Genotype-Specific Mitigation of Drought Stress in Myrtus communis L.: Synergistic Effects of CuO and MnO Nanoparticles on Growth, Antioxidant Defense, Membrane stability, and Essential Oil production

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Abstract

Background

Drought stress is a critical constraint on the growth and productivity of medicinal plants, including *Myrtus communis* L. This study investigated the potential of manganese oxide (MnO) and copper oxide (CuO) nanoparticles to alleviate drought induced damage in two Iranian myrtle genotypes (Khoraman and Fars). A factorial experiment, arranged in a completely randomized design with three replications, was conducted under three irrigation regimes (100%, 80%, and 60% of field capacity) and six nanoparticle treatments: distilled water (control), MnO (25, 50 mg L^{-1}), CuO (25, 50 mg L^{-1}), and combined MnO + CuO (25 mg L^{-1} each).

Results

Drought stress significantly reduced shoot and leaf dry weight and relative water content, while increasing electrolyte leakage, malondialdehyde, and proline accumulation. Antioxidant responses, including enzymatic activities (superoxide dismutase, peroxidase, catalase, and ascorbate peroxidase) and non-enzymatic compounds (total phenolics, flavonoids,nd proline), were elevated under water-deficit conditions. Although drought stress increased essential oil content, EO yield declined due to reduced biomass. Foliar application of CuO and MnO nanoparticle, particularly in combination, significantly alleviated the adverse effects of drought by reducing membrane damage (lower electrolyte leakage and malondialdehyde), enhancing osmolyte accumulation, and upregulating antioxidant defense systems. These changes led to improved biomass production and enhanced essential oil content and yield. Notably, CuO nanoparticle had a stronger effect on growth and essential oil yield in the Khoraman genotype, while MnO nanoparticle was more effective in the Fars genotype. The highest essential oil content was obtained under 50 mg L^{-1} treatments of either MnO or CuO, and under their combined application. Variations in essential oil composition were also observed in response to treatments, though without a consistent pattern.

Conclusions

Overall, MnO and CuO nanoparticles act synergistically as stress modulators, enhancing drought tolerance, optimizing physiological and biochemical responses, and ultimately promoting growth and secondary metabolite production in *M. communis*.

1. Introduction

Myrtus communis L., commonly known as myrtle, is an evergreen shrub in the family Myrtaceae, noted for its aromatic and medicinal traits. It grows naturally across the Mediterranean, North Africa, and parts of Western Asia, with notable populations in various climatic zones of Iran [1, 2]. Historically, myrtle has

been utilized in traditional remedies, including in the works of Avicenna, for treating inflammatory conditions, respiratory ailments, and digestive disorders [3, 4]. A key value of myrtle lies in its essential oils (EOs) and other secondary metabolites. The EOs, extracted from leaves, flowers, and fruits, are rich in monoterpenes and oxygenated constituents like *α*-pinene, 1,8-cineole, limonene, and linalool. These biochemical compounds possess a variety of bioactivities—antioxidant, antimicrobial, anti-inflammatory, among others—that support its uses in pharmaceutical, cosmetic, and food industries [3, 2]. Yet, the EO yield and its chemical profile can vary greatly depending on genetic background, environmental variables (such as soil, moisture, temperature), phenological stage, and harvest timing [5].

One of the environmental stresses that most severely affects medicinal and aromatic plants is drought. In arid and semi-arid regions like Iran, reduced rainfall and increasing evapotranspiration create prolonged periods of water deficit [6]. Drought stress compromises several physiological functions—photosynthesis, stomatal conductance, nutrient uptake, and water relations—leading to reduced growth, lowered biomass, and impaired production of both primary metabolites and valuable secondary compounds [1]. Under drought, plants tend to accumulate reactive oxygen species (ROS) such as superoxide radicals, hydrogen peroxide (H_2O_2), and hydroxyl radicals. While some ROS play signaling roles, excessive ROS can oxidize membrane lipids, denature proteins, disrupt cell walls, and damage DNA. To counterbalance oxidative stress, plants rely on intricate antioxidant systems—enzymatic components such as superoxide dismutase (SOD), catalase (CAT), peroxidases (POD), and ascorbate peroxidase (APX), along with non-enzymatic molecules including phenolics, flavonoids, and osmoprotectants like proline [7, 8]. However, when drought is severe or prolonged, these intrinsic mechanisms often fall short, resulting in reduced physiological performance and secondary metabolite production [9].

Given the limitations of traditional agronomic or breeding strategies for drought mitigation, recent research has turned to nanotechnology as a promising complement [10]. Nanoparticles (NPs), because of their small size and large surface area, can interact more efficiently with plant systems. Low-dose applications of certain metal oxide nanoparticle (NP) can boost antioxidant activity, enhance water-use efficiency, stabilize membranes, and sometimes stimulate secondary metabolite [11, 10, 12, 13]. However, beneficial effects strongly depend on NP type, size, concentration, and method of application; high doses or inappropriate particles may induce phytotoxicity [14, 15].

Among micronutrients, manganese (Mn) and copper (Cu) are central to many plants physiological and biochemical pathways. Mn serves crucial roles in photosynthetic reactions, nitrogen assimilation, ROS detoxification, and as a cofactor for enzymes involved in phenolic and flavonoid biosynthesis [12, 10, 13]. When delivered in nanoparticulate form (MnO NPs), Mn can provide more efficient uptake and reduced risk of toxicity compared to bulk forms [16, 17]. Similarly, Cu participates in photosynthetic electron transport, oxidative stress defense (e.g., via Cu/Zn SOD, polyphenol oxidase), respiration, and regulatory networks of gene expression [18]. Studies indicate that CuO-NPs, applied carefully, can enhance both growth and secondary metabolite synthesis under stress [19, 14].

Empirical work on medicinal plants demonstrates that treatments with MnO NPs or CuO NPs can increase concentrations of phenolics, flavonoids, and osmolytes; elevate antioxidant enzyme activities; improve membrane integrity; generally, they support better plant growth under drought or oxidative stress [16, 20, 21]. For example, nanoparticulate Mn has been shown to enhance SOD, CAT, and POD in species like *Atropa belladonna* and *Triticum aestivum* under water deficient stress [21, 20]. Similarly, CuO NPs enhance non-enzymatic and enzymatic antioxidant defenses in *Mentha arvensis* and *Dracocephalum moldavica*, increasing EO yield and affecting composition [22, 23]. What remains less well-explored is how combined application of MnO NPs and CuO NPs might act synergistically under drought stress, particularly in native medicinal plant genotypes. Genotypic variation often strongly influences how plants perceive and respond to drought and NP treatments—traits such as root architecture, water use efficiency, antioxidant capacity, and secondary metabolite pathways can differ considerably among genotypes [5]. In *M. communis*, previous studies have addressed how salinity, seasonal timing, or harvest time affect EO yield, antioxidant capacity, and phenolic content [5, 3]. Yet, drought stress combined with NP treatments, especially using MnO and CuO, remains largely uninvestigated in different genotypes of myrtle.

Given the dual challenges of water scarcity in arid and semi-arid agricultural zones and the increasing interest in nano-based amelioration strategies, our work focuses on two Iranian genotypes of *M. communis* (Khoraman and Fars). We aim to assess how foliar application of MnO and CuO NPs—separately and in combination—affects drought tolerance. Key measures include growth (shoot and leaf biomass), membrane integrity (electrolyte leakage, malondialdehyde), both enzymatic and non-enzymatic antioxidant defenses, and the yield and chemical profile of EOs. This study contributes in three main ways: (1) exploring genotype-specific responses to NP intervention under water-deficit; (2) investigating whether MnO and CuO have synergistic effects; (3) linking physiological, biochemical, and phytochemical metrics to inform optimized NP use in cultivation. Findings will have practical implications for enhancing medicinal plant productivity and secondary metabolite quality under limited irrigation—critical goals for sustainable agriculture in water-scarce regions.

2. Materials and Methods

2.1. Treatments and Experimental Design

This study was performed in the greenhouse of the Faculty of Agriculture, Lorestan University (Khorramabad, Iran), using a factorial arrangement based on a completely randomized design (CRD) with three replications. The experimental layout involved three main factors: genotype, drought stress intensity, and NP foliar treatments. Two genotypes of *M. communis* (Khoraman and Fars) were selected based on prior characterization of Iranian myrtle populations [2]. Drought stress was imposed at three levels: 100%, 80%, and 60% of field capacity (FC), simulating non-stressed, moderate, and severe drought conditions, respectively. The third factor comprised six foliar treatments: (1) distilled water as the control, (2) MnO NPs at 25 mg L^{-1}, (3) MnO NPs at 50 mg L^{-1}, (4) CuO NPs at 25 mg L^{-1}, (5) CuO NPs at 50 mg L^{-1}, and (6) a combined application of MnO and CuO NPs at 25 mg L^{-1} each.

Seedlings were initially raised in cocopeat and perlite (1:1) under controlled conditions. Once they reached a height of approximately 15 cm, they were transplanted into 10-kg plastic pots containing a 1:1:1 mixture of agricultural soil, river sand, and well-rotted farmyard manure. Soil physicochemical characteristics are summarized in Table 1. All plants were maintained under uniform irrigation and fertilization regimes for one year before treatments were applied. NPs sprays were administered in two intervals, with the first application occurring ten days prior to drought stress induction. The second spray followed 50 days later. Drought stress was initiated by maintaining soil moisture at target FC levels through daily pot weighing and water replenishment, continued until the plants reached full flowering. At this stage, four pots per treatment replication were randomly selected for physiological and biochemical analysis. Afterward, plants were

harvested and separated into aerial parts and leaves for dry biomass determination.

2.2. Relative Water Content (RWC)

RWC was assessed following the method described by Ritchie et al. [24]. Ten mature leaves were excised, and their fresh weight (FW) was recorded right away. Leaves were then immersed in distilled water for 24 h at room temperature. After blotting surface moisture, turgid weight (TW) was measured. Finally, the samples were oven-dried at 80°C for 48 h to obtain dry weight (DW).

2.3. Membrane Stability

2.3.1. Electrolyte Leakage (EL)

EL was measured following the approach described by Lutts et al. [25]. Fifteen uniform leaf discs of equal size (0.5 cm diameter) were washed with distilled water and placed into test tubes containing 10 mL of deionized water. After shaking at ambient temperature for 24 h, the first electrical conductivity (EC_1) was recorded. Samples were then autoclaved at 120°C for 30 min, cooled back to room temperature, and the final conductivity (EC_2) was measured.

2.3.2. Malondialdehyde (MDA) Content

Lipid peroxidation was estimated by quantifying MDA, a major end-product, using the method of Wang et al. [26]. About 0.1 g of fresh leaf material was frozen in liquid nitrogen, ground, and mixed with 5 mL of 0.5% thiobarbituric acid prepared in trichloroacetic acid. The mixture was heated to 100°C for 15 min, then quickly chilled on ice, and centrifuged at 4,000 rpm for 10 min. Absorbance was read at 450, 532, and 600 nm, and MDA content was calculated using appropriate extinction coefficients.

