International Journal of Horticultural Science and Technology
Journal homepage: https://ijhst.ut.ac.ir

Manganese Sulfate Foliar Application Improves Cold Stress Tolerance in Yaqouti Grapevines through Physiological Adaptations

Khaled Gavili Kilaneh[1], Mohammad Satari[1]*, Rouhollah Karimi[2]

1 Department of Biology, Faculty of Basic Sciences, Malayer University, Malayer, Iran
2 Department of Horticulture and Landscape Engineering, Faculty of Agriculture, Malayer University, Iran

*Corresponding author's email: m.satari@malayeru.ac.ir

ARTICLE INFO

Article history:

Received: 8 July 2025,
Received in revised form: 9 July 2025,
Accepted: 19 July 2025,

Article type:

Research paper

Keywords:

Cold adaptation,
Grapes,
Nutrition,
Soluble sugar,
Total phenols,

ABSTRACT

This study evaluated the efficiency of manganese sulfate ($MnSO_4$) foliar application in enhancing cold tolerance of Yaqouti grapevines by regulating antioxidant enzymes and osmolyte accumulation. Vines were treated with MnSO4 (0, 4000, or 8000 ppm) and exposed to no stress (24 °C) or chilling stress (4 °C) conditions. Physiological and biochemical parameters were analyzed. They included chlorophyll content, ion leakage, malondialdehyde (MDA), hydrogen peroxide (H2O2), osmolytes, phenolic compounds, and antioxidant enzymes. Results demonstrated that MnSO4 alleviated cold-induced damage. While chilling stress increased ion leakage and MDA (indicating membrane damage), $MnSO_4$ application significantly reduced these effects. The 4000 ppm $MnSO_4$ treatment enhanced soluble sugar content compared to cold-stressed samples with no MnSO4 treatment. Furthermore, it increased total phenolic compounds and flavonoids by 35 and 27%, respectively, with more pronounced effects at 8000 ppm. Notably, 4000 ppm MnSO4 boosted catalase (CAT) and guaiacol peroxidase (GPX) activities by 60 and 40% under cold stress compared to untreated stressed vines. These findings indicated that manganese sulfate improves cold resilience in Yaqouti grapevines by enhancing antioxidant defense systems and promoting adaptive metabolite accumulation, thereby stabilizing cellular membranes under low-temperature stress.

Introduction

Cold stress is a major crucial abiotic constraint on plant productivity. It substantially reduces yield and limits the geographical distribution of plants. As one of the most significant factors inhibiting plant growth and development, cold-induced cellular damage during sensitive phenological stages markedly compromises global crop performance. Plants activate adaptive responses to cold exposure through coordinated morphological, physiological, biochemical, and molecular adjustments (Jahed et al., 2023; Cao et al., 2023). These comprehensive responses effectively counteract cold-induced impairments, including oxidative stress, disruption of membrane structure, reduced photosynthetic efficiency, and disrupted nutrient/water uptake dynamics (Qari et al., 2022).

The cold acclimation mechanism comprises several interconnected processes: decreased tissue water content, accumulation of compatible osmolytes (particularly soluble carbohydrates and specific amino acids), modified phytohormone profiles (characterized by increased abscisic acid and decreased gibberellin concentrations), and enhanced deposition of protective phenolic compounds (notably suberin and lignin) in vulnerable tissues. Concurrently, plants significantly elevate the activity of crucial antioxidant enzymes, including superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), and ascorbate peroxidase (APX), which

COPYRIGHT
© 2027 The author(s). This is an openaccess article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other medium is permitted, provided the original author(s) and source are cited, in accordance with accepted academic practice. No permission is required from the authors or the publishers.

collectively function to scavenge reactive oxygen species (ROS), thereby safeguarding the photosynthetic apparatus from oxidative damage (Zhou et al., 2025).

One pivotal approach to enhancing plant cold tolerance involves strategic nutrient management. As a sustainable approach for cold stress mitigation, this method reduces physiological sensitivity to low temperatures, thereby enhancing stress adaptation capacity (Ihtisham et al., 2023; Karimi et al., 2014). Proper nutritional supplementation during the growing season augments carbohydrate reserves and improves pre-stress physiological status, consequently elevating cold tolerance in grapevines (Adhikari et al., 2022).

Notably, foliar application of micronutrients has shown significant benefits for plant growth across various species. Contemporary research demonstrates that applying essential micronutrients including boron, copper, iron, and zinc via foliar spraying enhances vegetative growth, fruit yield, and economic profitability in horticultural crops such as strawberry (Anchal et al., 2023). Among these micronutrients, manganese (Mn^{2+}) represents a crucial trace element that activates numerous enzymes indispensable for fundamental biochemical processes, encompassing oxidation, reduction, decarboxylation, and hydrolytic reactions (Zhou et al., 2025).

Manganese (Mn^{2+}) is a key nutrient that regulates both respiratory and photosynthetic enzymes while modulating nitrate accumulation in plant tissues. It acts as an essential cofactor for key enzymes involved in photosynthesis, nitrogen assimilation, and oxidative stress mitigation, playing a pivotal role in maintaining plant metabolic homeostasis (Khoshru et al., 2023). As a vital element for plant metabolism and development, Mn^{2+} serves as an indispensable cofactor, either directly or indirectly, for approximately 35 cellular enzymes. This micronutrient performs two primary biochemical functions: (1) acting as a catalytically active metal and (2) functioning as an enzymatic activator.

The catalytic roles include manganese-containing superoxide dismutase (Mn-SOD), which scavenges free radicals, as well as oxalate oxidase and the oxygen-evolving complex in photosystem II. As an activator, Mn^{2+} enhances the activity of key enzymes such as malic enzyme, isocitrate dehydrogenase, PEP carboxykinase, and phenylalanine ammonia lyase. This activation extends to enzymes involved in nitrogen metabolism (glutamine synthetase, arginase), gibberellic acid biosynthesis, RNA polymerase function, and fatty acid biosynthesis (Adamczyk-Szabela et al., 2020).

Furthermore, Mn^{2+} strengthens plant defense systems against abiotic stresses through multiple mechanisms. It precisely regulates the activity of photosynthetic and respiratory enzymes while controlling nitrate accumulation under stress conditions. Additionally, Mn^{2+} improves membrane stability, enhances chlorophyll retention, and boosts nitrate reductase activity during stress exposure (Parveen et al., 2024). Manganese (Mn^{2+}) applications have become increasingly prevalent for mitigating abiotic stresses due to their protective properties. Under stress conditions, Mn^{2+} enhances root system development while simultaneously decreasing lignin and protein accumulation. Furthermore, it mediates the redistribution of essential cations, including calcium (Ca^{2+}), manganese (Mn^{2+}), sodium (Na^{+}), and potassium (K^{+}), between root and shoot tissues (Faisal et al., 2021). Optimal Mn^{2+} supplementation under abiotic stress conditions not only alleviates manganese deficiency but also upregulates superoxide dismutase (SOD) expression at transcriptional levels. This dual action enhances ROS scavenging and promotes manganese-dependent protein synthesis, preserving cellular integrity (Ye et al., 2019). Empirical evidence supports the stress-protective role of Mn^{2+}: foliar application in mung bean plants markedly enhanced salt stress tolerance (Shahi and Srivastava, 2018). Correspondingly, salt-stressed Ornithogalum 'Shahpasand' displayed a 6-8% elevation in leaf manganese content relative to control plants, indicating a direct relationship between Mn^{2+} accumulation and salinity adaptation (Salachna and Piechocki, 2015). This beneficial pattern persists across diverse plant species, with numerous studies validating the effectiveness of Mn^{2+} in alleviating various abiotic stresses, including drought, elevated temperatures, and salinity (Parveen et al., 2024; El-Beltagi et al., 2024).

Previous investigations on grapevine cultivars have consistently demonstrated that low temperature exposure induces distinct temperature-dependent biochemical responses. Notably, chilling stress reportedly increased ion leakage, malondialdehyde accumulation, and reactive oxygen species production, while cold-tolerant cultivars consistently displayed heightened antioxidant activity (Beheshti Rooy et al., 2017). These findings establish crucial thresholds for membrane damage while emphasizing the fundamental role of antioxidant systems in cold tolerance mechanisms.

Comprehensive analysis of seasonal dynamics has revealed significant fluctuations in soluble proteins, total phenols, and malondialdehyde (MDA) content in relation to cold hardiness among grapevine cultivars (Karimi et al., 2014). The research identified clear cultivar-specific resistance patterns, with 'Red' and 'Khalili' demonstrating maximum cold tolerance during January, while 'Ruby' and 'Pearl' exhibited minimal resistance. Throughout cold acclimation, gradual accumulation of both soluble proteins and phenolic compounds was observed, reaching maximum concentrations during

mid-winter (January). Remarkably, cold-resistant cultivars (‘Red’ and ‘Khalili’) maintained substantially higher levels of these cryoprotective compounds compared to susceptible genotypes, establishing a direct positive correlation with frost tolerance. In contrast, MDA concentrations, a well-established biomarker of membrane lipid peroxidation, showed minimal levels at both dormancy onset and termination, but displayed significant increases during January across all cultivars, mirroring seasonal oxidative stress patterns.

