RESEARCH ARTICLE

Copper Oxide Nanoparticles Influenced Ionome, Reduced Growth, Altered Pigments and Stomatal Morphology in *Mentha arvensis* L.

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Abstract

Copper oxide nanoparticles (CuO NPs) are being enormously used in agriculture, aquaculture, textile, cosmetic, food and pharmaceutical industries. CuO NPs are reported to make their presence in soil water and air due to their release in the environment. *Mentha arvensis* L. (Lamiaceae) is commercially grown for essential oil across the world due to its medicinal value. The present study reports the responses of *M. arvensis* to CuO nanoparticles (0.0–5.0 µg mL^{-1}) after 18 d exposure. The build-up of high copper concentrations in *M. arvensis* plant tissues (roots and leaves) confirms the internalization and movement of CuO NPs in shoots. CuO NPs modified ionomes of roots and leaves of *M. arvensis*. Both roots and leaves of *M. arvensis* plants exposed to CuO NPs exhibit altered levels of micro (reduced Fe, Mn, and upregulated Zn and Mo contents) and macronutrients (decreased Ca, Mg and K levels). Further, CuO NPs decreased biomass, total chlorophyll, chlorophyll a, chlorophyll b and relative water contents and increased carotenoids and anthocyanin contents in *M. arvensis* leaves. Besides, CuO NPs caused alteration in stomatal morphology of test plant. The present study suggests sustainable use and environmental release of CuO NPs due to their possible bio-toxic consequences.

Keywords: CuO NPs; *Mentha arvensis*; Altered root and leaf ionome; Phytotoxic responses.

Highlights

- Internalization and upward movement of CuO NPs in *Mentha arvensis* L.
- Altered ionomes of roots and leaves
- Reduced growth, photosynthetic pigments and anthocyanin
- Distorted stomatal structure

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Introduction

The copper oxide nanoparticles (CuO NPs) are highly used metal oxide NPs in agri-environment (pesticide, fungicide, fertilizers), cosmetic, food, chemical, textile, electronic, aquaculture (antibacterial and antifouling agents) and paint industries, medical applications, wastewater remediation, and wood preservation worldwide (Wang *et al*., 2022; Rajput *et al*., 2018). The enormous application of CuO NPs in various sectors led to high environmental release of these nanoparticles (soil, fresh, marine waters and sediments) and their consequent adverse impacts on plants, microbes, aquatic animals and human health raise environmental safety issues globally (Gomes *et al*., 2012; Assadian *et al*., 2017; Wang *et al*., 2022). Several studies report the internalization of CuO NPs in plants after foliar/root exposure and their translocation and accumulation in various plant tissues end in phytotoxicity of the CuO NPs (Wang *et al*., 2023). Plant ionome is a quantitative profile of the elemental composition of plants and alterations in the elemental composition after exposure to environmental/physiological stress or genetic variations (Salt *et al.,* 2008). Reduction in root growth after the adsorption/internalization of CuO NPs by plant root may reduce the availability of micro and macro nutrient elements to plants. A few studies have documented that the reduced nutrient acquisition altered the leaf and root ionome of plants exposed to CuO NPs (Tan *et al*., 2018; Ahmed *et al*., 2021; Dumont *et al*., 2022; Kumar, 2023). Lower availability of nutrients reduced the growth and yield of essential oil plants of commercial importance (Kumar *et al*., 2023). However, the influence of CuO NPs on plant ionomers were species, route of exposure and size of CuO NPs specific (Ahmed *et al*., 2021; Kumar, 2023).

Essential oils (EOs) have a global demand of 226.9 kilotons in 2018 due to high demand in the pharmaceutical, cosmetic, food and pesticide industries (Makkar *et al*., 2018; Market analysis report, 2022; Petruzzi, 2022). Further, it has been stated that the global demand of EOs is speculated to reach 404.2 kilotons (CAGR of 9.57%) in the year 2028.

Mentha arvensis L, a commercial essential oil-yielding crop cultivated globally due to medicinal and economic value (Kumar *et al*., 2020). Hence, in this study influence of CuO NPs on leaf and root ionome of *Mentha arvensis* and their consecutive impact on growth, photosynthetic pigments and stomatal morphology has been explored.

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Materials And Methods

Characterization of CuO NPs

CuO NPs (size: <50 nm, catalogue No. 544868) were procured from Sigma-Aldrich (USA). CuO NPs were suspended (40 µg mL^{-1}) in filtered milli- Q water (0.22 micron) and Hoagland growth medium (5%, v/v) by sonication (30% amplitude, 20 minutes, 25W) using a probe sonicator (Sonics Vibra Cell, Sonics & Material Inc., New Town, USA) for determination of hydrodynamic size and particle charge (zeta potential) using a Zeta sizer (Model ZEN 3600, Malvern Instruments Ltd., UK).