2.4. Proline Content

Proline levels were determined using the protocol of Bates et al. [27]. Frozen leaf tissue was ground and mixed with 10 mL of 3% sulfosalicylic acid, then spun at 15,000 rpm for 15 min at 4°C. Two mL of the clear supernatant was combined with 2 mL of acid ninhydrin reagent (prepared from ninhydrin, glacial acetic acid, and orthophosphoric acid) and 2 mL of glacial acetic acid. This mixture was heated at 90°C

for 1 h, then cooled rapidly in ice. Four mL of toluene was added, and the chromophore-containing toluene phase was separated by vortexing. Absorbance was measured at 520 nm, and proline levels were determined from a standard curve, expressed in μmol g^{-1} fresh weight.

2.5. Enzymatic Antioxidants

2.5.1. Enzyme Extraction Buffer

The extraction buffer was made by dissolving 2.43 g of Tris in 100 mL of distilled water and adjusting the pH to 7.8 using HCl. After refrigeration for 24 h, 20 mL of glycerol was added, and the final volume was brought up to 200 mL. The buffer was stored in amber bottles to prevent light-induced degradation.

2.5.2. Protein and Enzyme Extraction

Antioxidant enzymes were extracted following Chang and Kao [28] with minor adaptations. Around 0.25 g of frozen leaf tissue was ground in 2.5 mL of extraction buffer and centrifuged at 13,000 rpm for 15 min at 4°C. The supernatant obtained was used for enzymatic assays and protein quantification.

2.5.3. SOD

SOD activity was determined by its ability to inhibit nitro blue tetrazolium chloride photoreduction under fluorescent light, following Beauchamp and Irwin [29]. The reaction mixture included 100 μL each of extraction buffer and enzyme extract. Samples were illuminated for 10 min, then immediately covered. Absorbance was measured at 560 nm. The enzyme activity was calculated based on enzyme units per mg of leaf tissue using the corresponding formula.

2.5.4. CAT

CAT activity was measured based on the decomposition rate of H_2O_2 following the method outlined by Aebi [30]. The reaction mixture included 3 mL of 50 mM phosphate buffer (pH 7.0), 100 μL enzyme extract, and 5 μL of 30% H_2O_2. Absorbance at 240 nm was recorded at 20-second intervals over a 2-minute period. The enzyme activity was expressed as the amount of H_2O_2 decomposed (μmol) per minute per mg protein.

2.5.5. POD

POD activity was assessed according to Drotar et al. [31]. The assay solution contained phosphate buffer (3 mL), 10 μL of H_2O_2 (30%), 3 μL of guaiacol solution (200 mM), and 100 μL enzyme extract. The formation of tetraguaiacol was monitored at 470 nm. Results were expressed as absorbance increase per minute per mg protein.

2.5.6. APX

APX activity was determined following the procedure of Ranieri et al. [32]. The assay mixture consisted of 1.5 mL of phosphate buffer (pH 7.0), 400 μL of 0.5 mM ascorbic acid, 600 μL of 0.1 mM EDTA, 400 μL

of 30% H_2O_2, and 100 µL of enzyme extract. The decline in absorbance at 290 nm, reflecting ascorbate oxidation, was tracked for a period of 4 minutes.

2.6. Non-Enzymatic Antioxidants

2.6.1. Total Phenolic Content (TPC)

Leaf extracts were prepared by macerating 10 g of finely powdered, oven-dried leaves in 100 mL of 80% methanol for 72 hours at ambient temperature under constant agitation. After filtration, the extract was concentrated under reduced pressure using a rotary evaporator and stored at 4°C in amber vials until further analysis [33].

TPC was determined using a modified Folin–Ciocalteu approach [34]. For the assay, 300 µL of the prepared extract was combined with 1.2 mL of 7% sodium carbonate and 0.5 mL of 10% Folin–Ciocalteu reagent. The mixture was kept in the dark for 20 minutes, and absorbance was read at 765 nm using a UV–Vis spectrophotometer. Results were expressed as milligrams of gallic acid equivalents (GAE) per gram of dry material.

2.6.2. Total Flavonoid Content (TFC)

TFC was estimated following the aluminum chloride colorimetric technique [35]. The reaction mixture contained 0.5 mL of methanolic plant extract, 0.1 mL of 1 M potassium acetate, 0.1 mL of 10% aluminum chloride, and 2.8 mL of distilled water. After 30 minutes of incubation in the dark, absorbance was recorded at 415 nm. TFC was calculated based on a standard curve of quercetin and expressed as mg quercetin equivalents (QE) per gram of dry weight.

2.6.3. Antioxidant Capacity

The antioxidant power of the plant extracts was evaluated using the ferric reducing antioxidant power (FRAP) assay, following Benzie and Strain [36]. The FRAP reagent was freshly prepared by mixing acetate buffer (300 mM, pH 3.6), TPTZ solution (10 mM in 40 mM HCl), and $FeCl_3$ (20 mM) at a 10:1:1 ratio and prewarmed to 37°C. In a 96-well plate, 20 µL of the methanolic extract was added to 180 µL of the reagent. After an 8-minute incubation at 37°C, absorbance was recorded at 593 nm. Antioxidant capacity was expressed as mmol Fe^{2+} equivalents per gram of dry weight.

2.7. EO Extraction

EO was extracted using hydrodistillation in a Clevenger-type apparatus. Twenty grams of dried, ground leaf material was subjected to distillation with distilled water for 3 hours. The collected EO was dehydrated using anhydrous sodium sulfate, and the EO content was measured gravimetrically. The oil was stored in dark, airtight glass vials at 4°C for further analysis [37].

2.8. EO Composition

Quantitative profiling of EO constituents was performed using a Shimadzu GC-2014 gas chromatograph with a Flame Ionization Detector (GC-FID) and an RTX-5 capillary column (30 m × 0.25 mm i.d., 0.25 µm film thickness). The oven program began at 50°C for 4 minutes, then increased by 5°C per minute until reaching 260°C, where it was held for 10 minutes. The injector was maintained at 250°C and the detector at 300°C. Nitrogen served as the carrier gas at a flow rate of 1 mL per minute.

For qualitative analysis, an Agilent 7890A GC system coupled with a mass spectrometer (GC-MS) and an HP-5 column (30 m × 0.25 mm i.d., 0.25 µm film thickness) was utilized. Helium served as the carrier gas at a constant flow of 1 mL minute. The temperature program matched that of the GC analysis. Electron ionization was carried out at 70 eV, and ion source was set to 270°C.

Identification of EO components was based on retention indices (Kovats index), comparison of mass spectra with NIST library data, and co-injection with authentic reference standards when available. The relative concentration of each constituent was determined from GC-FID peak areas using the normalization method, with no correction factors applied [38, 39].

2.9. Statistical Analysis

Experimental data were subjected to analysis of variance (ANOVA) under a factorial CRD structure using SAS software (version 9.4). When significant differences were detected, mean comparisons were conducted using Least Significant Difference (LSD) at the 5% level.

3. Results and Discussion

3.1. Plant Biomass

ANOVA results demonstrated that genotype, drought stress intensity, and foliar application of NPs each exerted significant influences on leaf dry weight and overall plant dry weight. Furthermore, interactions between genotype × drought stress, as well as genotype × NP treatment, significantly affected these biomass parameters. Notably, the interaction between drought stress × NPs application was significant solely for leaf dry weight (Table S1). Evaluating the genotype × drought stress interaction revealed that the Khoraman genotype consistently exhibited greater leaf and total biomass compared to the Fars genotype. Both genotypes experienced marked reductions in biomass under drought conditions; however, the decline was most pronounced in Khoraman under severe drought (60% FC), where total biomass and leaf dry weight decreased by 50.56% and 47.62%, respectively, relative to well-watered controls (Table 3).

The genotype × NPs treatment interaction highlighted differential responses between the two genotypes. In Khoraman, foliar application of MnO NPs and CuO NPs significantly enhanced total biomass, but increasing the concentration to 50 mg L^{-1} did not further improve plant dry weight compared to 25 mg L^{-1}. Conversely, in the Fars genotype, biomass improvements were more evident at the highest NPs concentration, with MnO demonstrating superior efficacy (Fig. 1A). Regarding leaf dry weight, CuO NPs

treatments benefited Khoraman more prominently, while MnO NPs alone showed no significant effect; however, combined MnO NPs and CuO NPs application exerted a synergistic enhancement. Foliar application of 50 mg L^{-1} MnO NPs significantly increased leaf dry weight in the Fars genotype, unlike other concentrations (Fig. 1B). The interaction between drought stress × NPs application further elucidated treatment-specific effects. Under optimal water condition, CuO NPs at 25 mg L^{-1} and combined MnO NPs and CuO NPs applications significantly boosted leaf dry weight compared to untreated controls. During mild drought stress (80% FC), only the combined NPs treatment maintained this positive effect, whereas under severe drought (60% FC), NPs treatments did not yield significant biomass improvements (Fig. 2).

Overall, Khoraman genotype exhibited superior biomass accumulation under both stress and non-stress conditions, reflecting inherent genetic variation and distinct drought coping mechanisms. The biomass reduction under drought aligns with previously documented findings [1, 40]. Typically, genotypes with higher growth potential under favorable conditions may undergo more substantial biomass decline under stress due to constrained resource allocation toward stress tolerance [41]. The pronounced biomass loss in Khoraman under severe drought likely results from stomatal closure limiting CO_2 uptake and photosynthesis, coupled with oxidative damage impairing membrane integrity, pigment stability, and enzymatic functions, culminating in decreased growth [42, 43]. Consistent with our results, foliar application of CuO and MnO NPs has been shown to alleviate drought-induced growth inhibition by enhancing antioxidant defenses and improving physiological processes [44, 20, 18]. Cu NPs aid in scavenging ROS and modulating auxin and nitrogen metabolism, whereas Mn NPs supports photosystem II function and nitrogen assimilation, thus sustaining energy production and carbon fixation under stress [17, 45].

3.2. Physiological and Biochemical Traits

3.2.1. RWC and Membrane Stability

Significant genotypic differences were observed in leaf RWC and MDA levels. Drought stress significantly influenced RWC, EL, and MDA content, while NPs treatments notably affected membrane stability indicators. The genotype × drought stress interaction significantly modulated MDA content, and drought × nanoparticle interactions influenced both EL and MDA accumulation (Table S1). The Khoraman genotype maintained approximately 9% higher leaf RWC than Fars across different drought conditions (Table 2). Drought progressively decreased RWC, with severe stress (60% FC) reducing it by nearly 44%. MDA content, a marker of lipid peroxidation, was consistently higher in Fars than Khorraman regardless of water availability, and drought intensified MDA accumulation in both genotypes (Table 3). Drought × NPs interactions revealed that foliar application of 50 mg L^{-1} MnO NPs and the combined MnO NPs + CuO NPs treatment mitigated EL and MDA under moderate drought (80% FC). Under severe drought, all NPs treatments substantially reduced EL, with MDA content also declining significantly, except for CuO NPs at 25 mg L^{-1} (Table 4).