The ongoing impacts of climate change and global warming have led to increased frequency of late spring frost events, creating substantial risks for grapevine reproductive structures and consequent yield reductions. Given the documented role of magnesium ions in enhancing cold tolerance across various plant species, manganese sulfate has emerged as a promising candidate for improving cold resilience in grapevines. Importantly, current literature reveals a critical knowledge gap: no studies to date have systematically evaluated the effects of foliar manganese sulfate application on spring frost tolerance in woody plants, including grapevines. We hypothesize that manganese sulfate supplementation significantly enhances the vine's antioxidant defense system under cold stress conditions. Therefore, this study specifically aims to assess the efficacy of foliar-applied manganese sulfate ($MnSO_4$) in improving cold tolerance in the Yaqouti grape cultivar through two primary mechanisms: modulation of key antioxidant enzyme activities and promotion of protective osmolyte accumulation.

Materials and Methods

Plant materials and growth conditions

This research was conducted in 2024 using rooted seedlings of the Yaqouti grape cultivar (*Vitis vinifera* L.) in a factorial (3 × 2) experiment arranged in a completely randomized design with three replications. The study was carried out in the educational-research greenhouse of Malayer University. The first factor consisted of three concentrations of manganese sulfate (4000 and 8000 ppm), and the second factor comprised two temperature regimes: 24 °C (control) and 4 °C (chilling stress). At the beginning of the experiment in March 2024, rooted cuttings were transferred into 10-liter plastic pots filled with a 1:1 mixture of cocopeat and perlite. Plants were irrigated and fertilized twice per week with a nutrient solution containing 0.5 g L^{-1} of NPK 20-20-20 fertilizer (Nutripod, Mersin Dasht Co., Karaj, Iran). During the vegetative growth phase, powdery mildew was managed by spraying liquid sulfur, and insect pests were controlled using Confidor. Foliar applications of manganese sulfate were performed at concentrations of 4 and 8 ppm. Spraying was conducted in three intervals, one day apart, using a 500 mL hand sprayer. Control plants were sprayed with distilled water containing an equal volume of liquid surfactant (referred to as “hairs” in the original). Three days after the final foliar application, half of the control vines and half of the manganese-treated vines (4 and 8 ppm) were subjected to low temperature (4 °C) in a controlled cold chamber for 12 h. The initial temperature of the cold chamber was adjusted to match ambient conditions at the time of pot transfer, then gradually reduced at a rate of 2 °C h^{-1} until it reached 4 °C, where it was maintained for 12 h. After the chilling treatment, the temperature was gradually increased and returned to room conditions over a 6 h period. Following an additional 3 h recovery at room temperature (approximately 24 °C), fully expanded upper leaves were harvested for subsequent physiological and biochemical analyses.

Laboratory treatments

In a separate but related study, additional experiments were conducted on two-year-old potted Yaqouti grapevines grown under controlled greenhouse conditions at Malayer University. The vines were planted in 10-liter pots filled with a growth medium consisting of 50% sandy loam soil, 25% peat, and 25% well-rotted animal manure. They were irrigated three times per week and fertilized with 0.5 g L^{-1} of NPK 20-20-20. Standard cultural practices were maintained until the vines reached the 15-leaf stage. The experimental design followed a factorial (3 × 2) arrangement in a completely randomized layout with three replications (each replication consisting of two pots). The treatments were the same as described earlier: three concentrations of manganese sulfate (0, 4, and 8 ppm) and two temperature regimes (24 °C and 4 °C). Three days after manganese application, a subset of each treatment group was exposed to 12 h of low temperature (4 °C) in a cold chamber with a light intensity of approximately 200 µmol m^{-2} s^{-1}. The temperature was reduced at a rate of 2 °C h^{-1} until it reached 4 °C, then gradually increased to the ambient level (24 ± 2 °C) over 6 h. After a 3-day recovery period in the greenhouse, fully developed upper leaves were sampled for physiological and biochemical analyses.

Chlorophyll index

The chlorophyll content of fully expanded leaves was measured using a chlorophyll meter (SPAD, Atago Co., Japan), following the manufacturer’s instructions.

Proline measurement

Since proline accumulation is a well-established physiological indicator of stress response, its content

was quantified following the method of Bates et al. (1973). Fresh leaf tissue (0.5 g) was accurately weighed (±0.001 g) using an analytical balance, flash-frozen in liquid nitrogen, and homogenized in a porcelain mortar with 10 mL of 3% sulfosalicylic acid. The homogenate was centrifuged at 10,000 rpm, and 2 mL of the resulting supernatant was reacted with 2 mL of glacial acetic acid and 2 mL of freshly prepared ninhydrin reagent (1.25 g ninhydrin dissolved in 30 mL glacial acetic acid and 20 mL of 6 M phosphoric acid). The reaction mixture was incubated at 40 °C for 15 min in a fume hood with constant stirring, then transferred to a boiling water bath (100 °C) for 1 h. After incubation, the samples were cooled in an ice-water bath for 10 min. Subsequently, 4 mL of toluene was added, and the mixture was vigorously vortexed to ensure complete biphasic separation. The upper toluene phase containing the chromophore was carefully collected, and absorbance was measured at 520 nm against a toluene blank using a UV-Vis spectrophotometer.

Total soluble sugar measurement

To quantify soluble sugar content, one fully expanded leaf was collected from each vine. A 0.5 g sample of fresh tissue was homogenized with 5 mL of 95% ethanol, and the supernatant was collected. This procedure was repeated twice with 70% ethanol, and all extracts were combined and centrifuged at 6000 rpm for 15 min. The resulting supernatant was stored at 4 °C until analysis. Soluble sugar content was determined using the anthrone method (Irigoyen et al., 1992). A 0.1 mL aliquot of the alcoholic extract was mixed with 3 mL of freshly prepared anthrone reagent (150 mg anthrone in 100 mL of 72% sulfuric acid). The mixture was heated in a boiling water bath for 10 min and then cooled to room temperature. Absorbance was measured at 625 nm using a spectrophotometer. A glucose standard curve (0–120 mg L^{-1}) was used for calibration, and sugar content was expressed as milligrams per gram fresh weight (mg g^{-1} FW), calculated using the following formula:

$$Soluble\ carbohydrates\ (mg\ g^{-1}\ FW) = \frac{Absorbance \times dilution\ factor \times 1000}{sample\ weight \times standard\ factor}$$

Determination of relative leaf water content (RWC)

Sampling was conducted using sterilized scissors to excise the most recently developed leaf (reference leaf) from each vine. Immediately after detachment, samples were placed in ice to prevent water loss, and fresh weight (FW) was recorded in the laboratory using a high-precision analytical balance (±0.0001 g), ensuring the leaves remained intact. Subsequently, the leaf samples were submerged in distilled water and kept at 4 °C for 24 h to achieve full turgidity. After incubation, turgid weight (SW) was measured, and samples were dried in an oven at 70 °C for 24 h to obtain dry weight (DW). Relative water content was calculated using the following formula (Ritchie et al., 1990):

$$RWC\ (\%) = [\frac{FW - DW}{SW - DW}] \times 100$$

Measurement of ion leakage

To evaluate membrane stability, ten leaf discs (1 cm^2 each) were excised from fully developed leaves of each treatment and immediately transferred to the laboratory. Discs were placed into 50 mL flasks containing 15 mL of double-distilled water and gently agitated on a rotary shaker at room temperature for 24 h. Electrical conductivity (EC_1) of the bathing solution was measured using a conductivity meter. The same flasks were then autoclaved at 121 °C and 1 atm for 15 min. After cooling to room temperature, the final conductivity (EC_2) was recorded. Relative electrolyte leakage was calculated using the formula (Sairam et al., 2004):

$$REL\ (\%) = (\frac{EC_1}{EC_2}) \times 100$$

Lipid peroxidation and malondialdehyde (MDA) content

Lipid peroxidation was quantified by measuring malondialdehyde (MDA) levels (Buege and Aust, 1978). Fresh leaf tissue (0.5 g) was homogenized in 5 mL of 20% trichloroacetic acid (TCA) containing 0.5% thiobarbituric acid (TBA). The mixture was centrifuged at 6000 ×g for 15 min. The supernatant was incubated in a water bath at 80 °C for 25 min, then rapidly cooled using ice. Following a second centrifugation, absorbance was read at 532 nm and corrected by subtracting nonspecific absorbance at 600 nm. MDA content was calculated using an extinction coefficient of 155 mM^{-1} cm^{-1} and expressed as μmol g^{-1} fresh weight (Buege and Aust, 1978).