Raising Plants for CuO NPs Treatment

Plants of *Mentha arvensis* L. (var. Kosi) were grown from suckers (uniform healthy) obtained from CSIR-CIMAP, Lucknow, U. P., India. The suckers were double sterilized (Firstly suckers immersed in 70% ethanol for 3 min. and repeatedly washed with sterilized water prior to second sterilization; secondly suckers soaked in 5% H_2O_2 for 5 min and washed thrice with sterilized double distilled water prior to planting). *Mentha arvensis* suckers were planted in earthen pots containing sand (0.01 M HCl washed), irrigated with sterilized Milli-Q water and positioned for germination in dark. *Mentha arvensis* suckers after germination (5d) were transferred in the experimental field (normal light and dark conditions, temperature and humidity). Plants were regularly irrigated by 5% Hoagland solution (H.S.). After 16 days of growth in the experimental field, plants exhibiting stunted growth patterns were discarded. The plants (approximately the same height) were allowed to grow further for nine days. A 100 plants of *Mentha arvensis* (9.5 cm, approximate weight: 3.8 g) were acclimatized (5% Hoagland containing 100 ml conical flasks) for 6 d in a growth chamber (light intensity, 210 µM cm^{-2} $s^{-1;}$ day : night, 16:8 h and humidity: 65%) prior to CuO NPs treatment.

CuO NPs Exposure

CuO NPs (80 µg mL^{-1}) were suspended in growth medium (5% Hoagland solution) for a stock solution, sonicated (Vibra cell Sonics) for 20 minutes (45 sec on 15 sec off, amplitude 30*%).* The plants of *M. arvensis* (36 d old) were treated in triplicates (biologically independent) to environmentally pertinent levels of CuO NPs (0-5 µg ml^{-1}) for 18d in growth chamber (light intensity, 210 µM cm^{-2} s^{-1} ; day : night, 16:8 h and humidity: 65%). *M. arvensis* plants placed in 5% H.S. served as control. The nutrient solution was changed every 48 hours. After 18 days plants were harvested, washed with double distilled water, blotted to remove water and then leaves and roots were separated for the analysis of various parameters.

CuO NPs Internalization, Upward Movement into Shoot and its Influence on Leaf and Root Ionome

Internalization of CuO NPs and upward translocation was determined in terms of total copper contents of leaves and roots compared to untreated control. Further, the influence of internalized CuO NPs on leaf and root ionome was determined by measuring the levels of metallic nutrients (micronutrients: Cu, Zn, Fe, Mo and Mn and macronutrients: Ca, Mg and K) in leaves and roots of CuO NPs (0.0-5µg ml^{-1}) treated *M. arvensis* plants. Briefly, *M. arvensis* plants treated by CuO NPs were harvested after 18d, repeatedly washed by Milli-Q water, roots of harvested repeatedly washed plants were placed in 0.01 N HCl (Gardea – Torresday *et al.*, 2004) for 6 seconds and washed five times with Milli-Q water to removed CuO NPs stuck to surface of roots. Roots and leaves were separated and dried in oven (70 ± 2°C for one week). CuO NPs (0.0–5 µg mL^{-1}) exposed roots/leaves (0.5 g) were placed in HNO_3: H_2O_2 (3:1, v/v) for microwave acid digestion (Mars 6^{TM}, CEM Corporation, Mathews, NC, US). The volume of the digested samples was increased to 50 mL by adding Milli-Q water and total copper content, micronutrients (Cu, Zn, Fe, Mo and Mn) and macronutrients (Ca, Mg and K) in roots and leaves were determined by Inductively Coupled Plasma Mass Spectrometry (ICP-MS 7700 series, Agilent, U.S) and expressed as µg g^{-1} DW (micronutrients) or mg g^{-1} DW (macronutrients).

Biomass and Pigments

The influence of CuO NPs (0.0-5.0 µg ml^{-1}) on biomass of *M. arvensis* plants was estimated by measuring biomass (g fresh weight basis) of complete plants, shoots and roots. Photosyntheic pigments (total chlorophyll, chl a, chl b) and carotenoid levels after 18d were determined in fresh leaves (0-0.5 µg ml^{-1}) according to Arnon (1949) and Duxbury and Yenstch (1956), respectively. Anthocyanin contents were determined as per method narrated by Nakata *et al.,* (2014). The input from chlorophyll and its degraded substances to OD_{530} was adjusted (Laby *et al.*, 2000). The anthocyanin levels in leaves of CuO NPs (0–5 µg mL^{-1}) treated *M. arvensis* plants has been reported as Absorbance 530nm/g FW.

Scanning Electron Microscopy (SEM) for Stomatal Morphology

The changes in stomatal morphology of *M. arvensis* leaves were determined using SEM in CuO NPs (0.0-5.0 µg ml^{-1}) exposed plants. Briefly, leaf sections (4x4 mm) leaves of *M. arvensis* plants grown in CuO NPs (0.0–5.0 µg mL^{-1}) containing H.S. were fixed (2.5% glutaraldehyde in 0.1 M sodium phosphate buffer, pH 7.2) for 12 hours at 4°C with repeated stirring, washed five times by sodium phosphate buffer, dehydrated by passing a graded series of acetone (10, 30, 40, 50, 60, 70, 90%, once for 10 minutes at each dehydration step and twice for 30 min at 100%). Leaf sections were then dried using a critical point dryer. Dehydrated leaf sections were then mounted on SEM stub using carbon tape (two-sided) for imaging through SEM (JEOL, Japan; model JSM-6490LV) at an accelerating voltage of 10 kV.