These findings corroborate previous reports highlighting drought-induced membrane destabilization through ROS-mediated lipid peroxidation, leading to increased EL and impaired water retention [1, 46]. Enhanced membrane integrity and maintenance of leaf water status are critical for drought resilience [47]. Biogenic metal NPs are known to mimic antioxidant functions by scavenging ROS, thereby preventing oxidative damage to membrane lipids [45]. Consistent with the findings of the present study, the application of Cu NPs [48, 18, 44] as well as different Mn sources in both salt and nano forms [12, 20] has been shown to enhance RWC, improve the drought tolerance index, and upregulate the expression of resistance-related genes in plants exposed to drought and salinity stress. Cu NPs enhance water uptake and cell membrane protection via increased SOD activity and aquaporin-mediated root hydraulic conductivity [49]. Mn supports Mn-SOD expression and activates Mn-dependent proteins essential for cellular stability under stress [50]. Notably, manganese NPs exhibit higher bioavailability and reduced toxicity compared to bulk or ionic forms, resulting in superior stress mitigation [45].

3.2.2. Proline

Both genotype and drought stress significantly influenced proline content, with a notable genotype × drought interaction (Table S1). The Fars genotype consistently accumulated more proline than Khoraman, exhibiting 76.6% higher levels under well-watered conditions. Drought stress markedly increased proline content in both genotypes, with approximately 67% elevation at severe stress (60% FC) relative to controls (Table 3). Proline accumulation is a well-established adaptive response facilitating osmotic adjustment and stabilization of cellular structures during water deficit [51]. Its biosynthesis is upregulated via enhanced transcription of key enzymes, such as pyrroline-5-carboxylate reductase, supporting drought acclimation [47]. In addition to osmoprotection, proline may reflect metabolic adjustments related to altered photosynthetic electron transport and mitochondrial activity under stress, serving as a secondary response to maintain cellular homeostasis [47]. Elevated proline synthesis likely contributes to drought resilience by mitigating oxidative damage and sustaining metabolic function [51].

3.2.3 Antioxidant Enzyme Activities

ANOVA results revealed that genotype, drought stress, and foliar application of NPs individually influenced the activity of key antioxidant enzymes including SOD, CAT, POD, and APX. Significant interactions between genotype × drought stress were observed for CAT, POD, and APX activities, whereas genotype × NPs foliar application significantly affected SOD and POD. Moreover, drought stress × NPs foliar application interaction significantly modulated all enzyme activities, with a three-way interaction (genotype × drought stress × NPs foliar application) evident for POD only (Table S1).

Regardless of drought stress, the Fars genotype exhibited higher APX activity than Kharman. Both genotypes showed increased CAT and APX activities under drought stress, with Fars displaying a more pronounced APX induction (Table 3). SOD activity was inherently greater in Fars compared to Kharman under non-stress conditions, by approximately 35.9%. NPs treatments demonstrated genotype- and concentration-dependent effects on SOD activity: in Fars, both MnO NPs and CuO NPs at 50 mg L^{-1}, individually and combined, significantly enhanced SOD activity. Khoraman responded strongly to CuO

NPs at both 25 and 50 mg L^{-1} concentrations and its combination with MnO NPs, yielding significant increases in SOD activity. The highest SOD activity overall was observed in Fars under combined NPs treatment (Fig. 3A).

Drought stress at 60% FC markedly elevated SOD, CAT, and APX activities by 24.6%, 35.4%, and 34.7%, respectively, compared to well-watered controls. NPs application under drought further modulated antioxidant enzyme activities, with CuO NPs at 50 mg L^{-1} and MnO NPs at both concentrations, alone or combined, significantly boosting SOD and APX activities relative to untreated plants (Table 4). POD activity was highest in Kharman, particularly under drought, where it increased by 100% at 60% FC, and in Fars, by 58.3% at 80% FC compared to controls. Foliar spray of NPs generally enhanced POD activity across genotypes and drought levels, with the maximum increase seen in Khoraman under 60% FC treated with combined NPs or 50 mg L^{-1} MnO NPs (Fig. 4).

These findings are consistent with previous studies demonstrating drought-induced upregulation of antioxidant enzymes as a defensive response to oxidative stress, caused by excessive ROS accumulation under water deficit [1, 40, 52]. SOD enzymes, as metalloenzymes, catalyze dismutation of superoxide radicals ($O_2^{\bullet -}$) to oxygen and H_2O_2, constituting the first enzymatic defense line. Subsequently, H_2O_2 is decomposed to water and oxygen by CAT, APX, and POD, thus mitigating oxidative damage [53, 54]. The distinct subcellular localization of APX (present in chloroplasts, mitochondria, cytosol) and CAT (restricted to peroxisomes) suggests complementary roles, with APX acting as a more effective intracellular ROS [33]. Enhanced enzymatic antioxidant activity reflects an adaptive mechanism in myrtle to maintain membrane integrity under drought-induced oxidative stress [40]. Copper and manganese NPs have been documented to enhance antioxidant enzyme activity under abiotic stress [48, 18, 50, 55]. Cu NPs stimulate ROS production that triggers enzymatic and non-enzymatic antioxidant defense pathways, thereby improving drought tolerance [18]. Mn functions as a cofactor in Mn-SOD isoforms, oxalate oxidase, and the Mn cluster of photosystem II, critical for photosynthetic electron transport, enabling Mn-based NPs to enhance enzyme activity and plant resilience [56, 39]. In sum, the observed enzyme activity increases following foliar NPs application likely arise from physiological roles of Mn and Cu in cellular ROS scavenging and stress signaling, corroborated by enhanced growth responses reported in other studies with Mn NPs [13].

3.2.4. Non-Enzymatic Antioxidants

ANOVA results showed significant genotype and drought stress effects on TPC, TFC, and antioxidant capacity. NPs foliar application significantly influenced antioxidant activity but did not significantly alter TPC and TFC (Table S1). The Khoraman genotype consistently exhibited higher TPC, TFC, and antioxidant activity than Fars by 27.9%, 23.9%, and 15.6%, respectively. Drought stress at 60% FC increased TPC, TFC, and antioxidant activity by 29%, 28.9%, and 10.2% compared to controls (Table 2). Mn NPs treatments, both singly and combined with Cu NPs, significantly enhanced antioxidant capacity (Fig. 5A).

Non-enzymatic antioxidants, including phenolics and flavonoids, play crucial roles in scavenging ROS generated under drought stress, constituting an essential component of the plant's defense arsenal [57]. However, secondary metabolite accumulation responses vary among species, genotypes, and environmental conditions [9]. The literature reports conflicting effects of drought on TPC; some species decrease phenolics under stress, while others accumulate them, indicating genotype-specific and stress-intensity-dependent regulation [58, 47]. Phenolic compounds function not only as antioxidants but also as signaling molecules and metal chelators, mitigating oxidative stress through multiple pathways [20]. Their biosynthesis is closely linked to phenylalanine ammonia-lyase (PAL) activity, a key enzyme upregulated under stress conditions [57]. Flavonoid accumulation similarly enhances drought tolerance by neutralizing free radicals [6].

Though NPs treatments did not significantly change phenolic and flavonoid levels in our study, the increased antioxidant capacity suggests activation of other non-enzymatic defense pathways. NPs can act as elicitors of secondary metabolism, stimulating biosynthesis of bioactive compounds through oxidative signaling cascades [59]. Studies on *A. belladonna*, *Ocimum basilicum*, *Stevia rebaudiana*, and *Brassica nigra* corroborate the stimulatory effects of Mn and Cu NPs on phenolic biosynthesis and antioxidant potential [20, 55, 60, 61]. NPs-induced "oxidative bursts" involving rapid H_2O_2 accumulation can upregulate PAL and polyphenol oxidase (PPO), enhancing phenolic metabolism [20]. Copper ions also influence PPO and other oxidases, while manganese stimulates PAL and other Mn-dependent enzymes, collectively enhancing antioxidant defense and secondary metabolite production [19, 45].

3.3. EO Content and Yield

Drought stress, genotype, and NPs application significantly affected EO content and yield in myrtle plants, with significant genotype × drought stress and genotype × NPs interactions (Table S1). NPs treatments—particularly Mn NPs and Cu NPs at 50 mg L^{-1} and their combination—increased EO content significantly (Fig. 5B). Khoraman genotype exhibited higher EO content and yield than Fars under non-stress conditions by 27% and 38.9%, respectively. Drought stress increased EO content in both genotypes but reduced overall EO yield due to biomass loss (Table 3). The genotype-specific response to NPs revealed that all treatments except 50 mg L^{-1} Mn NPs increased EO yield in Khoraman, while in Fars, only 50 mg L^{-1} Mn NPs and combined treatments were effective. Cu NPs had a stronger positive effect in Khoraman, whereas Mn NPs were more effective in Fars (Fig. 3B).

These findings align with prior reports in medicinal plants such as *M. communis*, *Artemisia dracunculus*, *O. basilicum*, and *Origanum majorana*, where drought induces a "concentration effect" by reducing biomass but increasing glandular trichome density and EO content [62, 63]. Drought also activates terpenoid biosynthesis pathways (MEP/MVA) via abscisic acid and ROS signaling, upregulating key enzymes and promoting EO accumulation [64]. NPs can elicit mild oxidative stress, stimulating secondary metabolism and EO production as protective antioxidants [65]. Genotype-specific responses play a key role in plant adaptation to drought stress. While some genotypes may increase EO percentage or maintain favorable composition under stress, most studies—including the present one—show that

overall EO yield declines. This reduction is largely due to severe biomass loss under intense drought, which outweighs gains in EO content and ultimately lowers EO productivity [58, 66].

Mn NPs have been recognized as effective growth stimulators, promoting both vegetative development and the biosynthesis of secondary metabolites. Supplementation with micronutrients such as manganese and copper has been shown to enhance plant growth, developmental processes, and biomass accumulation, ultimately supporting greater EO productivity [20]. Beyond improving nutrient acquisition, Mn NPs also stimulate key metabolic pathways involved in secondary metabolite formation and act as biocompatible agents that alleviate abiotic stress [45]. For instance, foliar application of Mn NPs at 25 mg L^{-1} in *A. belladonna* significantly improved growth and alkaloid production, effects largely attributed to their strong antioxidant potential [20]. Comparable positive influences of CuO NPs on EO yield and composition have likewise been reported in *O. basilicum* and *S. rebaudiana* [55, 67].