Quantification of hydrogen peroxide (H_2O_2)

Hydrogen peroxide concentration was determined following the method of Velikova and Loreto (Velikova et al., 2001). Fresh leaf tissue (0.3 g) was homogenized in 3 mL of 1% TCA and centrifuged at 6000 rpm for 10 min at 4 °C. Then, 0.75 mL of the supernatant was mixed with 0.75 mL of 10 mM potassium phosphate buffer (pH 7.0) and 1.5 mL of 1 M potassium iodide. Absorbance was recorded at 390 nm using a spectrophotometer. H_2O_2 concentration was calculated from a standard curve prepared with concentrations ranging from 100 to 1000 μmol mL^{-1}.

Total phenolic content

Total phenolic content was measured according to Velioglu et al. (1998). Fresh leaf tissue (0.5 g) was homogenized in 4 mL of ethanol, and the homogenate was centrifuged at 9500 rpm for 20 min. An aliquot of 300 µL of the supernatant was mixed with 1200 µL of 7% Na_2CO_3 and 500 µL of 10% Folin–Ciocalteu reagent. The mixture was incubated in darkness for 20 min, and absorbance was measured at 725 nm. Phenolic content was expressed as mg gallic acid equivalents (GAE) per gram fresh weight, based on a gallic acid standard curve.

Total flavonoid content

Total flavonoid content was determined using the aluminum chloride colorimetric method (Chang et al., 2002). Briefly, 0.1 mL of 10% aluminum chloride, 0.1 mL of 1 M potassium acetate, and 2.8 mL of distilled water were mixed in a test tube, followed by the addition of 0.5 mL of methanolic leaf extract. The mixture was incubated in the dark for 30 min, and absorbance was measured at 415 nm. Flavonoid content was expressed as mg quercetin equivalents (QE) per gram fresh weight.

Determination of soluble protein content

Soluble protein content was measured using the Bradford assay (Bradford, 1976). Fresh leaf tissue (0.5 g) was homogenized in 5 mL of 1 mM Tris buffer (pH 7.0). After centrifugation at 6000 rpm for 20 min, 100 µL of the supernatant was mixed with 5 mL of Bio-Rad reagent (containing 10% glacial acetic acid, 25% ethanol, 65% distilled water, and 0.1% Coomassie Brilliant Blue G-250). Absorbance was recorded at 595 nm. Protein concentrations were calculated from a standard curve prepared using bovine serum albumin and expressed as mg g^{-1} fresh weight.

Antioxidant enzyme activities

For enzymatic assays, frozen leaf tissues were ground in liquid nitrogen. A 0.1 g sample was transferred to a microtube containing 1 mL of extraction buffer, thoroughly mixed, filtered, and centrifuged at 10,000 rpm at 4 °C for 15 min. The resulting supernatant was used to determine the activities of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), and ascorbate peroxidase (APX) based on their specific spectrophotometric assay protocols.

Catalase (CAT) activity

Catalase activity was measured by mixing 50 µL of enzyme extract with 3 mL of extraction buffer containing 50 mM sodium phosphate (pH 7.0) and 2 mM EDTA. The reaction was initiated by adding 5 µL of 30% hydrogen peroxide. The decrease in absorbance at 240 nm was recorded for 1 min. One unit of catalase activity was defined as the amount of enzyme that decomposes 1 µmol of hydrogen peroxide per min. The enzyme activity was expressed as units per mg of leaf protein (Bergmeyer, 2012).

Guaiacol peroxidase (POD) activity

For POD activity, 50 µL of enzyme extract was added to 3 mL of extraction buffer (50 mM sodium phosphate, pH 7.0, and 2 mM EDTA). The reaction started by adding 5 µL of 30% H_2O_2 and 5 µL of guaiacol. The increase in absorbance at 465 nm was recorded for 1 min. One unit of POD activity was defined as the amount of enzyme that oxidizes 1 µmol of guaiacol per min. Results were expressed as units per mg of leaf protein (Herzog and Fahimi, 1973).

Ascorbate peroxidase (APX) activity

APX activity was determined based on the method of (Nakano and Asada, 1981), which is based on the decline in absorbance at 290 nm due to ascorbate oxidation. A 0.1 g frozen leaf sample was homogenized in 1 mL of extraction buffer and centrifuged at 14,000 rpm for 20 min at 4 °C. In both the sample and blank cuvettes, 3 mL of extraction buffer and 51.4 µL of 30% H_2O_2 were added. After zeroing the spectrophotometer, 50 µL of enzyme extract was added to the sample cuvette. Absorbance at 290 nm was recorded every 10 s for 120 s. One unit of APX activity was defined as the amount of enzyme that oxidizes 1 µmol of ascorbate per min, and activity was expressed as units per milligram of protein.

Leaf elemental analysis

Leaf nutrient content was determined using healthy mature leaves collected from the middle portion of shoots. Samples were washed with distilled water, oven-dried at 75 °C for 72 h, and ground to a fine powder. Wet digestion was performed following Abdel-Shafey et al. (1994). Briefly, 1 g of dried material was digested with 10 mL of 65% HNO_3 in a 65 °C water bath for 2 h, followed by the addition of 2.6 mL of 20% H_2O_2. The digests were filtered (Whatman No. 42) and diluted to 50 mL. Potassium content was determined by flame photometry (model G 405, Germany), while Fe, Zn, and Mn were measured using atomic absorption spectroscopy (model 220, Varian). Phosphorus and nitrogen concentrations were determined by standard colorimetric and Kjeldahl methods, respectively.

Statistical analysis

Data were analyzed by analysis of variance (ANOVA) using SAS software (version 1.9). Mean values from each treatment group were compared using Duncan's multiple range test at the 5%

significance level ($P < 0.05$). Graphs were generated with Microsoft Excel.

Results and Discussion

Ion leakage as an indicator of membrane stability

The effects of temperature, manganese sulfate, and their interaction affecting ion leakage showed statistical significance. The interaction effect was significant ($P \leq 0.05$), while the main effect of manganese sulfate was more significant ($P \leq 0.01$) (Table 1).

Figure 1 demonstrates that cold stress increased ion leakage in all treatments. However, vines treated with manganese sulfate, especially at 8000 ppm, displayed significantly lower ion leakage compared to untreated controls. Under low-temperature conditions, vines treated with 8000 ppm manganese sulfate showed 27 percent less ion leakage than the control group, indicating enhanced membrane stability.

Table 1. Analysis of variance of temperature and manganese sulfate effects on membrane stability indices in *Yaqouti* grape leaves under cold stress.

Source of changes	Degrees of freedom	Mean square		
		Ion leakage	Malondialdehyde	Hydrogen peroxide
Temperature(T)	2	847.2**	5911.46**	0.1013**
Manganese sulfate	1	70.1**	3372.13**	0.1959**
T×M	2	13.2*	1311.77**	1.0621*
Error	12	3.22	53.57	0.009
Coefficient of variation	-	6.33	5.12	8.77

Note: ns, *, ** denote non-significant, significant at $P < 0.05$, and $P < 0.01$, respectively.

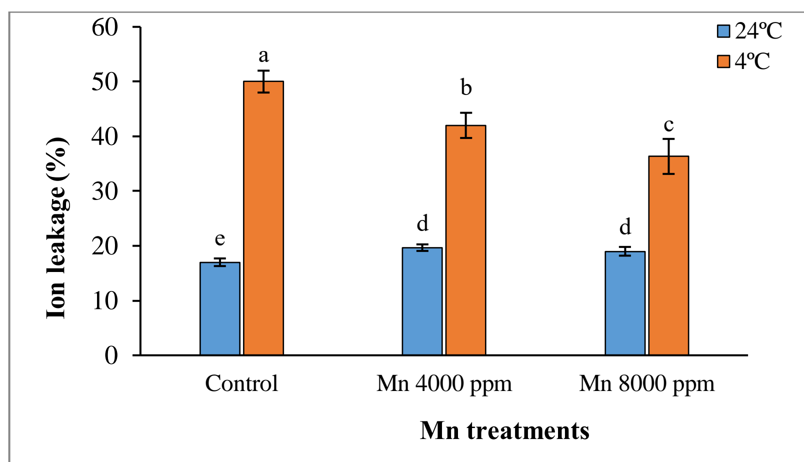

Fig. 1. Effect of foliar application of manganese sulfate (Mn; 4000 ppm and 8000 ppm) and temperature (4 °C and 24 °C) on ion leakage percentage in ‘Yaqouti’ grapevine leaves. Different letters indicate significant differences ($P<0.05$).

These findings agree with previous studies in strawberry (Turhan and Eris, 2004), pistachio and grapevine (Asgarian et al., 2022), where cold stress resulted in increased ion leakage. The reduced ion leakage observed in manganese-treated vines may result from improved antioxidant capacity and decreased reactive oxygen species (ROS) production, which helps minimize oxidative damage to cell membranes.