Statistical Analysis

The statistical analyses applied in this study are adopted from Gomez and Gomez (1984). The experiments were executed in CRB (complete randomized block) design. One-way ANOVA (analysis of variance) was performed independently for all the parameters assayed in different plant tissue (leaves/ roots) to determine the variability and validity of observed data sets. Duncan's multiple range test (DMRT) was done to explore the significant difference between treatments given to each tissue.

Results And Discussion

Characterization of CuO NPs

The CuO NPs used in the current study exhibit <50 nm TEM size, according to the supplier (Sigma Aldrich). However, The TEM size measured by us in this study was 10.2 to 43.5 nm (Table 1).

Table 1: Characterization of CuO NPs by hydrodynamic light scattering and transmission electron microscopy.

Hydrodynamic light scattering				*TEM size*
Suspension medium	Average hydrodynamic Size	PDI	ζ (zeta) potential (mV))	10.2–43.5 nm*
Milli-Q Water	154±7	0.139 ± .006	-35.6 ± 2	
5% Hoagland Solution	288 ±13	0.261 ± 0.011	-33.8 ± 1.4	

Value are Mean (n = 10) ±SD'* several small sized particles (> 6.7 nm) were also present

However, the presence of several small-sized particles (> 6.7 nm) of CuO NPs was also recorded. The purity (metal basis) of the test NPs determined by SEM EDAX in our laboratory was 100% (Kumar, 2023). However, the presence of several small-sized particles (> 6.7 nm) of CuO NPs was also recorded (Table 1). The hydrodynamic size and ζ (zeta) potential of the CuO NPs in Milli-Q water were 154 ± 7 nm and -35.6mV, respectively (Table 1). In 5% H.S. the hydrodynamic size and ζ (zeta) potential of the CuO NPs were 288 ± 13 nm and -33.8 mV, respectively.

The increased hydrodynamic size of CuO NPs in Milli-Q water than TEM was probably due to the formation of agglomerates of CuO NPs as revealed by ζ (zeta) potential (35.6 ± 2 mV) which is suggestive of weak surface charges on CuO NPs.

The agglomeration of CeO_2 NPs in distilled water due to weak surface charges on ceria NPs has also been documented by Ogunkunle *et al.*, (2023). Further, an increment in the size of the CuO NPs in H. S. observed compared to Milli-Q water might be ascribed to the presence of high minerals in H.S. Similar to our observations, enhanced hydrodynamic size of TiO_2 NPs in presence of minerals has been documented in some other recent studies (Gupta *et al.*, 2018; Kumar *et al.*, 2023).

CuO NPs Internalization Upward Translocation and its Influence on Leaf and Root Ionome

Results of the current study revealed internalization of CuO NPs by roots of *M. arvensis* which is evident from concentration-dependent increase in copper contents of roots compared to untreated control (Fig. 1). Besides, the upward movement of

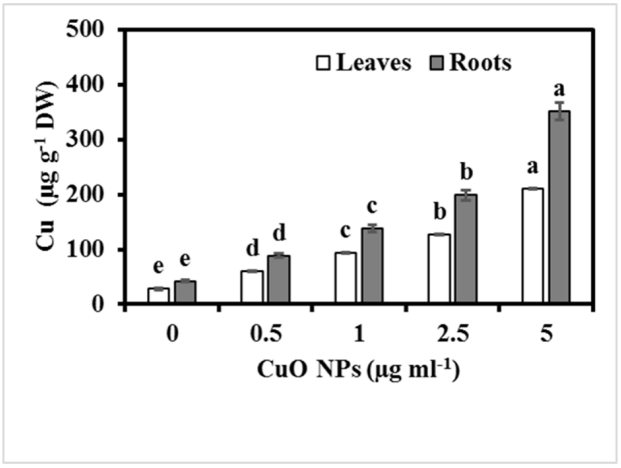

Fig. 1: Internalization of CuO NPs (0.0-5.0 µg mL^{-1}) by *M. arvensis* and its upward movement in shoot. Values are Mean (n = 3) ± SD. One way ANOVA, p < 0.05 for leaves and roots separately. Different superscripts on bars for root/leaves depict significant (p <0.05) difference between means according to DMRT