3.4. EO Composition

Genotype significantly influenced the relative proportions of most EO constituents, except *α*-terpineol acetate, while drought stress affected all compounds except (*E*)-caryophyllene. NPs application significantly modulated all analyzed components. A pronounced genotype × drought stress interaction was observed for several major metabolites, including isobutyl isobutyrate, *α*-pinene, 1,8-cineole, linalool, *α*-terpineol acetate, geranyl acetate, and (*E*)-caryophyllene. Moreover, the interaction effects of genotype × NPs treatment, drought × NPs treatment, as well as their combined three-way interaction were statistically significant across all quantified compounds (Table S2). Khoraman genotype exhibited higher 1,8-cineole, whereas Fars produced more linalool and linalyl acetate. Drought stress induced irregular changes in EO composition without consistent trends across genotypes. The application of Mn NPs and Cu NPs also induced genotype-specific and treatment-dependent shifts in EO profiles. Notably, the highest linalool content (34.02%) was detected in the Fars genotype under 60% FC when treated with 50 mg L^{-1} Mn NPs. Similarly, the greatest levels of geranyl acetate (21.5%) and linalyl acetate (10.61%) were found in Fars under moderate drought conditions (80% FC) following foliar application of 25 mg L^{-1} Cu NPs. Highest *α*-terpineol (6.51%) occurred in Khoraman at 80% FC with 50 mg L^{-1} Mn NPs. Other components such as geranyl acetate and linalyl acetate peaked in Fars under 80% FC with 25 mg L^{-1} Cu NPs (Table 5).

Extensive research has documented the chemical diversity of *M. communis* EO across various regions, including Iran [2, 63], Italy [68], and Tunisia [69]. Common major constituents identified in these studies include *α*-pinene, 1,8-cineole, limonene, linalool, *α*-terpineol, and linalyl acetate. Consistent with these findings, our results confirm significant chemical polymorphism among Iranian myrtle genotypes, highlighting the combined influence of genetic variation and environmental factors on EO composition. It is well established that while genetic makeup predominantly governs the biosynthesis of secondary metabolites, environmental stresses such as drought profoundly modulate both the quantity and profile of bioactive compounds [70]. Plants often respond to abiotic stress by producing a range of secondary metabolites, including phenolics, terpenoids, and alkaloids, which serve protective roles [71]. In this study, drought stress and foliar application of Cu NPs and Mn NPs elicited significant but genotype- and

compound-specific alterations in EO composition. These changes did not follow a uniform trend, underscoring the complexity of plant metabolic responses under stress. Similar patterns have been reported in other medicinal species; for example, drought increased EO content and modified its composition in *Lavandula angustifolia*, with elevated borneol and reduced camphor levels [72]. Likewise, in *A. dracunculus*, drought induced both quantitative shifts and the emergence of novel EO components [41]. These dynamic responses likely result from drought-mediated modulation of key enzymatic activities within secondary metabolite biosynthetic pathways [73].

NPs also influenced EO profiles by enhancing the relative abundance of certain constituents while suppressing others. Although the precise mechanisms remain to be fully elucidated, CuO NPs have shown potential as abiotic elicitors that stimulate the biosynthesis of bioactive metabolites [55]. However, excessive concentrations of CuO NPs and MnO NPs may exert phytotoxic effects, disrupting cellular metabolism and growth [60]. Recent evidence suggests Mn NPs mitigate drought stress by improving nutrient uptake, reducing oxidative damage, and modulating enzyme activities involved in secondary metabolism, thereby enhancing EO yield and composition in species such as *O. basilicum* and *L. angustifolia* [74, 75]. Supporting these findings, Nekoukhou et al. [23] reported that 80 mg/L CuO NPs increased EO yield and key components like geranyl acetate in *D. moldavica*. Similarly, in *O. basilicum*, CuO NPs treatments altered major EO constituents, including caryophyllene and eugenol, indicating a shift in EO quality [76, 77]. Overall, these results underscore the multifaceted influence of drought and NPs treatments on EO biosynthesis, highlighting the need for further mechanistic studies to optimize NPs use for enhancing phytochemical profiles under stress conditions.

4. Conclusion

This study highlights the genotype-specific physiological and biochemical responses of *M. communis* to drought stress and the modulatory effects of CuO NPs and MnO NPs. Among the two Iranian genotypes examined, ‘Khoraman’ consistently outperformed ‘Fars’ across various growth and stress tolerance parameters under both well-watered and drought-stressed conditions. Notably, Khoraman exhibited higher biomass accumulation, RWC, TPC, TFC, antioxidant capacity, and EO yield—particularly 1,8-cineole, a major pharmacologically active compound. In contrast, the Fars genotype demonstrated a more pronounced oxidative stress response, with elevated levels of MDA, proline, and higher activities of antioxidant enzymes such as SOD, APX, POD, along with greater proportions of linalool and linalyl acetate in its EO. Drought stress, particularly at 60% FC, led to marked reductions in growth parameters and leaf RWC, while increasing membrane injury (EL and MDA). In response, both genotypes activated their enzymatic (SOD, CAT, POD, APX) and non-enzymatic (phenolics, flavonoids, proline) antioxidant defenses. Although EO content increased under drought, the overall yield declined due to reduced biomass. Additionally, drought-induced modifications in EO composition were observed but did not follow a consistent or genotype-specific trend, suggesting a complex regulation of secondary metabolism under abiotic stress.

Importantly, foliar application of CuO NPs and MnO NPs—alone or in combination—significantly mitigated the adverse effects of drought by enhancing stress tolerance mechanisms. These NPs reduced membrane damage, improved osmotic adjustment, and amplified both enzymatic and non-enzymatic antioxidant systems. However, their effects were strongly influenced by genotype, drought intensity, NPs type, and application concentration. In Khoraman, CuO NPs, particularly at 50 mg L^{-1} and in combination with MnO, significantly enhanced leaf biomass and EO yield. In contrast, MnO NPs were more effective in Fars, particularly at 50 mg L^{-1}, indicating a genotype-specific NPs responsiveness. Furthermore, NPs treatments improved EO yield and modified its composition, likely through the stimulation of enzymes involved in secondary metabolite biosynthesis. While CuO appeared more effective in enhancing EO yield in Khoraman, MnO was better suited to Fars. The combination of both NPs often produced synergistic effects, especially under moderate to severe drought stress. In conclusion, the findings underscore the potential of CuO NPs and MnO NPs as innovative agro-nanotechnological tools for enhancing drought resilience and secondary metabolite production in medicinal plants. By modulating key physiological and biochemical pathways—including antioxidative defense, osmotic regulation, and EO biosynthesis—these NPs offer promising strategies to sustain productivity and phytochemical quality of *M. communis* under water-limited conditions. Further research is warranted to elucidate the molecular mechanisms underlying these responses and to optimize NPs formulations for field applications in medicinal plant cultivation.

Abbreviations

EOs
Essential Oils
ROS
Reactive Oxygen Species
H_2O_2
Hydrogen Peroxide
SOD
Superoxide Dismutase
CAT
Catalase
POD
Peroxidases
APX
Ascorbate Peroxidase
NPs
Nanoparticles
NP
Nanoparticle
Mn

Manganese
Cu
Copper
CRD
Completely Randomized Design
FC
Field Capacity
RWC
Relative Water Content
FW
Fresh Weight
TW
Turgid Weight
DW
Dry Weight
EL
Electrolyte Leakage
EC
Electrical Conductivity
MDA
Malondialdehyde
TPC
Total Phenolic Content
GAE
Gallic Acid Equivalents
TFC
Total Flavonoid Content
QE
Quercetin Equivalents
FRAP
Ferric Reducing Antioxidant Power
GC-FID
Gas Chromatograph Flame Ionization Detector
GC-MS
Gas Chromatography System Coupled with a Mass Spectrometer
ANOVA
Analysis of Variance
LSD
Least Significant Difference
$O_2^{\bullet -}$

Superoxide Radicals
PAL
Phenylalanine Ammonia Lyase
PPO
Polyphenol Oxidase

Declarations

CRediT authorship contribution statement

Reza Yarahmadi: Investigation, Writing original draft, Resources; Hasan Mumivand: Project administration, Validation, Methodology, Conceptualization, planned the experiments, and Writing–review & editing; Mohamad Reza Raji: Writing -Review & Editing, and Visualization; Abdollah Ehtesham Nia: Writing – review & editing, and Software; Ferdinando Branca: Writing – review & editing, and Data curation; Sergio Argento: Writing – review & editing, and Formal analysis.

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Author Contribution

Reza Yarahmadi: Investigation, Writing original draft, Resources; Hasan Mumivand: Project administration, Validation, Methodology, Conceptualization, planned the experiments, and Writing–review & editing; Mohamad Reza Raji: Writing -Review & Editing, and Visualization; Abdollah Ehtesham Nia: Writing – review & editing, and Software; Ferdinando Branca: Writing – review & editing, and Data curation; Sergio Argento: Writing – review & editing, and Formal analysis.

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Availability of data

No external datasets were generated or analyzed during the course of this study.

References

1. Bektaş Ü, Isak MA, Bozkurt T, Dönmez D, İzgü T, Tütüncü M, Simsek O. Genotype-specific responses to in vitro drought stress in myrtle (*Myrtus communis* L.): integrating machine learning techniques. PeerJ. 2024;12:e18081. doi.org/10.7717/peerj.18081.
2. Yarahmadi R, Mumivand H, Ehtesham Nia A, Raji MR, Argento S. Natural Diversity in Total Phenol, Flavonoids, Antioxidant Properties, and Essential Oil Composition of Iranian Populations of *Myrtus communis* L. Plants. 2024;13(24):3458. doi.org/10.3390/plants13243458.
3. Ghafouri F, Rahimmalek M. Genetic structure and variation in different Iranian myrtle (*Myrtus communis* L.) populations based on morphological, phytochemical and molecular markers. Ind Crop Prod. 2018;123:489–99. doi.org/10.1016/j.indcrop.2018.06.086.
4. Bajalan I, Pirbalouti AG. Variation in antibacterial activity and chemical compositions of essential oil from different populations of myrtle. Ind Crop Prod. 2014;61:303–7. doi.org/10.1016/j.indcrop.2014.07.023.
5. Rahimmalek M, Mirzakhani M, Pirbalouti A. Essential oil variation among 21 wild myrtle (*Myrtus communis* L.) populations collected from different geographical regions in Iran. Ind Crop Prod. 2013;51:328–33. doi.org/10.1016/j.indcrop.2013.09.010.
6. Mumivand H, Ebrahimi A, Shayganfar A, Khoshro HH. Screening of tarragon accessions based on physiological and phytochemical responses under water deficit. Sci rep. 2021b;11(1):17839. doi.org/10.1038/s41598-021-97388-z.
7. Hasanuzzaman M, Borhannuddin B, Faisal Z, Ali R, Sayed Mohammad M, Jubayer M, Masayuki F, Vasileios F. Reactive oxygen species and antioxidant defense in plants under abiotic stress: Revisiting the crucial role of a universal defense regulator. Antioxidants. 2020;9(8):681. doi.org/10.3390/antiox9080681.
8. Toscano S, Ferrante A, Romano D. Response of Mediterranean ornamental plants to drought stress. Hortic. 2019;5:6. doi.org/10.3390/horticulturae5010006.
9. Albergaria ET, Oliveira AFM, Albuquerque UP. The effect of water deficit stress on the composition of phenolic compounds in medicinal plants. S Afr J Bot. 2020;131:12–7. doi.org/10.1016/j.sajb.2020.02.002.
10. Djalovic I, Prasad P. The comparative effects of manganese nanoparticles and their counterparts (bulk and ionic) in *Artemisia annua* plants via seed priming and foliar application. Front Plant Sci. 2023;13:1098772. 10.3389/fpls.2022.1098772.
11. Kathirvelan P, Vaishnavi S, Manivannan V, Djanaguiraman M, Thiyageshwari S, Parasuraman P, Kalarani MK. Response of Maize (*Zea mays* L.) to Foliar-Applied Nanoparticles of Zinc Oxide and Manganese Oxide Under Drought Stress. Plants. 2025;14:732. doi.org/10.3390/plants14050732.
12. Ye Y, Keni CR, José A, Carolina Valdes IA, Medina-Velo RS, Turley Jose R, Peralta V, Jorge L, Gardea T. Manganese nanoparticles control salinity-modulated molecular responses in *Capsicum annuum* L. through priming: A sustainable approach for agriculture. ACS Sustain Chem Eng. 2020;8(3):1427–36. doi.org/10.1021/acssuschemeng.9b05615.

