosynthetic organ of green plants and leaves is called the plant's "food factory." For a plant to engage in photosynthetic activity, it needs more and healthier leaves. Poor leaf growth may have resulted from toxic conditions in the leaves brought on by the salt stress. Furthermore, plants' enzymatic activity may also be negatively impacted (Li et al., 2017), leading to the production of fewer leafy plants. These results are consistent with those of Mola et al. (2017), who found that irrigating lettuce plants with salt water had a significant impact on leaf area, number of leaves, and shoot biomass. Consistent with previous research on tomatoes (Tanveer et al., 2020), sorghum (Netondo et al., 2004), and other plants (Kataria et al., 2019), our study found a significant decrease in the leaf area of petunia with increasing salinity. This decrease in leaf size is correlated with a rise in leaf thickness, which can lead to higher chlorophyll content (Heidari et al., 2014). This aligns with our findings suggesting that reduced leaf area under NaCl stress conditions may result in enhanced chlorophyll content in plants.

The present study found that salinity stress significantly decreased plant surface area, leaf area, and shoot and root dry weight. These results are in agreement with those of Rita and Gherbin (2006), who showed that the Romsun HS90 sunflower cultivar under 150 mM salinity saw an 85% reduction in leaf area and a 77% decrease in total shoot dry weight. The dry weights of both shoots and roots decreased by 17% at 100 mM salt. Additionally, several current research studies have shown that salt stress reduces photosynthesis (Qiu et al., 2003). Research has indicated that using modest amounts of nanoparticles based on copper can improve the growth of plants. For example, in transgenic and traditional cotton cultivars, 10 mg L^{-1} CuO NPs enhanced root biomass (van et al., 2016). Similarly, it has been demonstrated that low concentrations of nano-copper dioxide particles enhance maize seedling growth (Adhikari et al., 2016).

CONCLUSION

This study highlights the potential of copper nanoparticles (CuNPs) in mitigating the adverse effects of salinity stress on *Petunia hybrida*. Applying CuNPs significantly improved plant growth, physiological performance, and overall stress tolerance. These findings underline the promising role of nanotechnology in enhancing plant resilience under abiotic stress conditions, offering sustainable solutions for agriculture and horticulture. Further research is recommended to explore the long-term implications and mechanisms of nanoparticle applications in different plant species and environmental conditions.

Declaration of competing interests

The authors declare no competing interests or personal relationships affecting the work reported in this article.

Author contribution statement

Sana Batool, **Iram Naz;** Project idea, data taking, analysis and write up, **Maryium;** Supervision and revision of the project and draft.

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