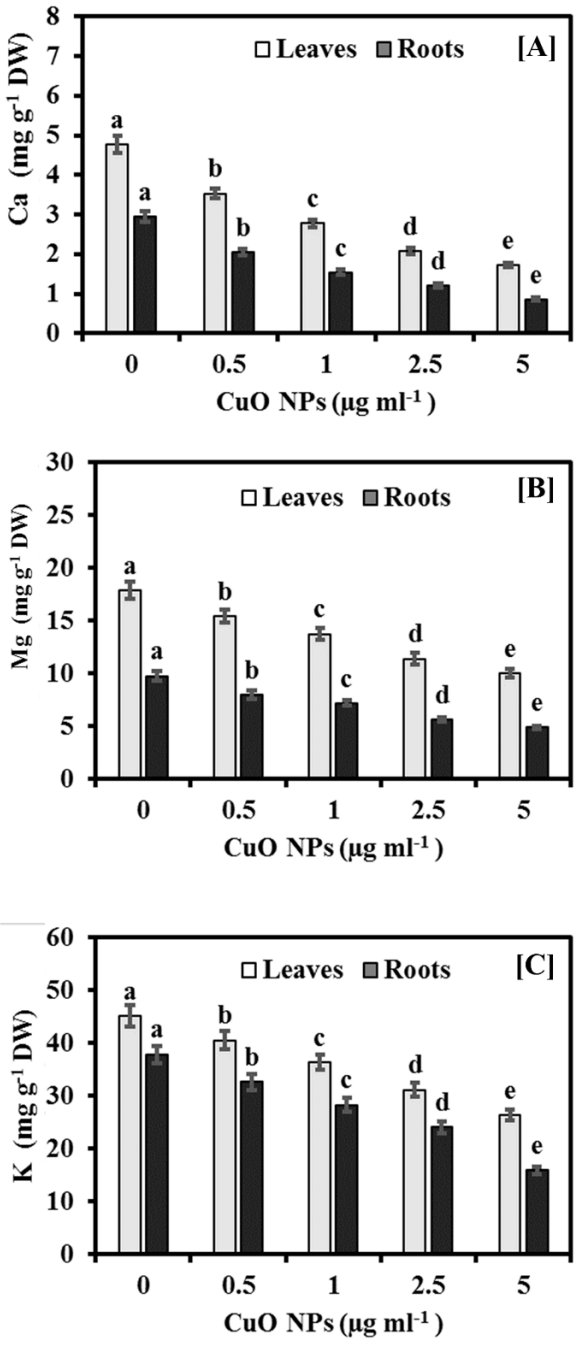

Fig. 2: Effect of CuO NPs (0.0-5.0 µg mL^{-1}) on macronutrients of *M. arvensis* plant tissues (roots and leaves). Values are mean (n = 3) ± SD. One-way ANOVA, p <0.05 for leaves and roots separately. Different superscripts on bars for root/leaves depict significant (p <0.05) differences between means according to DMRT

the CuO NPs in shoots of *M. arvensis* plants was confirmed by significantly (one way ANOVA, p <0.05) upregulated copper contents in leaves of CuO NPs treated plants compared to unexposed control (Fig. 1). Roots and leaves of *M. arvensis* plants the accumulated differential amount of the copper (Fig. 1) The roots of *M. arvensis* treated by highest concentration (5.0 µg mL^{-1}) of CuO NPs concentrated maximum amount of copper (350.62 µg g^{-1} DW). However, leaves of *M. arvensis* plants exposed to same concentration accumulated copper (209.58 µg g-1 DW) after 18 d treatment.

A previous study has also reported significantly high accumulation of copper in leaves of high anthocyanin and low anthocyanin varieties of *O. basilicum* after foliar exposure of Cu $(OH)_2$ nanowires (Tan *et al.*, 2018). Similarly, another study on *Brassica oleracea* and *Lactuca sativa* has also documented high accumulation of copper in leaves and translocation of CuO NPs in roots after foliar application of CuO NPs (Xiong *et al.,* 2017). High copper levels in roots and shoots of *Elsholtzia splendens* , *Lactuca sativa*, *Medicago sativa, Coriandrum sativum* and *Zea mays* after exposure to different nano copper forms also indicate root to shoot translocation of nanoforms of copper (Shi *et al.,* 2014; Hong *et al.,* 2015; Zuverza-Mena *et al.,* 2015; Ahmed *et al.,* 2021; Kumar, 2023). However, the copper accumulation from different nano forms was species and type of nanoparticle specific (Hong *et al.* 2015; Dumont *et al.*, 2022; Di *et al.*, 2023).

CuO NPs influenced the ionome of leaves and roots differentially in this study (Fig. 2 A-C, Fig. 3 A-D). The exposure of CuO NPs significantly (one way, ANOVA, p < 0.05) declined the levels of K, Mg and Ca in both leaves and roots of *M. arvensis* plants (Fig. 2 A, B and C). The reduction in concentrations of K, Ca and Mg was more pronounced in roots than in leaves (Fig. 2A, B and C). The CuO NPs (5 µg mL^{-1}) reduced 63.92, 36.22 and 41.64% Ca, Mg and K in *M. arvensis* leaves compared to untreated control, respectively. However, the same concentration of CuO NPs reduced 70.68, 49.95 and 58.08% Ca, Mg and K in roots of *M. arvensis* compared to unexposed control. In this study CuO NPs adversely impact the Mn and Fe contents of *M. arvensis* plants (Fig. 3A, B).