Membrane lipid peroxidation and hydrogen peroxide accumulation

Both malondialdehyde (MDA) and hydrogen peroxide (H_2O_2) levels were significantly influenced by temperature, manganese sulfate treatment, and their interaction ($P < 0.01$; Table 1). Cold stress consistently elevated MDA and H_2O_2 concentrations across all experimental groups (Figs. 2 and 3). Notably, vines receiving manganese sulfate supplementation, particularly at 8000 ppm, showed substantially reduced accumulation of these oxidative stress markers compared to untreated controls under low-temperature conditions.

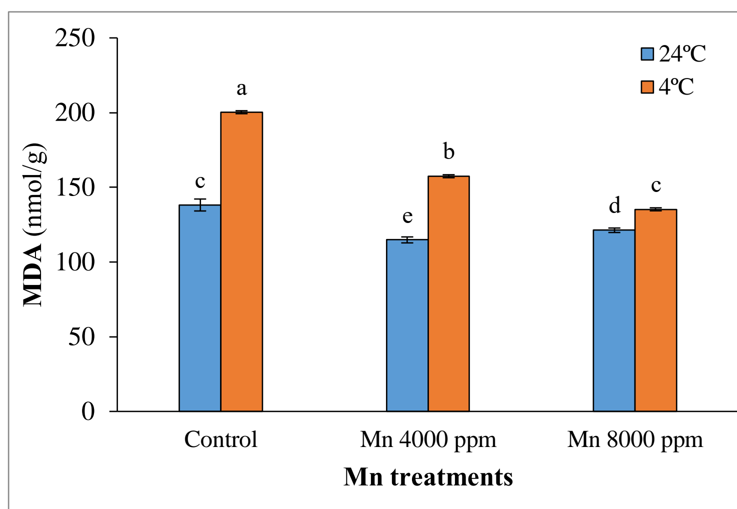

Fig. 2. Effect of foliar application of manganese sulfate (Mn; 4000 ppm and 8000 ppm) and temperature (4 ℃ and 24 ℃) on malondialdehyde (MDA) content in 'Yaqouti' grapevine leaves. Different letters indicate significant differences (*P*<0.05).

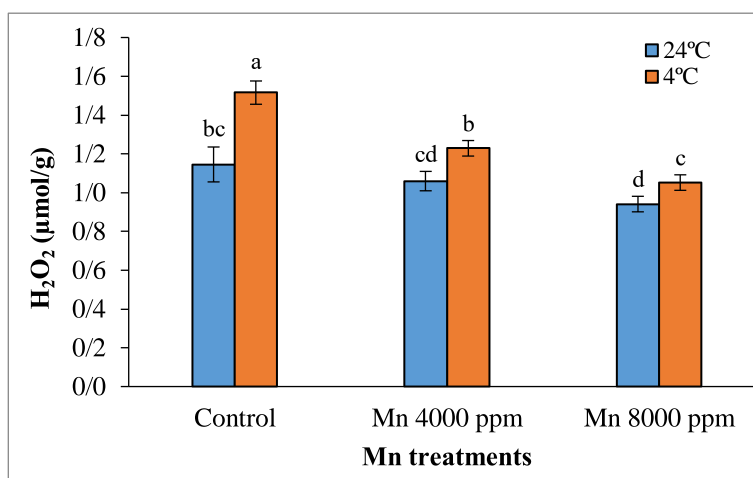

Fig. 3. Effect of foliar application of manganese sulfate (Mn; 4000 ppm and 8000 ppm) and temperature (4 ℃ and 24 ℃) on hydrogen peroxide (H_2O_2) content in 'Yaqouti' grapevine leaves. Different letters indicate significant differences (*P*<0.05).

In greenhouse-grown vines, manganese sulfate application similarly reduced MDA and H_2O_2 levels compared with control plants. These results indicate that manganese sulfate contributes to preserving membrane integrity and mitigating oxidative damage during chilling stress. Similar protective effects have been reported in other plant species, where cold-induced oxidative stress is associated with increased ion leakage and lipid peroxidation (Koca et al., 2006; Abdelgawad et al., 2016). Cold stress–induced ROS accumulation primarily impairs photosystem II function and disrupts Calvin cycle processes, ultimately compromising membrane integrity and selective permeability (Karimi et al., 2016; 2020). Our findings suggest that manganese sulfate alleviates these detrimental effects through two complementary mechanisms: stabilization of the photosynthetic apparatus and enhancement of the endogenous antioxidant defense system.

Accumulation of compatible osmolytes

Table 2 showed significant effects of cold stress and its interaction with manganese sulfate on leaf proline content (P<0.01), whereas manganese sulfate alone had no significant effect. These findings align with previous reports in grapevine (Karimi et al., 2021), confirming that cold stress induces proline accumulation. This response is likely linked to a stress-driven redirection of glutamine metabolism toward proline biosynthesis, which occurs concurrently with reduced chlorophyll synthesis (Fozouni et al., 2012).

Table 2. Analysis of variance of temperature and manganese sulfate effects on proline, soluble sugar, and soluble protein content in grapevines under cold stress.

Source of changes	Degrees of freedom	Mean square		
		Proline	Soluble sugar	Soluble protein
Temperature (T)	1	0.250**	19.73*	4.37**
Manganese sulfate	1	0.078**	0.91**	8.37**
T×M	2	0.012**	1.87**	0.18**
Error	12	0.009	0.011	0.003
Coefficient of variation	-	15.10	9.39	7.90

Note: ns, *, ** denote non-significant, significant at $P < 0.05$, and $P < 0.01$, respectively.

Cold stress, manganese sulfate treatment, and their interaction similarly affected soluble sugar content (Table 3). Maximum sugar accumulation occurred in cold-stressed vines treated with 4000 ppm manganese sulfate, while minimum levels appeared in untreated vines at normal temperatures. As established osmoprotectants, soluble sugars stabilize cellular membranes and act as ROS scavengers (Gupta and Huang, 2014; Jiang and Zhang, 2004), with their accumulation in manganese-treated vines potentially enhancing cold tolerance (Karimi et al., 2022).

Table 3. Mean values of proline, soluble sugar, and soluble protein in *Yaqouti* grapevines treated with manganese sulfate under different temperature regimes.

Treatment		Proline (µmol g^{-1})	Soluble sugar (mg g^{-1})	Soluble protein (mg g^{-1})
Fertilizer (ppm)	Temperature (°C)			
Control	24	0.397^{f}	0.82^{c}	0.96^{d}
Manganese sulfate 4000	24	0.485^{e}	1.63^{b}	1.32^{d}
Manganese sulfate 8000	24	0.593^{d}	1.63^{b}	2.15^{b}
Control	4	0.635d^{c}	1.64^{b}	1.38^{c}
Manganese sulfate 4000	4	0.793^{b}	2.07^{ab}	2.19^{b}
Manganese sulfate 8000	4	0.853^{a}	2.31^{a}	2.25^{a}

Different letters within columns indicate significant differences ($P < 0.05$, Duncan's test)

Significant effects were also observed for leaf protein content (Table 3). Cold stress elevated soluble proteins across all treatments, peaking in vines that received 8000 ppm manganese sulfate. This response likely involves upregulated synthesis of cold-responsive proteins, particularly antifreeze proteins and membrane-associated enzymes mediating lipid remodeling (Juurakko et al., 2021), which collectively improve membrane fluidity and stress adaptation.

Phenolic compounds: phenols and flavonoids

Temperature and manganese sulfate independently influenced total phenolic content ($P < 0.01$), though their interaction was non-significant (Table 4). Cold stress markedly increased phenolics, with maximal accumulation in 8000 ppm manganese sulfate-treated vines (Fig. 4). Manganese application consistently enhanced phenolic levels regardless of temperature regime.

Table 4. Analysis of variance of temperature and manganese sulfate effects on phenolic compounds in grapevines under cold stress.

Source of changes	Degrees of freedom	Mean square	
		Total phenol	Total flavonoids
Temperature(T)	2	40.83**	33.56**
Manganese sulfate	1	29.23**	13.97**
T×M	2	0.14ns	0.305*
Error	12	0.151	0.040
Coefficient of variation	-	6.43	5.18

Note: ns, *, ** denote non-significant, significant at $P < 0.05$, and $P < 0.01$, respectively.