13. Dimkpa CO, Singh U, Adisa IO, Bindraban PS, Elmer WH, Gardea-Torresdey JL, White JC. Effects of manganese nanoparticle exposure on nutrient acquisition in wheat (*Triticum aestivum* L). Agron. 2018;8(9):158. doi.org/10.3390/agronomy8090158.
14. Ibrahim MH, Chee Kong Y, Mohd Zain NA. Effect of cadmium and copper exposure on growth, secondary metabolites and antioxidant activity in the medicinal plant Sambung Nyawa (*Gynura procumbens* (Lour.) Merr). Mol 22(10): 1623. doi.org/10.3390/molecules22101623
15. Cumplido-Nájera CF, González-Morales S, Ortega-Ortíz H, Cadenas-Pliego G, Benavides-Mendoza A, Juárez-Maldonado A. The application of copper nanoparticles and potassium silicate stimulate the tolerance to *Clavibacter michiganensis* in tomato plants. Scientia Hortic. 2019;245:82–9. doi.org/10.1016/j.scienta.2018.10.007.
16. Kasote DM, Lee JH, Jayaprakasha GK, Patil BS. Manganese oxide nanoparticles as safer seed priming agent to improve chlorophyll and antioxidant profiles in watermelon seedlings. Nanomater. 2021;11(4):1016. doi.org/10.3390/nano11041016.
17. Pradhan S, Prasun P, Shouvik M, Kushal K, Sneha J, Samapd S, Shuvrodeb R, Pratip P, Arunava G. Manganese nanoparticles: impact on non-nodulated plant as a potent enhancer in nitrogen metabolism and toxicity study both in vivo and in vitro. J Agric Food Chem. 2014;62(35):8777–85. doi.org/10.1021/jf502716c.
18. Van Nguyen D, Nguyen HM, Le NT, Nguyen KH, Nguyen HT, Le HM, Van Ha C. Copper nanoparticle application enhances plant growth and grain yield in maize under drought stress conditions. J Plant Growth Regul. 2022;41(1):364–75. doi.org/10.1007/s00344-021-10301-w.
19. Siddiqi K, Salahuddin A, Azamal H. Current status of plant metabolite-based fabrication of copper/copper oxide nanoparticles and their applications: a review. Biomater Res. 2020;24(1):1–15. 10.1186/s40824-020-00188-1.
20. Tian H, Mansour G, Khalil K. Manganese oxide nanoparticle-induced changes in growth, redox reactions and elicitation of antioxidant metabolites in deadly nightshade (*Atropa belladonna* L). Ind Crop Prod. 2018;126:403–14. doi.org/10.1016/j.indcrop.2018.10.042.
21. Sieprawska A, Rudolphi-Szydło E, Skórka M, Telk A, Filek M. Assessment of the oxidative stress intensity and the integrity of cell membranes under the manganese nanoparticles toxicity in wheat seedlings. Sci Rep. 2024;14:3121. https://doi.org/10.1038/s41598-024-53697-7.
22. Lafmejani ZN, Jafari AA, Moradi P, Moghadam AL. Impact of foliar application of copper sulphate and copper nanoparticles on some morpho-physiological traits and essential oil composition of peppermint (*Mentha piperita* L). Herba Pol. 2018;64(2). 10.2478/hepo-2018-0006.
23. Nekoukhou M, Fallah S, Pokhrel LR, Abbasi-Surki A, Rostamnejadi A. Foliar co-application of zinc oxide and copper oxide nanoparticles promotes phytochemicals and essential oil production in dragonhead (*Dracocephalum moldavica*). Sci Total Environ. 2024;906:167519. https://doi.org/10.1016/j.scitotenv.
24. Ritchie S, Henry W, Nguyen T, Scott Holaday A. Leaf water content and gas-exchange parameters of two wheat genotypes differing in drought resistance. Crop sci. 1990;30(1):105–11.

doi.org/10.2135/cropsci1990.0011183X003000010025x.

25. Lutts S, Kinet J, Jules B. NaCl-induced senescence in leaves of rice (*Oryza sativa* L.) cultivars differing in salinity resistance. Ann bot. 1996;78(3):389–98. doi.org/10.1006/anbo.1996.0134.
26. Wang F, Zeng B, Sun Z, Zhu C. Relationship between proline and Hg. Arch Environ Contam Toxicol. 2009;56:723–31. doi.org/10.1007/s00244-008-9226-2.
27. Bates L, Waldren RP, Treare ID. Rapid determination of free proline for water stress studies. Plant Soil. 1973; 39:205–207, 1973. doi.org/10.1007/BF00018060
28. Chang CJ, Koa CH. H_2O_2 metabolism during sense scene of rice leaves changes in enzyme activities in light and darkness. Plant Growth Regul. 1988;25:11–5. https://doi.org/10.1023/A:1005903403926.
29. Beauchamp C, Irwin F. Superoxide dismutase: improved assays and an assay applicable toacrylamide gels. Anal biochem. 1971;44(1):276–87. doi.org/10.1016/0003-2697(71)90370-8.
30. Aebi H. Catalase in vitro. In Methods in enzymology. Acad press. 1984;105:121–6.
31. Drotar A, Phelps P, Fall R. Evidence for glutathione peroxidase activities in cultured plant cells. Plant Sci. 1985;42(85):35–40. https://doi.org/10.1016/0168-9452.
32. Ranieri A, Castagna A, Pacini J, Baldan B, Mensuali Sodi A, Soldatini GF. Early production and scavenging of hydrogen peroxide in the apoplast of sunflower plants exposed to ozone. J Exp Bot. 2003;54:2529–40. https://doi.org/10.1093/jxb/ erg270.
33. Babaei-Rad S, Mumivand H, Mollaei S, Khadivi A, Postharvest. UV-B and UV-C treatments combined with fermentation enhance the quality characteristics of *Capparis spinosa* L. fruit, improving total phenols, flavonoids, anthocyanins, phenolic acids, and antioxidant activity. Food Chem. 2025b;483:144306. https://doi.org/10.1016/j.foodchem.2025.144306.
34. Velioglu Y, Mazza G, Gao L, Oomah B. Antioxidant activity and total phenolics in selected fruits, vegetables, and grain products. J Agric Food Chem. 1998;46:10: 4113–7. doi.org/10.1021/jf9801973.
35. Chang C, Ming-Hua Y, Hwei-Mei W, Jiing-Chuan C. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. J food drug anal. 2002;10:3.
36. Benzie I, Strain J. The ferric reducing ability of plasma (FRAP) as a measure of antioxidant power: the FRAP assay. Anal Biochem. 1996;70–6. doi.org/10.1006/abio.1996.0292.
37. Khanizadeh P, Mumivand H, Morshedloo MR, Maggi F. Application of Fe_2O_3 nanoparticles improves the growth, antioxidant power, flavonoid content, and essential oil yield and composition of *Dracocephalum kotschyi* Boiss. Front. Plant Sci. 2024;15:1475284. 10.3389/fpls.2024.1475284.
38. Khanizadeh P, Mumivand H, Morshedloo MR, Di Bella MC. Nanoceria versus bulk cerium oxide: differential effects on growth, antioxidants, pigments, and essential oil of *Dracocephalum kotschyi* Boiss. Chem Biol Technol Agric. 2026;13:17. https://doi.org/10.1186/s40538-026-00926-y.
39. Aghamirzaei H, Mumivand H, Nia AE, Raji MR, Maroyi A, Maggi F. Effects of micronutrients on the growth and phytochemical composition of basil (*Ocimum basilicum* L.) in the field and greenhouse

(hydroponics and soil culture). Plants. 2024;13(17):2498. https://doi.org/10.3390/plants13172498.

40. Tafreshi S, Aghaie P, Momayez H, Hejaziyan S. Response of in vitro-regenerated *Myrtus communis* L. shoots to PEG-induced water stress. Biocatal Agric Biotechnol. 2021;34(1):102033. 10.1016/j.bcab.2021.102033.
41. Mumivand H, Ebrahimi A, Morshedloo MR, Shayganfar A. Water deficit stress changes in drug yield, antioxidant enzymes activity and essential oil quality and quantity of Tarragon (*Artemisia dracunculus* L). Ind Crops Prod. 2021a;164:113381. doi.org/10.1016/j.indcrop.2021.113381.
42. Gorgini Shabankareh H, Khorasaninejad S, Soltanloo H. Physiological response and secondary metabolites of three lavender genotypes under water deficit. Sci rep. 2021;11(1):19164. doi.org/10.1038/s41598-021-98750-x.
43. Kapoor D, Bhardwaj S, Landi M, Sharma A, Ramakrishnan M, Sharma A. The impact of drought in plant metabolism: how to exploit tolerance mechanisms to increase crop production. Appl sci. 2020;10:5692. doi.org/10.3390/app10165692.
44. Linh TM, Mai NC, Hoe PT, Lien LQ, Ban NK, Hien L, Chau N, Van N. Metal-Based Nanoparticles Enhance Drought Tolerance in Soybean. J Nanomater. 2020;4056563. doi.org/10.1155/2020/4056563.
45. Ye Y, Illya A, Medina V, Keni C, Fabiola M, Jorge L, Gardea-Torresdey. Can abiotic stresses in plants be alleviated by manganese nanoparticles or compounds? Ecotoxicol environ saf. 2019;184:109671. doi.org/10.1016/j.ecoenv.2019.109671.
46. Sharma L, Dalal M, Verma R, Kumar S, Yadav S, Pushkar S, Kushwaha S, Bhowmik A. Chinnusamy V Auxin protects spikelet fertility and grain yield under drought and heat stresses in rice. Environ Exp Bot. 2018;150:9–24. doi.org/10.1016/j.envexpbot.2018.02.013.
47. Gören HK, Tan U. Unraveling the complexities of drought stress in cotton: a multifaceted analysis of selection criteria and breeding approaches. PeerJ. 2024;12:e17584. https://doi.org/10.7717/peerj.17584.
48. Ahmed F, Bilal J, Abdul R, Zia-ur-Rehman M. Applications of copper and silver nanoparticles on wheat plants to induce drought tolerance and increase yield. IET nanobiotechnol. 2021;15(1):68–78. doi.org/10.1049/nbt2.12002.
49. Etesami H, Fatemi H, Rizwan M. Interactions of nanoparticles and salinity stress at physiological, biochemical and molecular levels in plants: A review. Ecotoxicol Environ Saf 2021 225:112769. doi.org/10.1016/j.ecoenv.2021.112769
50. Landa P. Positive effects of metallic nanoparticles on plants: Overview of involved mechanisms. Plant Physiol Biochem. 2021;161:12–24. doi.org/10.1016/j.plaphy.2021.01.039.
51. Yang X, Lu M, Wang Y, Wang Y, Liu Z, Chen S. Response mechanism of plants to drought stress. Hortic. 2021;7(3):50. doi.org/10.3390/horticulturae7030050.
52. Azizi S, Kouchaksaraei M, Hadian J, Abad A, Sanavi S, Ammer C, Bader M. Dual inoculations of arbuscular mycorrhizal fungi and plant growth-promoting rhizobacteria boost drought resistance

and essential oil yield of common myrtle. Ecol Manag. 2021;497(123–138):119478. 10.1016/j.foreco.2021.119478.