In the current study, CuO NPs (5 µg ml^{-1}) reduced Mn levels 60.42 and 64.34 % in leaves and roots of *M arvensis* plants, respectively. However, Zn and Mo levels were upregulated in leaves and roots of CuO NPs treated *M. arvensis* plants compared to untreated control. Previous studies reported that impact of CuO NPs on leaf and root ionome was species, plant part and nanoform specific (Dumont *et al.,* 2022; Di *et al.,* 2023). A recent study has reported enhanced Fe, Mn and Zn levels in CuO NPs exposed *Medicago polymorpha* L. plants (Ji *et al.,* 2022).

Similar to the present study CuO NPs induced high accumulation of Zn and Mo and a reduction in Fe content in wheat has also been reported by Lung *et al.,* (2021). However, in contrast to this study they have reported augmentation of Ca, Mg and K contents of *T. aestivum* plants by CuO NPs (Lung *et al.*, 2021). Root and shoot-specific impact of CuO NPs in *Origanum vulgare* has been documented by Du *et al.*, (2018). CuO NPs reduced Fe and Mn levels in *Origanum vulgare* shoots

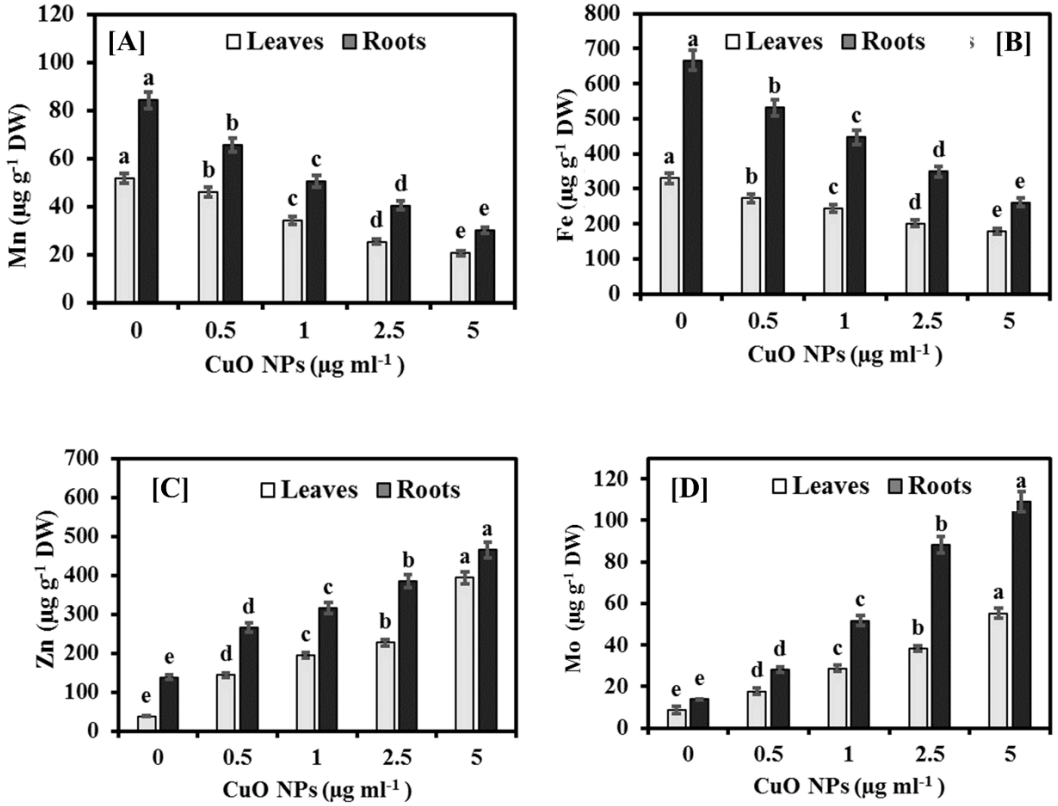

Fig. 3: Effect of CuO NPs (0.0–5.0 µg mL^{-1}) on micronutrients of *M. arvensis* plant tissues (roots and leaves). Values are mean (n = 3) ± SD. One-way ANOVA, p <0.05 for leaves and roots separately. Different superscripts on bars for root/leaves depict significant (p <0.05) differences between means according to DMRT

but enhanced Fe and decreased Mn levels in roots (Du *et al.*, 2018). The differential effect of Cu NPs and CuO NPs on mineral contents of roots and edible parts of *Brassica chinensis* L. (Di *et al.*, 2023) has been documented. They have reported that Cu NPs treatment decreased the levels of Mg, Ca and Mn in edible parts of *Brassica chinensis*. However, CuO NPs enhanced level of the Ca in root and K and Mn levels in edible portions of same plant (Di *et al.*, 2023). The CuO NPs are reported to influence the nutrient levels of grains yielded from two rice varieties: weedy and cultivated (Deng *et al.*, 2022). CuO NPs induced reduction in Mg, K and Ca contents in grains of weedy rice has been documented (Deng *et al.*, 2022). Interestingly, macronutrients were not influenced by CuO NPs in cultivated rice variety (Deng *et al.*, 2022). Results of the present study also advocate that CuO NPs induced effects on macro and micronutrients in *M. arvensis* were metal-specific.