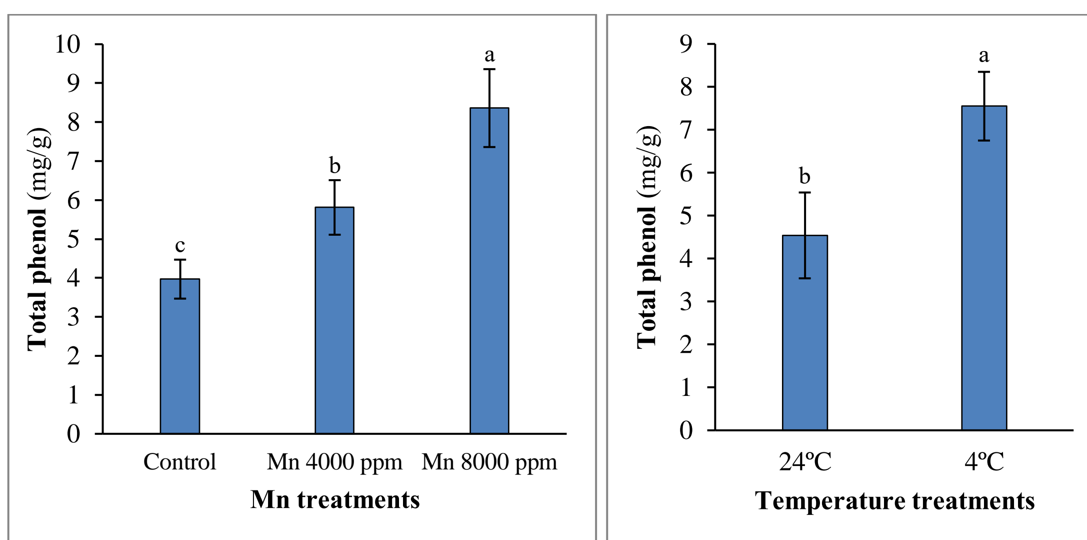

Fig. 4. Effect of foliar application of manganese sulfate (Mn; 4000 ppm and 8000 ppm) and temperature (4 °C and 24 °C) on phenolic content in 'Yaqouti' grapevine leaves. Different letters indicate significant differences (P<0.05).

Regarding flavonoids, all treatment effects were significant (Table 4). The highest concentrations occurred in cold-stressed vines supplemented with 8000 ppm manganese sulfate, indicating manganese's role in stimulating flavonoid biosynthesis during cold stress as part of non-enzymatic antioxidant defenses (Fig. 5).

Phenolics and flavonoids function as potent oxidative stress mitigators through free radical neutralization (Karimi et al., 2021; Sharma et al., 2023). Their production is governed by phenylalanine ammonia-lyase (PAL), which initiates the phenylpropanoid pathway via phenylalanine deamination (Parr and Bolwell, 2000). Cold stress prominently activates this pathway, increasing flavonoid production as an adaptive mechanism (Munns and Tester, 2008).

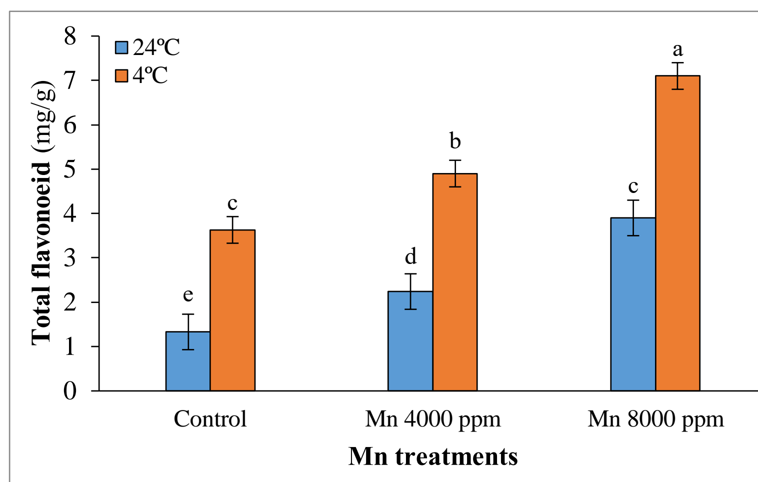

Fig. 5. Effect of foliar application of manganese sulfate (Mn; 4000 ppm and 8000 ppm) and temperature (4 ºC and 24 ºC) on total flavonoid content in 'Yaqouti' grapevine leaves. Different letters indicate significant differences (P<0.05).

Antioxidant enzyme activities

Table 5 revealed that the main effects of temperature and manganese sulfate concentration, as well as their interaction, significantly influenced catalase (CAT) activity at the 1% probability level. Likewise, the activities of guaiacol peroxidase (POD) and ascorbate peroxidase (APX) were significantly affected by temperature and manganese sulfate at the 1% level, and their interaction effects were significant at the 5% level.

Table 5. Analysis of variance of temperature and manganese sulfate effects on antioxidant enzyme activities in Yaqouti grape leaves under cold stress.

Source of changes	Degrees of freedom	Mean square		
		Catalase	Guaiacol peroxidase	Ascorbate peroxidase
Temperature(T)	2	19.11**	44.05**	42.19**
Manganese sulfate	1	38.39**	21.63 **	6.80 **
T×M	2	0.426 **	0.197 *	0.197*
Error	12	0.041	0.047	0.78
Coefficient of variation	-	3.75	3.53	5.71

Note: ns, *, ** denote non-significant, significant at $P < 0.05$, and $P < 0.01$, respectively.

Catalase activity increased progressively with rising manganese sulfate concentration across both temperature regimes, reaching a peak in vines treated with 8000 ppm. Under cold stress, CAT activity in these vines was approximately 60% greater than in untreated controls (Fig. 6). A similar trend was observed for POD activity, which also showed significant enhancement in response to the combined treatment of cold and 8000 ppm manganese sulfate, thereby exceeding the value recorded for the control treatment by nearly 40% (Fig. 7).

Under normal (greenhouse) temperature, manganese sulfate significantly increased APX activity, although there was no statistical difference between the 4000 and 8000 ppm concentrations. Under cold stress, APX activity increased significantly in response to higher manganese levels, with the highest activity recorded in vines treated with 8000 ppm (Fig. 8).

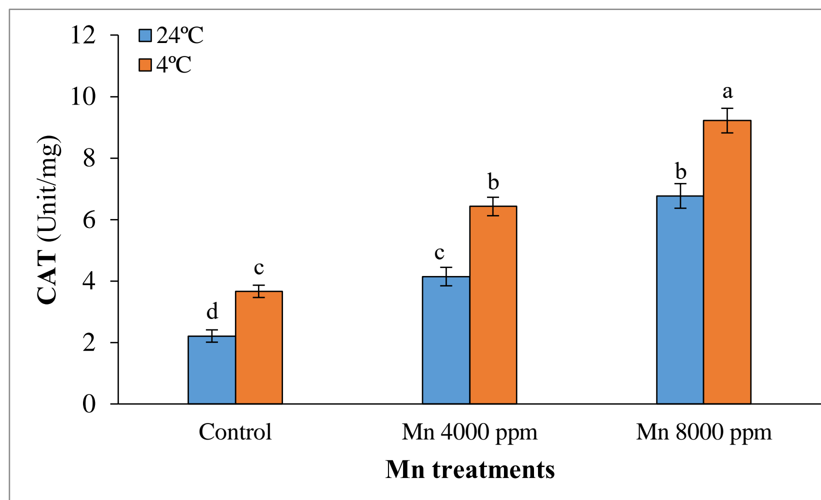

Fig. 6. Effect of foliar application of manganese sulfate (Mn; 4000 ppm and 8000 ppm) and temperature (4 ºC and 24 ºC) on catalase (CAT) activity in 'Yaqouti' grapevine leaves. Different letters indicate significant differences (P<0.05).

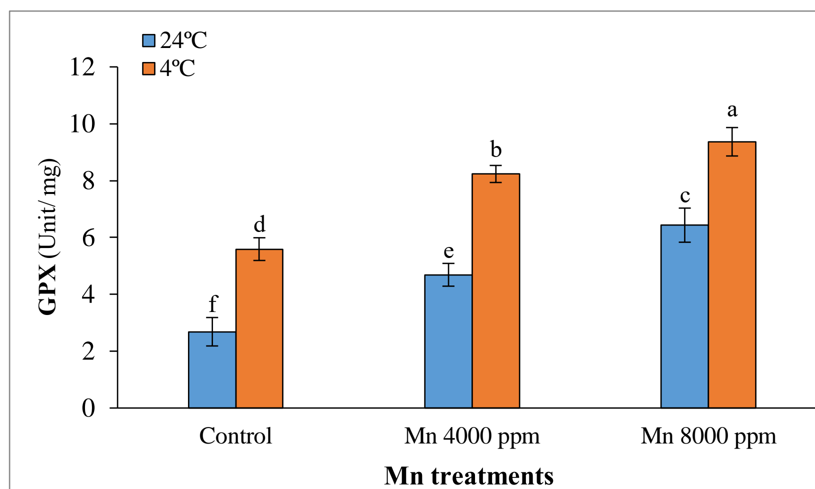

Fig. 7. Effect of foliar application of manganese sulfate (Mn; 4000 ppm and 8000 ppm) and temperature (4 ºC and 24 ºC) on guaiacol peroxidase (GPX) activity in 'Yaqouti' grapevine leaves. Different letters indicate significant differences (P<0.05).

