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ARTICLE

Alleviation of Salt Stress on the Growth and Active Constituents of Chamomile (*Matricaria chamomilla* L.) Using Glutathione and Hydrogen Peroxide

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ABSTRACT: Water salinity is a growing environmental concern that significantly impacts soil health, agricultural productivity, and freshwater sustainability, especially in arid regions. This study evaluated the comparative effects of foliar-applied glutathione (GSH) and hydrogen peroxide (H_2O_2) on growth, flower yield, essential oil composition, and physiological responses of *Matricaria chamomilla* L. under water salinity levels. The experiment was conducted during the 2022/2023 and 2023/2024 seasons at the Experimental Farm of El-Quassassin, Ismailia, Egypt, using a factorial randomized complete block design with three replicates. Foliar applications of GSH (1, 2, and 3 mM) and H_2O_2 (5, 10, and 20 mM) were tested under varying salinity levels (340 ppm as control, 1500, 3000, and 4500 ppm). Salinity severely impaired vegetative growth, flower yield, and key nutrients like nitrogen, protein, potassium, and carbohydrates, while increasing proline, sodium and essential oil percentages, especially at 3000 and 4500 ppm. Foliar application of GSH and H_2O_2 alleviated these adverse effects in a concentration-dependent manner. Among the tested concentrations, GSH at 2 mM showed superior performance under moderate salinity (1500 and 3000 ppm), enhancing plant growth, flower biomass, essential oil yield, and oil quality. Improvements were associated with increased oxygenated sesquiterpenes, particularly α-bisabolol oxide A and chamazulene. Such results revealed the possibility of applying the osmotic regulation agents to enhance the production of medicinal plants under saline irrigation conditions. Mechanistically, glutathione (GSH) enhances antioxidant capacity and regulates cellular redox balance. In contrast, hydrogen peroxide (H_2O_2) improves plant performance in a concentration-dependent manner by activating antioxidant defense pathways. To the best of our knowledge, this is the first study to provide novel insights into the comparative and concentration-dependent effectiveness of GSH and H_2O_2 in mitigating salinity stress, suggesting a practical approach to improving *Matricaria chamomilla* L. productivity and essential oil quality under saline conditions. So, this study recommends foliar spraying with 2 mM GSH during moderate saline irrigation (1500 ppm) as the most effective strategy for enhancing chamomile growth, flower yield, and essential oil quality.

KEYWORDS: *Matricaria chamomilla* L.; salinity stress; glutathione (GSH); hydrogen peroxide (H_2O_2); redox regulation; antioxidant signaling; oxidative stress markers; osmotic adjustment; essential oil composition; α-bisabolol oxide A; chamazulene

1 Introduction

Salinity is a significant environmental stressor that negatively impacts plant growth, productivity, and ionic balance, particularly in semi-arid and arid regions [1–3]. Excessive Na^+ and Cl can disrupt the electron transport system and increase the production of reactive oxygen species (ROS). This ultimately damages cellular components, proteins, lipids, and nucleic acids, disturbing photosynthesis, membrane stability, and metabolic processes. To mitigate this damage, plants employ antioxidant defense systems to regulate ROS levels [4]. Enhancing crops' resistance to salt stress is vital for their survival and yield optimization, both of which are vital for global food security. While the general effects of salinity stress on plant physiology are well established, its effect on medicinal and aromatic plants, particularly regarding the quality of bioactive compounds, remains insufficiently understood.