Biomass and Pigments

During the present study concentration-regulated retardation of *M. arvensis* plants compared to untreated control was observed (Fig. 4A-B). Retardation of plant growth in *Zea mays* and *Ocimum basilicum* (Kumar *et al.*, 2023; Roy *et al.,* 2024). It has been observed that CuO NPs inhibited the fresh biomass of *M. arvensis* plants compared to untreated control (Fig. 4 B). Similarly, reduced fresh biomass in CuO NPs exposed *Cicer arietinum, Brassica rapa* ssp. rapa seedlings has been documented (Nair and Chung 2015; Chung *et al.,*2019). It was obvious that root biomass was more affected than shoot (Fig. 4). Root biomass *M. arvensis* plants was significantly reduced in comparison to the untreated control as concentration of CuO NPs enhanced in nutrient solution. Significant inhibition of root growth in *Elsholtzia splendens* plants exposed to CuO NPs has been reported (Shi *et al.,* 2014). Similarly, Khaldari *et al.*, (2021) has also reported that root and shoot elongation, root growth was negatively influenced by the CuO NPs in *Lactuca sativa* L, and *Solanum lycopersicum* L. The CuO NPs reduced the growth of *H. sativum* plants by adversely influencing the lengths of root, shoot along with photosystem II (inhibition of maximal quantum yield and transpiration rate).

CuO NPs (0.5-5.0 µg ml^{-1}) significantly (DMRT, $p < 0.05$) decreased total chlorophyll, chla and chl b contents in *M. arvensis* leaves in concentration regulated manner (Fig. 5A). Reduced total chlorophyll chl a, chl b and carotenoids have been reported in *Oryza sativa* (Da Costa *et al.*, 2020; Yang *et al.*, 2020 and Ahmed *et al.,* 2021), *Brassica rapa* (Siddiqui and Husen, 2020), *Triticum aestivum* (Lung *et al.,* 2021), *Zea mays* (Roy *et al.*, 2022) and *O. basilicum* (Kumar *et al.*, 2023). The findings of the current study on chlorophylls in CuO NPs exposed *M. arvensis* leaves are consistent

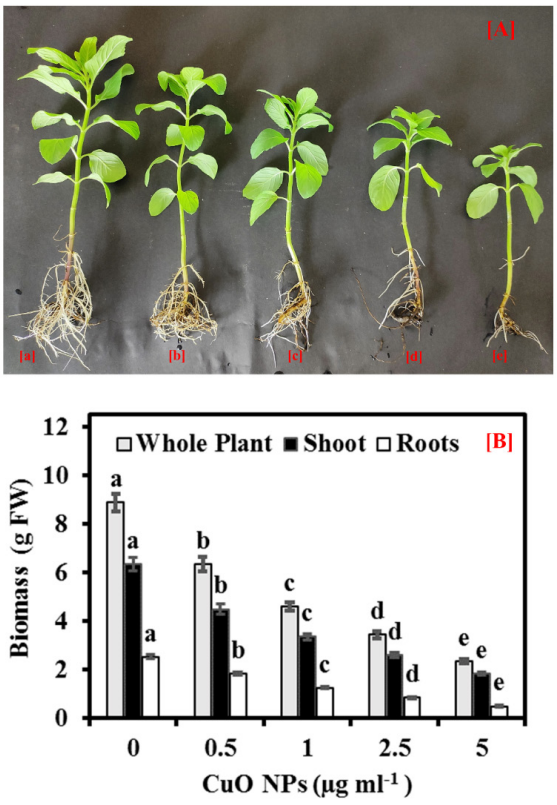

Fig. 4: Impact of CuO NPs (0.0-5.0 µg ml^{-1}) on growth (A) and biomass (B) of *M. arvensis* plants tissues. Values are mean (n = 3) ± SD. One way ANOVA performed separately for biomass of whole plant, shoot and roots were significant at $p < 0.05$. Different superscripts on bars for whole plant/shoot/roots depict significant ($p < 0.05$) difference between means. ($p < 0.05$) according to DMRT