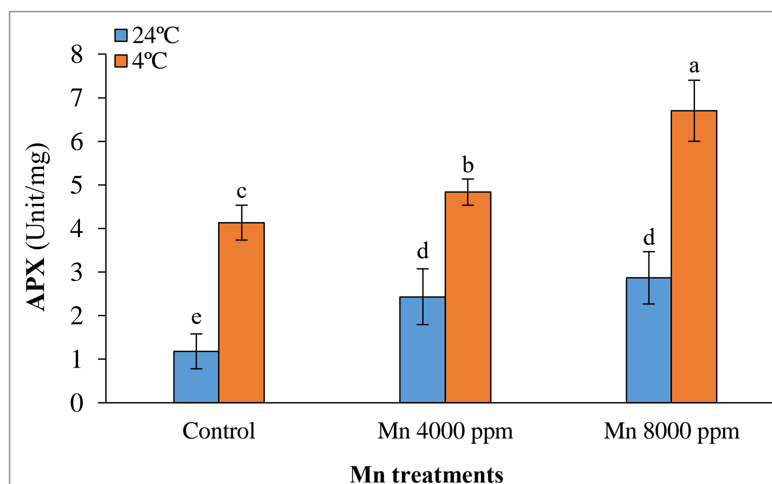

Fig. 8. Effect of foliar application of manganese sulfate (Mn; 4000 ppm and 8000 ppm) and temperature (4 ºC and 24 ºC) on ascorbate peroxidase (APX) activity in 'Yaqouti' grapevine leaves. Different letters indicate significant differences (P<0.05).

These results align with earlier findings that highlight the essential role of manganese in the function of hydrogen peroxide-detoxifying enzymes (Marschner, 2012). Also reported that foliar-applied manganese enhanced antioxidant enzyme activities in grapevines. Manganese contributes to cellular redox homeostasis by protecting membrane proteins and lipids from free radical damage. Additionally, as a cofactor for Mn-superoxide dismutase (Mn-SOD) and Mn-catalase, manganese is directly involved in free radical detoxification (Alloway, 2008; Humphries et al., 2007).

Chlorophyll index (SPAD)

Temperature and manganese sulfate concentration significantly affected chlorophyll index (SPAD) at the 1% level, while their interaction was not significant (Table 6). Cold stress resulted in a marked decrease in chlorophyll content in all vines. However, manganese-treated vines, particularly those receiving 8000 ppm, exhibited significantly higher chlorophyll levels compared to untreated controls, demonstrating improved resistance to chlorophyll degradation (Fig. 9).

Table 6. Analysis of variance of temperature and manganese sulfate effects on chlorophyll content (SPAD) and relative water content (RWC) in grape leaves.

Source of changes	Degrees of freedom	Mean square	
		Chlorophyll index (SPAD)	Relative water content (RWC)
Temperature(T)	2	79.57**	37.7**
Manganese sulfate	1	21.63**	26.14**
T×M	2	0.12^{ns}	2.50^{ns}
Error	12	0.77	0.804
Coefficient of variation	-	8.36	1.07

Note: ^{ns}, *, ** denote non-significant, significant at $P < 0.05$, and $P < 0.01$, respectively.

Cold conditions impair chlorophyll stability by disrupting the ultrastructure of chloroplasts and photosystem II, reducing stomatal conductance, and impeding photosynthesis and gas exchange (Karimi et al., 2016). The protective effect of manganese under cold stress may be attributed to its role in antioxidant defense, stabilization of magnesium in the chlorophyll structure, and involvement in oxalate oxidase and water-splitting systems within photosystem II (Adamczyk-Szabela et al., 2020).

Relative leaf water content (RWC)

Based on Table 6, the main effects of temperature and manganese sulfate on RWC were significant at the 1% level, while their interaction effect was not. Cold stress reduced RWC in all vines; however, this reduction was mitigated in vines treated with 8000 ppm manganese sulfate. The highest RWC was observed in control vines under ambient temperature and in those treated with 8000 ppm manganese sulfate without cold exposure (Fig. 10).

The reduction in RWC under cold stress is likely due to osmotic constraints in the root zone that limit water uptake. In cold conditions, free cellular water often binds to macromolecules such as sugars and proteins, contributing to cellular dehydration but also offering protection by reducing the risk of ice crystal formation through osmotic adjustment.

Mineral nutrient content in leaves

The main effects of cold and manganese sulfate were significant for nitrogen, phosphorus, potassium, and magnesium contents at the 1% level. The interaction effects of temperature and manganese sulfate were also significant for nitrogen and phosphorus (at 1%) and for potassium (at 5%), while no significant interaction effect was observed regarding magnesium.

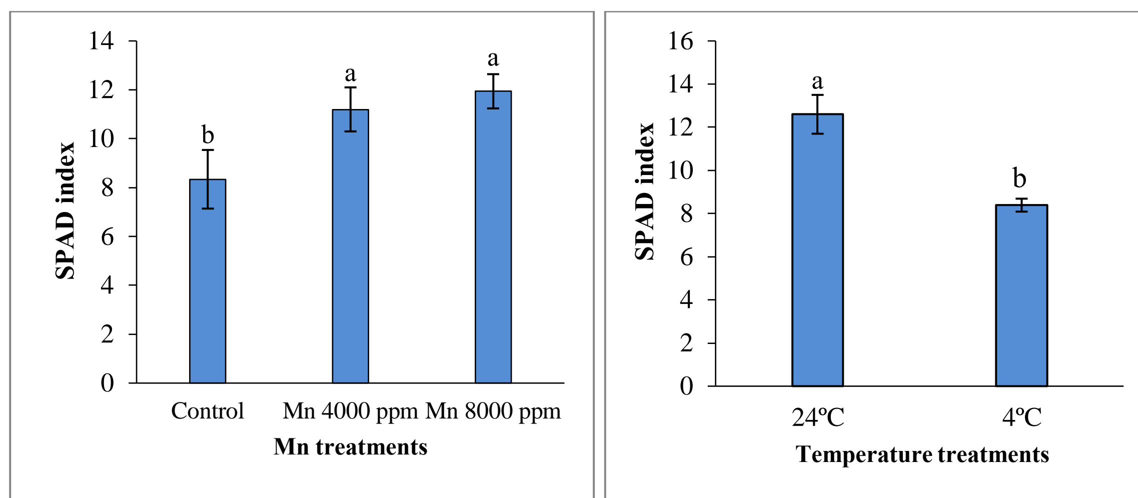

Fig. 9. Effect of foliar application of manganese sulfate (Mn; 4000 ppm and 8000 ppm) and temperature (4 °C and 24 °C) on SPAD index in 'Yaqouti' grapevine leaves. Different letters indicate significant differences (P<0.05).

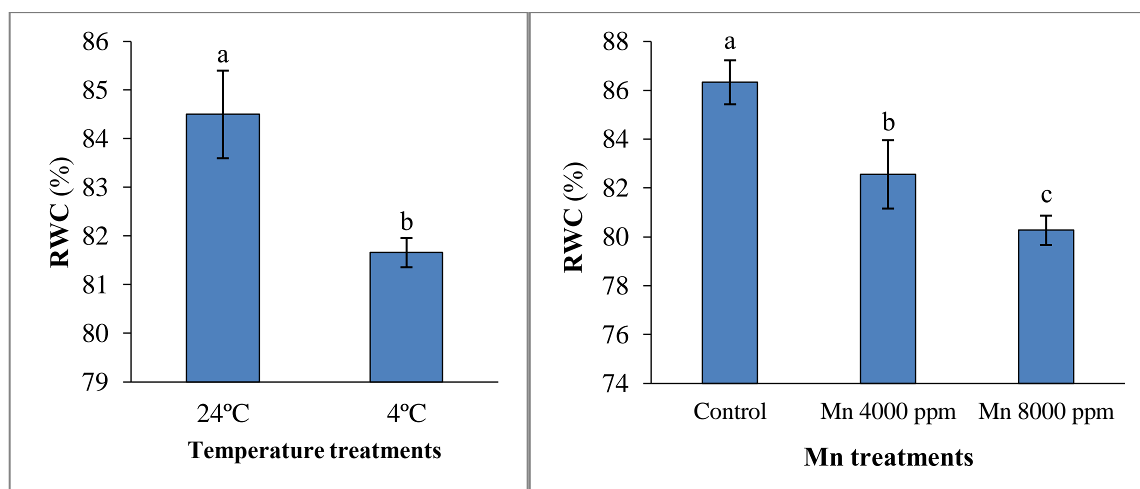

Fig. 10. Effect of foliar application of manganese sulfate (Mn; 4000 ppm and 8000 ppm) and temperature (4 °C and 24 °C) on relative water content (RWC) in 'Yaqouti' grapevine leaves. Different letters indicate significant differences (P<0.05).

Table 7. Analysis of variance of temperature and manganese sulfate effects on macronutrient content in *Yaqouti* grape leaves.

Source of changes	Degrees of freedom	Mean square			
		Nitrogen	Phosphorus	Potassium	Magnesium
Temperature(T)	2	1.52**	0.83 **	135.2**	0.012**
Manganese sulfate	1	0.19**	0.21**	196.5**	0.021**
T×M	2	0.03**	0.07**	40.3*	^{ns}0.00
Error	12	0.004	0.001	10.17	0.001
Coefficient of variation	-	6.20	3.18	6.17	4.99

Note: ^{ns}, *, ** denote non-significant, significant at $P < 0.05$, and $P < 0.01$, respectively.