53. Gupta A, Rico-Medina A, Caño-Delgado AI. The physiology of plant responses to drought. Science. 2020;368(6488):266–9. 10.1126/science.aaz7614.
54. Babaei Rad S, Mumivand H, Mollaei S, Khadivi A. Effect of drying methods on phenolic compounds and antioxidant activity of *Capparis spinosa* L. fruits. BMC Plant Biolo. 2025a;25(1):133. https://doi.org/10.1186/s12870-025-06110-y.
55. Nazir S, Jan H, Zaman G, Khan T, Ashraf H, Meer B, Abbasi BH. Copper oxide (CuO) and manganese oxide (MnO) nanoparticles induced biomass accumulation, antioxidants biosynthesis and abiotic elicitation of bioactive compounds in callus cultures of *Ocimum basilicum* (Thai basil). Artif Cells Nanomed Biotechnol. 2021;49(1):625–33. doi.org/10.1080/21691401.2021.1984935.
56. Schmidt SB, Husted S. The biochemical properties of manganese in plants. Plants. 2019;8(10):381. doi.org/10.3390/plants8100381.
57. Talbi S, Rojas J, Sahrawy M, Rodríguez-Serrano M, Cárdenas K, Debouba M, Sandalio L. Effect of drought on growth, photosynthesis and total antioxidant capacity of the Saharan plant *Oudeneya africana*. Environ Exp Bot. 2020;176(4):104099. 10.1016/j.envexpbot.2020.104099.
58. Beiranvandi M, Akbari N, Ahmadi A, Mumivand H, Nazarian F. Biochar and super absorbent polymer improved growth, yield, and phytochemical characteristics of Satureja rechingeri Jamzad in water-deficiency conditions. Ind Crops Prod. 2022;183:114959. https://doi.org/10.1016/j.indcrop.2022.114959.
59. Grodetskaya TA, Peter M, Evlakov A, Fedorova V, Mikhin O, Zakharova E, Kolesnikov A, Evtushenko A, Alexander Gusev A. Influence of Copper Oxide Nanoparticles on Gene Expression of Birch Clones In Vitro under Stress Caused by Phytopathogens. Nanomaterials. 2022;12(5):864. doi.org/10.3390/nano12050864.
60. Javed R, Mohamed A, Yücesan B, Gürel E, Kausar R, Zia M. CuO nanoparticles significantly influence in vitro culture, steviol glycosides, and antioxidant activities of *Stevia rebaudiana* Bertoni. Plant Cell Plant Cell Tissue Organ Cult. 2017;131:611–20. doi.org/10.1007/s11240-017-1312-6.
61. Zafar H, Ali A, Zia M. CuO nanoparticles inhibited root growth from Brassica nigra seedlings but induced root from stem and leaf explants. Appl Biochem Biotechnol. 2017;181(1):365–78. doi.org/10.1007/s12010-016-2217-2.
62. Nooshkam A, Mumivand H, Hadian J, Alemardan A, Morshedloo MR. Drug yield and essential oil and carvacrol contents of two species of *Satureja* (*S. khuzistanica* Jamzad and *S. rechingeri* Jamzad) cultivated in two different locations. J. Appl. Res. Med. Aromat. Plants. 2017; 6:126–130. https://doi.org/10.1016/j.jarmap.2017.04.002
63. Shahbazian D, Karami A, Raouf Fard F, Eshghi S, Maggi F. Essential Oil Variability of Superior Myrtle (Myrtus communis L.) Accessions Grown under the Same Conditions. Plants. 2022;11:3156. doi.org/10.3390/plants11223156.

64. Dalvand S, Mumivand H, Zahedi B, Nia AE, Argento S. Modulation of growth, oxidative stress damage, and trigonelline accumulation in drought-stressed fenugreek by nitrogen-containing biostimulants. Ind Crops Prod. 2026;247:123554. https://doi.org/10.1016/j.scienta.2026.114893.
65. Roy A, Khan A, Ahmad I, Alghamdi S, Rajab B, Babalghith A, Islam M. Flavonoids a bioactive compound from medicinal plants and its therapeutic applications. Biomed Res Int. 2022;1:5445291. doi.org/10.1155/2022/5445291.
66. Akbarzadeh S, Morshedloo MR, Behtash F, Mumivand H, Maggi F. Exogenous β-Aminobutyric Acid (BABA) Improves the Growth, Essential Oil Content, and Composition of Grapefruit Mint (*Mentha suaveolens × piperita*) under Water Deficit Stress Conditions. Hortic. 2023;9:354. doi.org/10.3390/horticulturae9030354.
67. Hernández-Fuentes AD, López-Vargas ER, Pinedo-Espinoza JM, Campos-Montiel RG, Valdés-Reyna J, Juárez-Maldonado A. Postharvest behavior of bioactive compounds in tomato fruits treated with Cu nanoparticles and NaCl stress. Appl Sci. 2017;7:980. doi.org/10.3390/app7100980.
68. Usai M, Marchetti M, Culeddu N, Mulas M. Chemotaxonomic evaluation by volatolomics analysis of fifty-two genotypes of *Myrtus communis* L. Plants. 2020;9(10):1288. doi.org/10.3390/plants9101288.
69. Messaoud C. Fruit color, chemical and genetic diversity and structure of *Myrtus communis* L. var. italic Mill morph populations Biochem Syst Ecol. 2011;39:570–80. doi.org/10.1016/j.bse.2011.08.008. Béjaoui ABoussaid M.
70. Benlarbi KH, Elmtili N, Macías FA, Galindo JG. Influence of in vitro growth conditions in the production of defence compounds in *Mentha pulegium* L. Phytochem. Lett. 2014;8:233–44. https://doi.org/10.1016/j.phytol.2014.03.007.
71. Mumivand H, Ebrahimi A, Bakhshipoor F. Unlocking *Petroselinum crispum* (Mill.) Fuss's potential: A study of morphological, agronomic, biochemical, and nutritional diversity, alongside essential oil composition, to strengthen breeding programs. Sci. Hortic. 2026; 363:114893. https://doi.org/10.1016/j.scienta.2026.114893
72. García-Caparrós P, Romero MJ, Llanderal A, Cermeño P, Lao MT, Segura ML. Effects of drought stress on biomass, essential oil content, nutritional parameters, and costs of production in six Lamiaceae species. Water. 2019;11(3):573. https://doi.org/10.3390/w11030573.
73. Jadidi M, Mumivand H, Nia AE, Shayganfar A, Maggi F. UV-A and UV-B combined with photosynthetically active radiation change plant growth, antioxidant capacity and essential oil composition of Pelargonium graveolens. BMC Plant Biol. 2023;23(1):555.
74. El-Naggar HM, Osman AR. Enhancing growth and bioactive metabolites characteristics in Mentha pulegium L. via silicon nanoparticles during in vitro drought stress. BMC Plant Biol. 2024;24(1):657. 10.1186/s12870-024-05313-z.
75. Salehi H, Cheheregani Rad A, Raza A, Djalovic I, Prasad PV. The comparative effects of manganese nanoparticles and their counterparts (bulk and ionic) in *Artemisia annua* plants via seed priming and foliar application. Front Plant Sci. 2023;13:1098772. 10.3389/fpls.2022.1098772.

76. Kumar S, Kaur V, Dwivedi A, Dubey A, Vajpayee P. Impact of copper oxide nanoparticles on uptake, cellular distribution, growth, ionome, antioxidant defense system and essential oil composition of *Ocimum basilicum* L. Environ. Sustain. 2025;1–19. https://doi.org/10.1007/s42398-025-00366-8.
77. Vijayakumar G, Kesavan H, Kannan A, Arulanandam D, Kim JH, Kim KJ, Rangarajulu SK. Phytosynthesis of copper nanoparticles using extracts of spices and their antibacterial properties. Processes. 2021; 9(8): 1341. https://www.mdpi.com/2227-9717/9/8/1341

Tables

Tables are available in the Supplementary Files section.