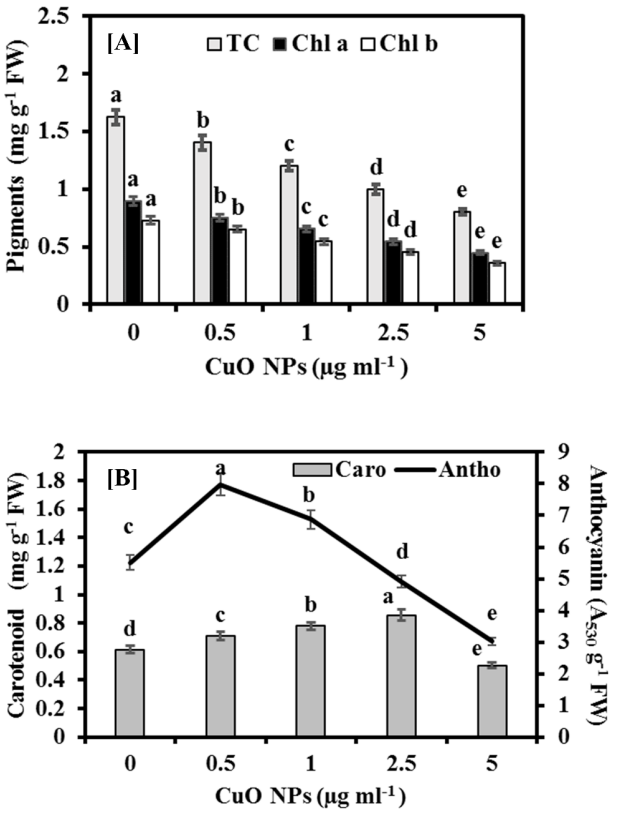

Fig. 5: CuO NPs (0.0–5.0 µg mL^{-1}) modified pigments (A: total chlorophyll, TC, chlorophyll a, Chl a and chlorophyll b, chl b, B: carotenoid and anthocyanin) of *M. arvensis*. Values are mean (n = 3) ± SD. One way ANOVA, $p < 0.05$ for each pigment. Different superscripts on bars for each pigment denote significant difference between means according to DMRT ($p < 0.05$).

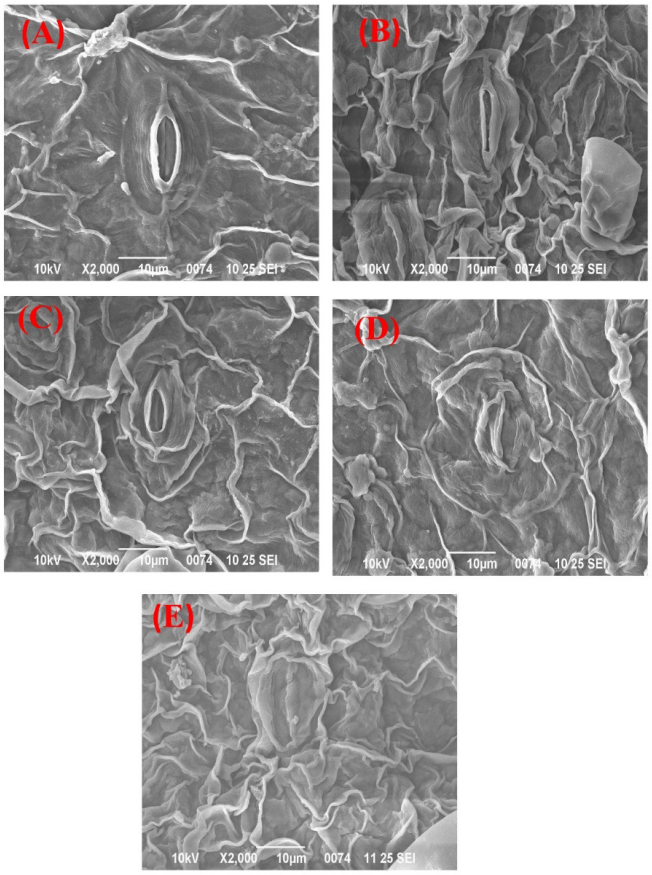

Fig. 6: SEM micrographs showing CuO NPs (A: 0.0, B 0.5, C :1.0, D: 2.5 and E: 5.0 µg ml^{-1}) induced alteration in stomatal morphology

with previous studies. Further, the decrease in levels of total chlorophyll, chl a, chl b is indicative of constrained photosynthesis resulting in reduced plant growth of *M. arvensis*. CuO NPs distorted cystoid membrane after accumulation of NPs in chloroplasts resulted in reduced growth and photosynthesis of rice seedlings (Da Costa *et al.*, 2016, 2020; Hassan *et al.,* 2021; Xu *et al.*, 2023).

However, in contrast to the previous reports CuO NPs significantly enhanced (one way ANOVA, $p < 0.05$) carotenoid contents in *M. arvensis* leaves compared to untreated control (Fig. 5 B). It has been documented that excess copper accumulated from nano and other forms of copper generates reactive ion species in plants (Roy *et al.*, 2022). The augmentation of carotenoids levels in CuO NPs exposed leaves is suggestive of their role as antioxidant for mitigation of oxidative stress induced by CuO NPs in *M. arvensis*. TiO_2 NPs induced increment in carotenoids contents for amelioration of oxidative stress in *Glycine max* and *M. arvensis* leaves has also been documented by Leopold *et al.*, (2022) and Kumar *et al.,* (2023). It has been documented that excess Cu and Fe produce ROS in thylakoids through Fenton and Haber-Weiss type reactions (Connolly and Guerinot, 2002; Zhao *et al.*, 2016). In this study an CuO NPs (0.5-5µg ml^{-1}) concentration mediated increase in anthocyanin level was recorded (Fig. 5B). Anthocyanins are synthesized by phenylpropanoid pathway and act as metal chelator and ROS mitigator (Kitamura *et al.*, 2002). The upregulation of anthocyanidin synthase ANS gene and accumulation of