The economic importance of medicinal and aromatic plants in pharmaceuticals, food, and cosmetics highlights the need to expand their cultivation in diverse environments, even under challenging conditions [5]. Among these, *Matricaria chamomilla* L. (German chamomile) is a high-value medicinal plant widely recognized for its pharmacological properties. It contains over 120 secondary metabolites, with azulene and α-bisabolol as the main components of the volatile oils [6–8]. Furthermore, chamomile is effective against microbial infections and oxidative damage. It is famous for its anti-inflammatory, antispasmodic, and antioxidant properties [6–10]. It is also valued as an ornamental plant, where flower yield and uniformity are important commercial traits [11]. However, salinity stress can negatively impact floral development and the plant's biochemical composition, ultimately diminishing both its therapeutic benefits and aesthetic value [12,13]. In Egypt, chamomile ranked as the second most significant medicinal crop in 2005, cultivated on approximately 9500 acres. The total production reached 8000 t, with 3000 t exported, generating around $5 million in revenue [14,15]. So, salinity stress not only threatens plant growth but also affects the quality of its bioactive constituents, directly impacting its commercial and medicinal value.

Understanding how plants tolerate salt stress is crucial for enhancing chamomile's salt tolerance and cultivation. Under salinity stress, plants produce reactive oxygen species (ROS), including singlet oxygen ($^1O_2$), superoxide anion ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), and hydroxyl radical (•OH), leading to oxidative damage and disruption of cellular functions [16,17]. To mitigate this damage, plants employ several antioxidant mechanisms [18]. Among these, the ascorbic acid (AsA)-glutathione (GSH) cycle, which detoxifies ROS and eliminates H_2O_2 from the cytoplasm and various organelles, thereby maintaining cellular redox homeostasis and preserving photosynthetic efficiency [19]. Improving this system is an effective strategy to enhance photosynthetic efficiency, stabilize membranes, and manage ROS signaling under saline conditions [20]. Additionally, various amino acids accumulate as antioxidants in plant cells during salt stress, supporting growth, productivity, and adaptation. They regulate stomatal openings, facilitate ion transport, and serve as osmolytes, affecting enzyme activity, detoxifying heavy metals, and gene expression [21]. In this context, applying redox-active compounds externally may strengthen the intrinsic antioxidant system, enhancing the plant's ability to keep reactive oxygen species (ROS) at signaling levels rather than harmful levels under salinity conditions. Glutathione is a natural antioxidant made up of three amino acids:

glutamic acid, cysteine, and glycine. Also, it is an essential non-enzymatic antioxidant that plays a crucial role in maintaining cellular redox balance, protecting cells from oxidative stress, and aiding detoxification. Additionally, it supports plant growth under stress and plays an important role in essential oil production, metabolism, and constituents and fractions [21–25].

On the other hand, hydrogen peroxide (H_2O_2) is a reactive oxygen species (ROS) that functions as an elicitor, produced from molecular oxygen (O_2). In chloroplasts, it is generated during metabolism to combat oxidative stress. It enhances the production of phenolic compounds and stimulates plant defenses against environmental stressors, microbes, and herbivores, while attracting pollinators. In low concentrations, H_2O_2 boosts antioxidant enzyme activity, strengthens cell walls, and promotes plant growth [26,27]. However, at high concentrations, it can trigger programmed cell death. H_2O_2 also regulates stomatal closure and root development and decreases lipid peroxidation and electrolyte leakage, aiding plant adaptation to saline conditions. Additionally, it enhances antioxidant activity and stimulates phenylalanine ammonia-lyase (PAL), impacting flavonoid content and stress responses. Leaf applications of H_2O_2 can improve defenses and reduce saline toxicity in plants [28,29].

While glutathione (GSH) and hydrogen peroxide (H_2O_2) have been individually reported to enhance plant stress tolerance, their comparative effectiveness as foliar treatments under different salinity levels has not yet been investigated in *Matricaria chamomilla* L. Furthermore, the extent to which each compound modulates redox regulation, antioxidant defense activation, and metabolic responses in this medicinal plant remains unclear. Therefore, this study aims to evaluate the effects of foliar application of glutathione (GSH) or hydrogen peroxide (H_2O_2) at different concentrations under varying salinity levels on growth, flower yield, and essential oil of *Matricaria chamomilla* L. Consequently, this study hypothesizes that foliar application of GSH or H_2O_2 may differentially modulate plant responses to salinity stress through redox regulation and stress signaling, resulting in distinct effects on growth performance, flower yield, and essential oil quality under saline conditions.