Table 8. Analysis of variance of temperature and manganese sulfate affecting micronutrient content in grapevine leaves.

Source of changes	Degrees of freedom	Mean square		
		Iron	Zinc	Magnesium
Temperature(T)	2	1.45^{**}	0.68^{**}	1441.2^{**}
Manganese sulfate	1	0.19^{**}	0.17^{**}	1976.7^{**}
T×M	2	0.03^{**}	0.05^{**}	77.4^{*}
Error	12	0.004	0.002	10.23
Coefficient of variation	-	7.12	5.24	8.06

Note: ^{ns}, *, ** denote non-significant, significant at $P < 0.05$, and $P < 0.01$, respectively.

Leaf macronutrient concentrations varied across treatments. Vines sprayed with 4000 ppm manganese sulfate had lower macronutrient levels than the controls. The lowest nitrogen and potassium concentrations were observed in vines exposed to cold stress alone, whereas the highest values were recorded in vines treated with 8000 ppm manganese sulfate under cold conditions (Table 9). Although cold stress generally reduced phosphorus content, the decline was significantly less pronounced in manganese-treated vines. Under ambient conditions, control plants (with and without manganese) exhibited the highest phosphorus concentrations.
Regarding micronutrients, the effects of cold, manganese sulfate, and their interaction on leaf zinc content were significant at the 1% level (Table 10). Zinc concentration peaked in vines treated with 8000 ppm manganese sulfate, particularly in those grown under non-stress conditions.

Cold stress disrupts nutrient uptake and induces oxidative stress through excessive ROS production. Foliar application of manganese may mitigate these effects by enhancing antioxidant activity and stabilizing cellular membranes (Waraich et al., 2012). The accumulation of osmoprotectants and activation of antioxidant defenses likely improve nutrient uptake efficiency under cold stress, particularly in manganese-treated plants.

Table 9. Mean macronutrient content in Yaqouti grape leaves treated with manganese sulfate under temperature stress.

Treatment		Nitrogen (%)	Phosphorus (%)	Potassium (%)	Magnesium (%)
Fertilizer (ppm)	Temperature (°C)				
Control	24	1.25^{b}	0.18^{c}	1.25^{ab}	1.8^{a}
Manganese sulfate 4000	24	1.46^{a}	0.33^{ab}	1.52^{a}	1.7^{a}
Manganese sulfate 8000	24	1.49^{a}	0.38^{a}	1.47^{a}	1.4^{b}
Control	4	0.98^{c}	0.14^{d}	1.15^{d}	1.0^{c}
Manganese sulfate 4000	4	1.23^{b}	0.29^{b}	1.33^{b}	1.9^{a}
Manganese sulfate 8000	4	1.24^{b}	0.28b	1.28^{b}	1.7^{a}

Different letters within columns indicate significant differences ($P < 0.05$).

Table 10. Mean micronutrient content in *Yaqouti* grape leaves under manganese sulfate and temperature treatments.

Treatment		Iron (ppm)	Zinc (ppm)	Magnesium (ppm)
Fertilizer (ppm)	**Temperature (°C)**			
Control	24	98.24[b]	60.47[c]	40.11[c]
Manganese sulfate 4000	24	109.76[a]	67.58[a]	61.30[b]
Manganese sulfate 8000	24	107.87[a]	67.78[a]	68.27[a]
Control	4	79.34[d]	56.98[d]	39.25[c]
Manganese sulfate 4000	4	96.33[c]	62.29[b]	58.23[b]
Manganese sulfate 8000	4	93.24[c]	60.49[c]	59.18[b]

Different letters within columns indicate significant differences ($P < 0.05$).

Conclusion

The findings of this study demonstrate that foliar application of manganese sulfate significantly modulates physiological and biochemical responses of Yaqouti grapevines under cold stress. Although the 4000 ppm manganese sulfate treatment resulted in higher chlorophyll content than the 8000 ppm treatment, both concentrations effectively mitigated cold-induced damage. Cold stress markedly increased ion leakage, hydrogen peroxide levels, and malondialdehyde content in grapevine leaves, reflecting oxidative damage to cellular membranes. However, manganese-treated vines, particularly those receiving 4000 ppm, exhibited lower ion leakage and reduced oxidative markers compared with untreated plants.

Application of 4000 ppm manganese sulfate under cold stress also led to the highest accumulation of soluble sugars, whereas untreated vines maintained at optimal temperatures had the lowest sugar content. Total phenolic and flavonoid levels were significantly enhanced by manganese application under cold stress, increasing by approximately 35% and 27%, respectively, compared with untreated controls. These secondary metabolites likely contributed to the observed enhancement of antioxidant capacity.

Furthermore, key antioxidant enzymes, specifically catalase and guaiacol peroxidase, showed substantial increases in activity in response to manganese sulfate, particularly at 4000 ppm under cold stress. These treatments resulted in 60% and 40% higher enzyme activity, respectively, compared with cold-stressed controls. Overall, these results suggest that manganese sulfate protects grapevines from low-temperature stress by enhancing antioxidant defenses, maintaining membrane stability, and improving overall physiological resilience.

Acknowledgments

The authors sincerely appreciate the Department of Biology, Faculty of Basic Sciences at Malayer University for their academic and logistical support. We are also grateful to the Educational and Research Greenhouse of Malayer University and its technical staff for providing the necessary facilities and support during this research.

Author Contributions

Conceptualization, MS and RK; Methodology, MS and RK; Validation: KG, MS, and RK; Formal Analysis, RK; Investigation: KG; Data Curation, RK and MS; Resources, MS; Writing – Original Draft: KG; Writing – Review and Editing, MS; Supervision, MS and RKi; Project Administration, MS. All authors have read and agreed to the published version of the manuscript.

Funding

This research was funded by the authors' institutional research grant (Grant Number 193-84/9). No external or corporate funding was received for this study. The Article Processing Charge (APC) was covered by the authors' institutional research funds.

Conflict of Interest

The authors indicate no conflict of interest in this work.

References

Abdel-Shafey HI, Hegemann W, Teiner A. 1994. Digestion with concentrated HNO_3 and H_2O_2. Environment Management and Health 5, 21–24.

Adamczyk-Szabela D, Lisowska K, Romanowska-Duda Z, Wolf WM. 2020. Combined cadmium-zinc interactions alter manganese, lead, copper uptake by Melissa officinalis. Scientific Reports 10(1), 1675. https://doi.org/10.1038/s41598-020-58491-9

Adhikari L, et al. 2022. Cold stress in plants: Strategies to improve cold tolerance in forage species. Plant Stress 4, 100081. https://doi.org/10.1016/j.stress.2022.100081

Alloway, B. J. (2008). Zinc in soils and crop nutrition (2nd ed.). International Zinc Association (IZA) and International Fertilizer Industry Association (IFA). ISBN 978-90-813327-1-1.

Anchal A, Singh DB, Kumar R, Sharma A & Sharma SK. (2023). The impact of micronutrients applied through foliar application on the growth, yield and benefit cost ratio of strawberry (Fragaria × *ananassa* Duch.) cv. 'Camarosa' under polyhouse condition. The Pharma Innovation Journal 12(8), 389–394. https://doi.org/10.22271/tpi.2023.v12.i8e.21740

Asgarian ZS, Karimi R, Ghabooli M, Maleki M. 2022. Biochemical changes and quality characterization of cold-stored 'Sahebi' grape in response to postharvest application of GABA. Food Chemistry 373, 131401. https://doi.org/10.1016/j.foodchem.2021.131401

Babst-Kostecka A, Przybyłowicz WJ, Seget B, Mesjasz-Przybyłowicz J. 2020. Zinc allocation to and within *Arabidopsis halleri* seeds: Different strategies of metal homeostasis in accessions under divergent selection pressure. Plant-Environment Interactions 1(3), 207–220. https://doi.org/10.1002/pei3.10023

Bates LS, Waldren RP, Teare ID. 1973. Rapid determination of free proline for water-stress studies. Plant and Soil 39, 205–207.

Beheshti Rooy M, Aghaei K, Moradi F. 2017. Effect of gradual and shock chilling stress on abscisic acid, soluble sugars and antioxidant enzymes changes in 'Sultana' grapevine. Iranian Journal of Plant Physiology 8(1), 2331–2340.

Bergmeyer HU. 2012. Methods of Enzymatic Analysis (3rd ed.). Wiley. https://doi.org/10.1002/9783527644506.