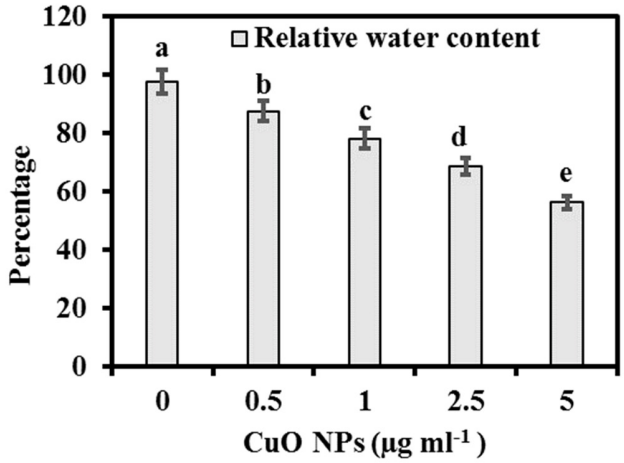

Fig. 7: Relative water contents of leaves of *M. arvensis* as affected by CuO NPs (0.0 - 5.0 µg ml^{-1}). Values are mean (n= 6) ± SD. One way ANOVA, $p < 0.05$. Different superscripts denote significant differences between means according to DMRT ($p < 0.05$) induced alteration in stomatal morphology

anthocyanin in *Brassica rapa* ssp. rapa seedlings has been reported by Chung *et al.*, (2019). Therefore, we suggest that induced anthocyanin contents in the current study were probably to scavenge reactive oxygen species generated by CuO NPs in *M. arvensis* leaves.

Impact on Stomatal Morphology and Relative Water Content

CuO NPs (0.5-5 µg ml^{-1}) affect stomatal morphology in leaves of *M. arvensis* plants (Fig. 6 A). SEM micrographs (Fig. 6 A) revealed dose dependent distortion of stomata (Distorted guard cells, inner wall of stomata and structural alteration in shape of stomatal aperture). Our results are in agreement with earlier reports documenting altered stomatal morphology due to phytotoxic impact of deposited CuO NPs (Xiong *et al.* 2017; Rajput *et al.*, 2018; Kumar. 2023). We also suggest that CuO NPs pave their way to stomata with water current, gets deposited and caused mechanical damage to stomata.

CuO NPs reduced relative water content in *M. arvensis* leaves in dose-dependent manner (Fig. 7). Metallic NPs adhered or deposited on surface of the roots intervene with water uptake leading to conditions similar to drought (Silva *et al.*, 2017; Kumar *et al.*, 2023). It has been suggested earlier that CuO NPs adhere on roots reduced the trans-root potential causing high osmotic stress and decreased water and ion uptake in *Oryza sativa* plants (Da Costa and Sharma, 2016).

We also advocate that the reduced root growth of *M. arvensis* under CuO NPs stress decreased surface area for water and ion uptake, generating negative water potential and osmotic stress. Further, CuO NPs adhered on root surface of *M. arvensis* also contribute to reduced water and ion uptake by enhancing osmotic stress.

Conclusion

The present study documents responses of *Mentha arvensis* plants grown in the presence of CuO NPs (0.0- 5.0 µg ml^{-1}).

Bio-accumulation of copper in the root and leaves of CuO NPs exposed *M. arvensis* plants was observed. Internalized CuO NPs modified the ionome of *M. arvensis* plant tissues by altering the accumulation of micro and macronutrients in roots and shoots of *M. arvensis*. CuO NPs exposure to *M. arvensis* reduced fresh biomass, pigments, and RWC of leaves. Phytotoxic consequences of CuO NPs resulted in distortion of stomatal morphology. The upregulation of carotenoids and anthocyanin in *M. arvensis* leaves suggests their role in the homeostasis of CuO NPs generated ROS. The present study advocates that the adverse impact of CuO NPs on growth, total chlorophyll, chla and chl b and RWC of *M. arvensis* was due to collective impact of internalization of CuO NPs, dissolved copper ions from leaching of CuO NPs, altered macro and mironutrient acquisition and oxidative stress induced by enhanced copper contents in plant tissues. This may reduce the pharmaceutical efficiency of the essential oil obtained from CuO NPs exposed plants.

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Authors Contribution

Archana Dwivedi: Conceptualization, Methodology, Validation, Investigation, Data curation, Original draft preparation; Subodh Kumar: Investigation, Data curation, Statistical analyses, Poornima Vajpayee: Funding acquisition, Conceptualization, Supervision review and editing of the manuscript.

Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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