Figures

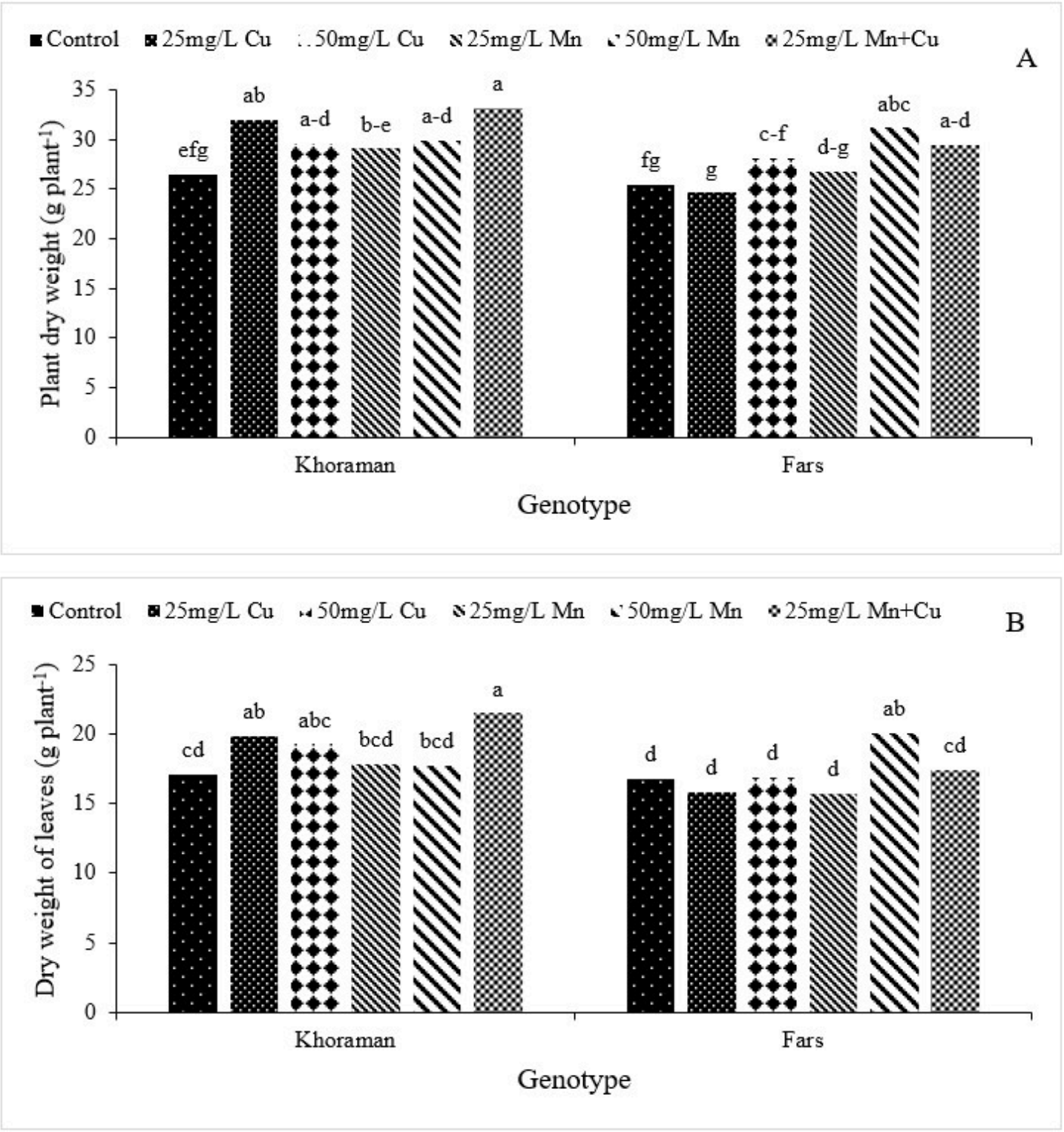


Figure 1

Interaction effect of genotype and foliar application of Mn and Cu nanoparticles on A) plant dry weight and B) dry weight of leaves.

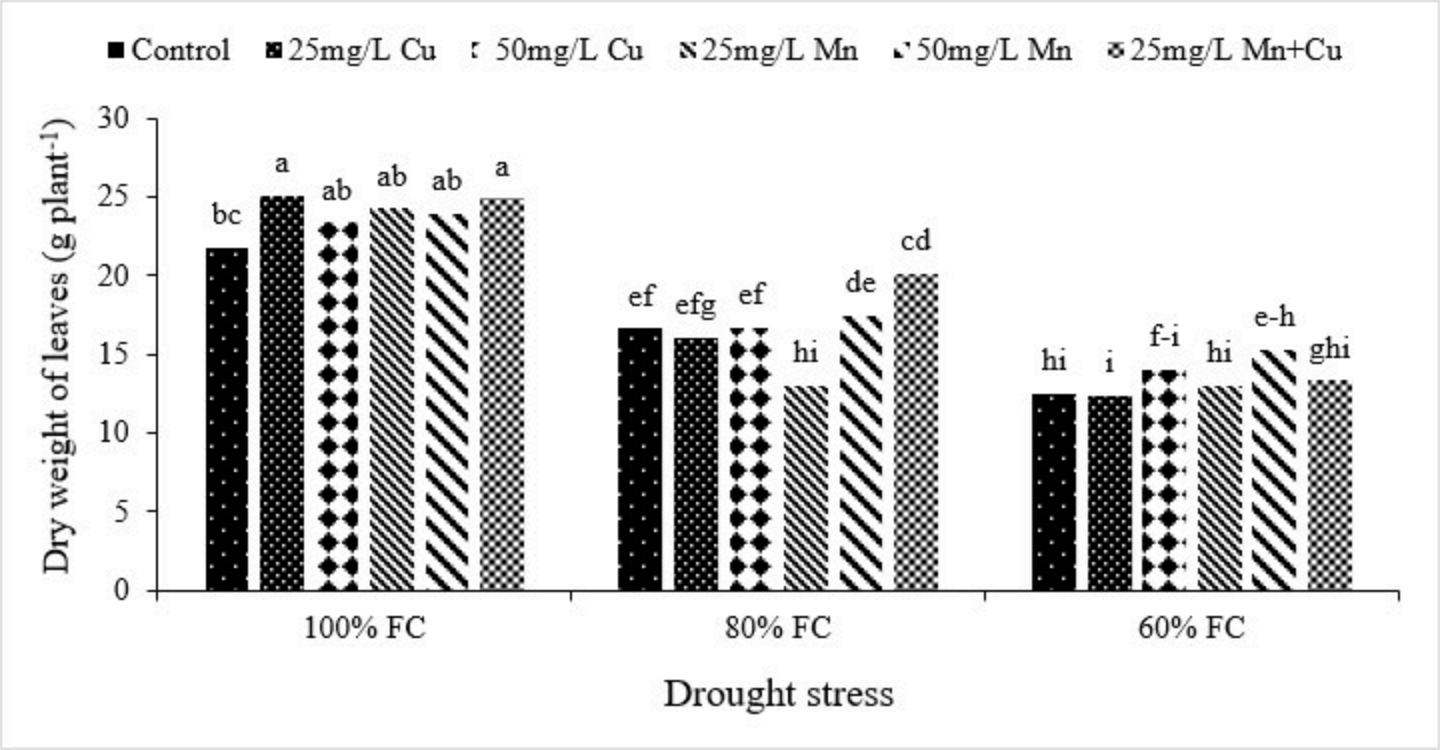
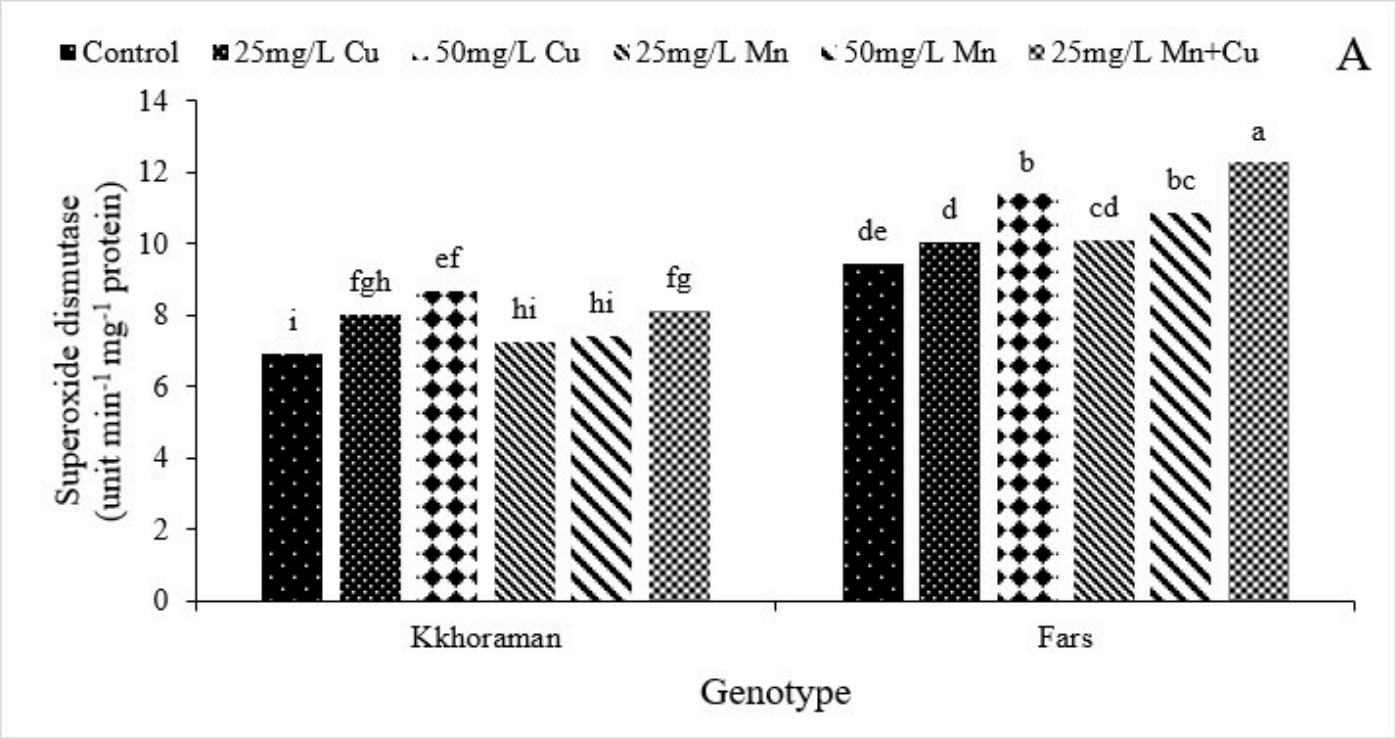


Figure 2

Interaction effect of drought stress and foliar application of Mn and Cu nanoparticles on dry weight of leaves.



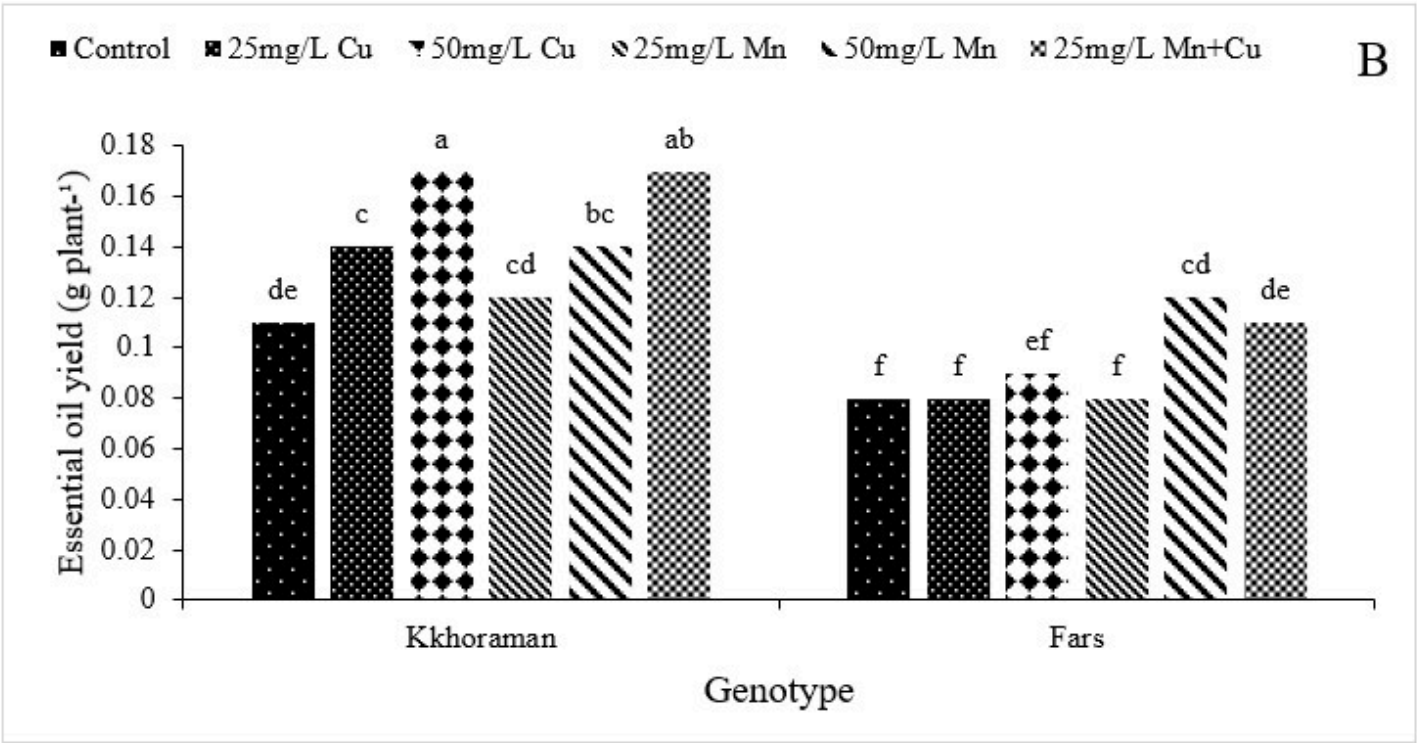


Figure 3

Interaction effect of genotype and foliar application of Mn and Cu nanoparticles on A) superoxide dismutase activity and B) essential oil yield.