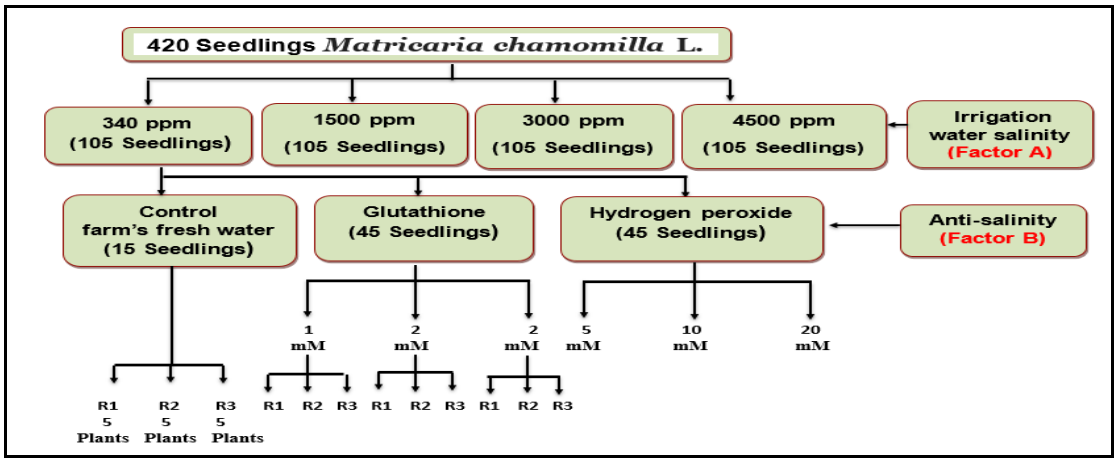
2 Materials and Methods

2.1 Experimental Layout and Treatments

This study was carried out at the El-Quassassin Horticulture Research Station Experimental Farm in Ismailia Governorate, Egypt (30°33′ N latitude, 31°56′ E longitude) during two successive growing seasons (2022/2023 and 2023/2024). The research aimed to evaluate the impact of salinity stress and its interaction with foliar applications of glutathione (GSH) and hydrogen peroxide (H_2O_2) on the production, growth, and bioactive constituents of *Matricaria chamomilla* L. The experimental setup included 28 treatment combinations, (4 salinity levels × 7 foliar treatments). Each combination was replicated three times, using fifteen pots/replicate. The experiment focused on two main factors: Irrigation water, using Nile River water as a control and three concentrations of well water, Irrigation water salinity, and anti-salinity treatments, which comprised different concentration of hydrogen peroxide and glutathione from Sigma Chemical (USA, St. Louis, MO), along with an untreated control group.

Scheme 1 demonstrated that a total of 420 uniform seedlings of *Matricaria chamomilla* L. were used to investigate the interactive effects of irrigation water salinity and anti-salinity treatments. The seedlings were divided into four irrigation water salinity levels (Factor A): 340 ppm (fresh farm water, serving as the control), 1500 ppm, 3000 ppm, and 4500 ppm, with 105 seedlings assigned to each salinity level. Within each salinity treatment, the plants were further subjected to anti-salinity applications (Each one was 45 seedlings, Factor B), which included glutathione (GSH) at concentrations of 1, 2, and 3 mM, and hydrogen peroxide (H_2O_2) at concentrations of 5, 10, and 20 mM as a foliar spray in addition to untreated control plants. Each

treatment consisted of three replicates, with five pots per replicate. Each pot contained one plant and was considered an experimental unit. The pots were organized using a randomized complete block design (RCBD), and their positions were periodically re-randomized to reduce spatial environmental variability. The effects of exogenous antioxidant applications and salinity stress on plant performance were assessed separately and in combination using this experimental setup.