Bradford MM. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry 72(1–2), 248–254. https://doi.org/10.1016/0003-2697(76)90527-3

Buege JA, Aust SD. 1978. Microsomal lipid peroxidation. Methods in Enzymology 52, 302–310. https://doi.org/10.1016/S0076-6879(78)52032-6

Cao Y, Hwarari D, Radani Y, Guan Y, Yang L. 2023. Molecular Mechanism Underlying Plant Response to Cold Stress. Phyton – International Journal of Experimental Botany 92(9), 2665–2683. https://doi.org/10.32604/phyton.2023.029210

Chang CC, Yang MH, Wen HM, Chern JC. 2002. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. Journal of Food and Drug Analysis 10(3), 178–182. https://doi.org/10.38212/2224-6614.2748

El-Beltagi HS et al. 2024. Exogenous application of manganese and arginine alleviates the adverse effects of salinity stress on pea (*Pisum sativum* L.). Cogent Food & Agriculture 10(1), 2389445. https://doi.org/10.1080/23311932.2024.2389445

Faisal M, Saqib AH, Hussain S, Saleem MH, Alshaya H, El-Sheikh MA & Wang, X. 2021. Manganese oxide nanoparticles (MnO-NPs) improve growth and salinity tolerance in soybean (*Glycine max* L.) by regulating ionic balance, antioxidant defense, and redox homeostasis. Frontiers in Plant Science 12, 759958. https://doi.org/10.3389/fpls.2021.759958

Fozouni M, Abbaspour M, Doulati Baneh H. 2012. Leaf water potential, photosynthetic pigments and compatible solutes alterations in four grape cultivars under salinity. VITIS 51(4):147–152. https://doi.org/10.5073/vitis.2012.51.147-152

Gupta B & Huang B. 2014. Mechanism of salinity tolerance in plants: physiological, biochemical, and molecular characterization. Genomics 1, 18. https://doi.org/10.1016/j.ygeno.2014.02.001

Herzog V, Fahimi HD. 1973. Determination of the activity of peroxidase. Analytical Biochemistry 55, 554–562. https://doi.org/10.1016/0003-2697(73)90144-8

Humphries JM, Stangoulis JCR, Graham RD. 2007. Manganese. In: Handbook of Plant Nutrition. CRC Press, 351–374.

Ihtisham M, et al. 2023. Primary plant nutrients modulate the reactive oxygen species metabolism and mitigate the impact of cold stress in overseeded perennial ryegrass. Frontiers in Plant Science 14, 1149832. https://doi.org/10.3389/fpls.2023.1149832

Irigoyen JJ, Emerich DW, Sánchez-Díaz M. 1992. Water stress induced changes in concentrations of proline and total soluble sugars in nodulated alfalfa (Medicago sativa L.) plants. Physiologia Plantarum 84, 55–60. https://doi.org/10.1111/j.1399-3054.1992.tb08764.x

Jahed KR, Saini AK, Sherif SM. 2023. Coping with the cold: unveiling cryoprotectants, molecular signaling pathways, and strategies for cold stress resilience. Frontiers in Plant Science 14,

1246093. https://doi.org/10.3389/fpls.2023.1246093

Jiang MY, Zhang JH. 2004. Abscisic acid and antioxidant defense in plant cells. Chinese Bulletin of Botany 46(1), 1–9.

Juurakko CL, Walker VK. 2021. Cold acclimation and prospects for cold-resilient crops. Plant Stress 2, 100028. https://doi.org/10.1016/j.stress.2021.100028

Karimi R, Amini H & Ghabooli M. 2022. Root endophytic fungus *P. indica* and zinc attenuate cold stress in grapevine by influencing leaf phytochemicals and minerals. Scientia Horticulturae 293, 110665. https://doi.org/10.1016/j.scienta.2021.110665

Karimi R, Ershadi A, Ehtesham Nia A, Sharifani M, Rasouli M, Ebrahimi A, Vahdati K. 2014. Morphological and molecular evaluation of Persian walnut populations in northern and western regions of Iran. Journal of Nuts 5(2), 21–31.

Karimi R, Ershadi A, Rezaei Nejad A, Khanizadeh S. 2016. Abscisic acid alleviates the deleterious effects of cold stress on 'Sultana' grapevine (*Vitis vinifera* L.) plants by improving the anti-oxidant activity and photosynthetic capacity of leaves. The Journal of Horticultural Science and Biotechnology 91(4), 386-395. https://doi.org/10.1080/14620316.2016.1160546

Karimi R, Saberi A, Khadivi A. 2021. Effects of foliar spray of agricultural grade mineral oil in springtime, in combination with potassium and calcium sulfates on the phenological and biophysical indices of clusters, and foliar nutritional levels in grapevine (*Vitis vinifera* L.) cv. Sultana (Id. Thompson seedless, Sultanina). Biological Research 54, 25. https://doi.org/10.1186/s40659-021-00348-0

Khoshru B, Mitra D, Nosratabad AF, et al. 2023. Enhancing manganese availability for plants through microbial potential: A sustainable approach for improving soil health and food security. Bacteria 2(3), 129–141. https://doi.org/10.3390/bacteria2030010

Koca H, Ozdemir F, Türkan I. 2006. Effect of salt stress on lipid peroxidation and superoxide dismutase and peroxidase activities of *Lycopersicon esculentum* and *L. pennellii*. Biologia Plantarum 50, 745–748. https://doi.org/10.1007/s10535-006-0117-y.

Marschner H. 2012. Mineral nutrition of higher plants. 3rd edition. Academic Press.

Munns R, Tester M. 2008. Mechanisms of salinity tolerance. Annual Review of Plant Biology 59, 651–681. https://doi.org/10.1146/annurev.arplant.59.032607.092911

Nakano Y, Asada K. 1981. Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. Plant and Cell Physiology 22, 867–880. https://doi.org/10.1093/oxfordjournals.pcp.a076232

Parr AJ, Bolwell GP. 2000. Phenols in the plant and in man: the potential for possible nutritional enhancement of the diet by modifying the phenols content or profile. Journal of the Science of Food and Agriculture 80(7), 985–1012. https://doi.org/10.1002/(SICI)1097-0010(20000515)80:7

Parveen A, et al. 2024. Protective role of manganese, proline and rice straw extract in wheat against drought-driven oxidative stress. Acta Physiologiae Plantarum 46, 28. https://doi.org/10.1007/s11738-024-03658-4

Qari SH, Hassan MU, Chattha MU, Mahmood A, Naqve M, Nawaz M, Barbanti L, Alahdal MA, Aljabri M. 2022. Melatonin induced cold tolerance in plants: physiological and molecular responses. Frontiers in Plant Science 13, 843071. https://doi.org/10.3389/fpls.2022.843071

Ritchie JT, Nguyen HT, Cassel DK. 1990. Leaf water content and leaf water potential. In: Stewart BA (ed), Advances in Soil Science. Volume 11. Springer, 111–135.

Sairam RK, Tyagi A. 2004. Physiology and molecular biology of salinity stress tolerance in plants. Current Science 86(3), 407–421.

Salachna P, Piechocki R. 2015. Response of speckled spur-flower to salinity stress and salicylic acid treatment. Journal of Ecological Engineering 16(2), 68–75. https://doi.org/10.12911/22998993/1855

Shahi S, Srivastava M. 2018. Influence of foliar application of manganese on growth, pigment content, and nitrate reductase activity of *Vigna radiata* (L.) R. Wilczek under salinity. Journal of Plant Nutrition 41(11), 1397–1404. https://doi.org/10.1080/01904167.2018.1450420

Sharma A, Kumar V, Shahzad B. 2023. Recent advances in phenolic compounds and their multifunctional role in abiotic stress tolerance in plants: Mechanisms, prospects, and challenges. Antioxidants 12(3), 518. https://doi.org/10.3390/antiox12030518

Turhan E, Eris A. 2009. Changes of growth, amino acids, and ionic composition in strawberry plants under salt stress conditions. Communications in Soil Science and Plant Analysis 40(21–22), 3308–

3322. https://doi.org/10.1080/00103620903325961

Velioglu YS, Mazza G, Gao L, Oomah BD. 1998. Antioxidant activity and total phenolics in selected fruits, vegetables, and grain products. Journal of Agricultural and Food Chemistry 46(10), 4113–4117. https://doi.org/10.1021/jf9801973

Velikova V, Loreto F. 2005. The relationship between isoprene emission and thermotolerance in Phragmites australis leaves exposed to high temperatures and during the recovery from a heat stress. Plant, Cell & Environment 28(3), 318–327. https://doi.org/10.1111/j.1365-3040.2004.01314.x

Waraich EA, Ahmad R, Halim A, Aziz T. 2012. Alleviation of temperature stress by nutrient management in crop plants: a review. Journal of Soil Science and Plant Nutrition 12(2), 221–244. https://doi.org/10.4067/S0718-95162012000200003

Ye Y, Medina-Velo IA, Cota-Ruiz K, Moreno-Olivas F, Gardea-Torresdey JL. 2019. Can abiotic stresses in plants be alleviated by manganese nanoparticles or compounds? Ecotoxicology and Environmental Safety 184, 109671. https://doi.org/10.1016/j.ecoenv.2019.109671

Zhou L, Ullah F, Zou J, Zeng X. 2025. Molecular and physiological responses of plants that enhance cold tolerance. International Journal of Molecular Sciences 26(3), 1157. https://doi.org/10.3390/ijms26031157