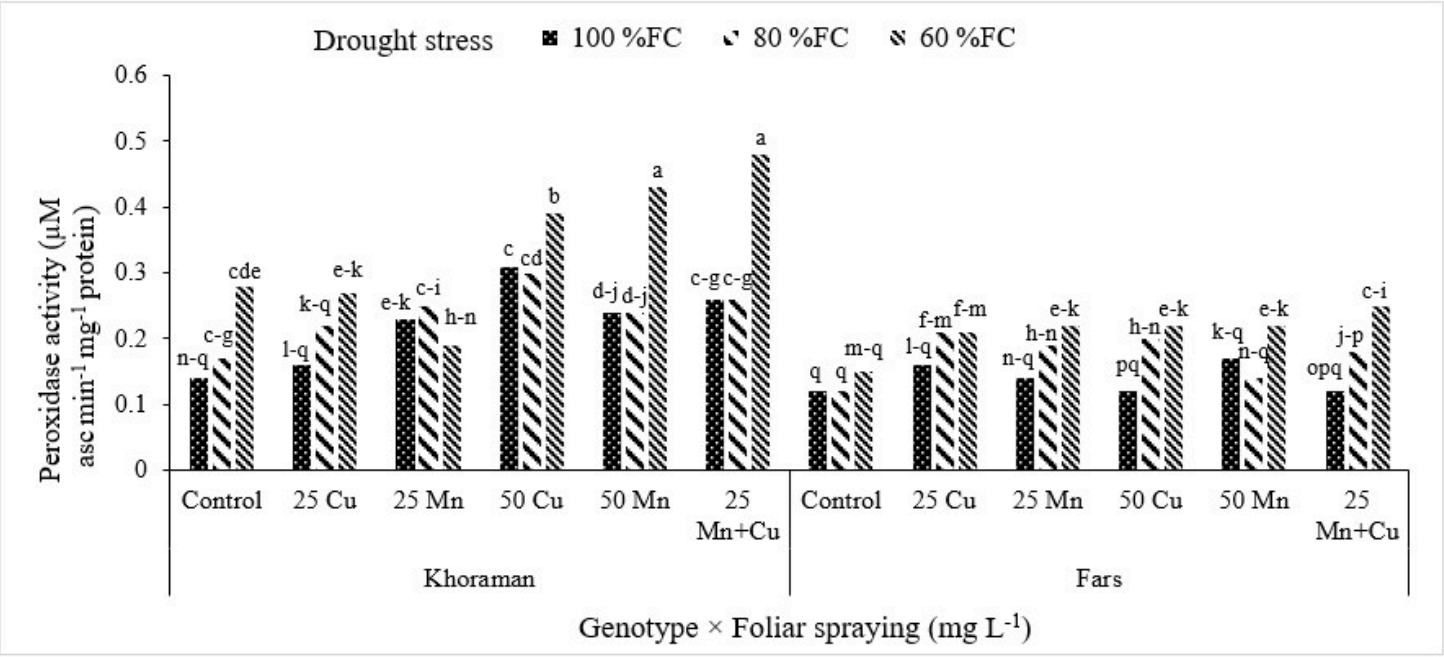
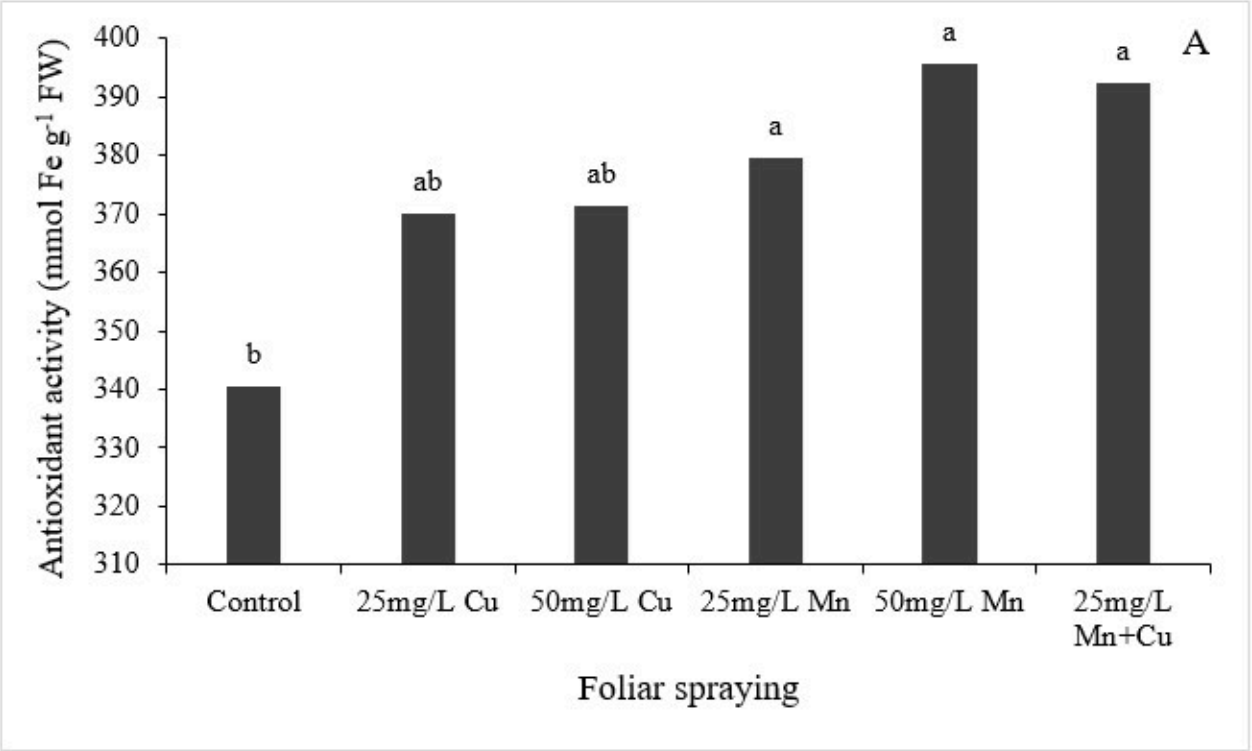


Figure 4

Interaction effect of genotype × drought stress × foliar application of Mn and Cu nanoparticles peroxidase enzyme activity



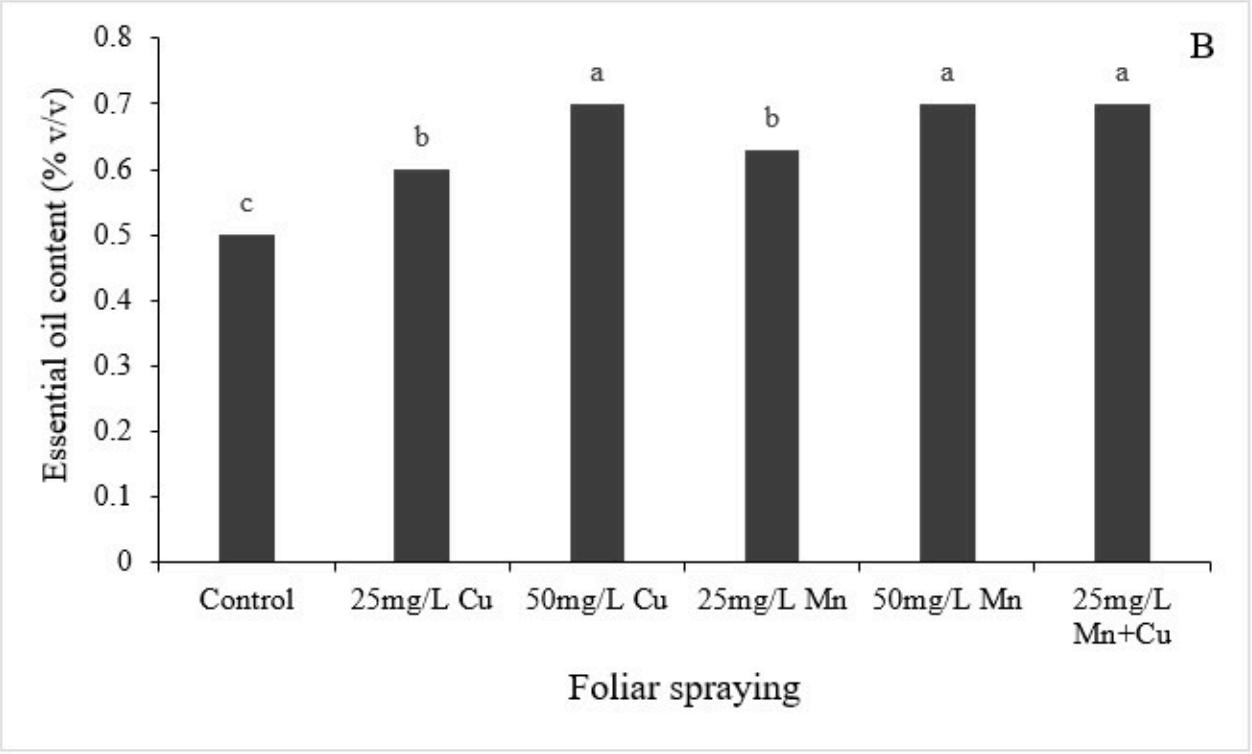


Figure 5

Effect of foliar spraying of Mn and Cu nanoparticles on A) the antioxidant activity and B) essential oil content

Supplementary Files

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- Table.docx
- SupplumentaryData.docx