Scheme 1: Schematic representation of the experimental design, demonstrating the interaction between irrigation salinity levels (Factor A) and anti-salinity foliar treatments (Factor B) applied to *Matricaria chamomilla* L. seedlings.

Before sowing, the chemical and physical characteristics of the soil were examined using the procedure outlined by Page et al. [30] and the well water chemical characteristics was carried out according to the methods described by Fishman and Friedman [31], at the Soil and Water Laboratory, ARC, as shown in Table 1.

Table 1: The experimental soil's physical and chemical characteristics and well water chemical characteristics.

		Soil's Physical and Chemical Characteristics
Physical characteristics	**Soil texture**	Sand 79.6%
		Sandy loam
		Clay 4.2%
		Silt 16.2%
		pH 7.53
		E.C 0.41 ds/m
Chemical characteristics	**Soluble cations**	
		Na^{+} 0.41 meq/L
		Mg^{++} 2.11 meq/L
		K^{+} 0.16 meq/L
		Ca^{++} 3.12 meq/L
	Soluble anions	
		SO_4^{-} 2.71 meq/L
		Cl^{-} 2.27 meq/L
		HCO^{-}_3 0.82 meq/L

Table 1: *Cont.*

Soil's Physical and Chemical Characteristics		
Well water chemical characteristics		
Well water characteristics	pH	7.5
	E.C	7.31 ds/m
	SAR	14.29
	Soluble cations	
	Ca^{++}	15 meq/L
	Mg^{++}	10.76 meq/L
	Na^{+}	51.30 meq/L
	K^{+}	0.25 meq/L
	Soluble anions	
	CO_3^{-}	-
	HCO^{-}_3	0.66 meq/L
	Cl^{-}	40.32 meq/L
	SO_4^{-}	36.3 meq/L

To prepare saline irrigation water, well water from Sorbium City in Ismailia Governorate, Egypt, with electrical conductivity of 7.31 ds/m (4678.4 ppm) was diluted with the farm's fresh water (340 ppm) according to the following equation [32].

$$V = \left[\frac{S}{F}\right] \times \left[\frac{Q}{S+F}\right] \times TV / \left[\left(\left[\frac{S}{F}\right] \times \left[\frac{Q}{S+F}\right]\right) + 1\right] \quad (1)$$

$$FV = TV - SV \quad (2)$$

SV : *volume from saline source in total volume TV*

- *Q*: required final salinity

FV : *volume from fresh source in total volume TV*

- *S*/*F*: ratio of salinity content from both sources
- *S* + *F*: sum of salinity of both sources

The experimental region has been classified as secure based on data from the Bilbiesed meteorological station. This data indicates an arid climate, characterized by low winter precipitation and hot, dry summers. Supplementary Table S1 and Fig. 1 illustrates the agrometeorological data for this region, located at a latitude of 30.66°, a longitude of 31.95°, and an elevation of 24.8 m. The data presented showcases the average monthly weather conditions for two growing seasons.

2.2 Experimental Procedures

Chamomile (*Matricaria chamomilla* L.) seeds were kindly provided by the Department of Medicinal and Aromatic Plants, Egypt's Ministry of Agriculture, Agricultural Research Center. Nursery beds made of sandy loam soil were used to plant seeds on August 23rd during the winter of 2022/2023 and 2023/2024. After 45 days, uniform seedlings (10–15 cm tall) were individually transplanted into plastic pots.

Four hundred twenty plastic pots, each 30 cm in diameter and 24 cm in height, were filled with 12 kg of sandy loam soil and had drainage holes. Polyethylene sheets were placed beneath the pots to prevent root penetration into the soil and to control weed growth. After transplanting, plants were irrigated with

Nile River water for approximately 20 days to ensure proper root system establishment and adequate shoot growth. Subsequently, saline water treatments were applied via irrigation.

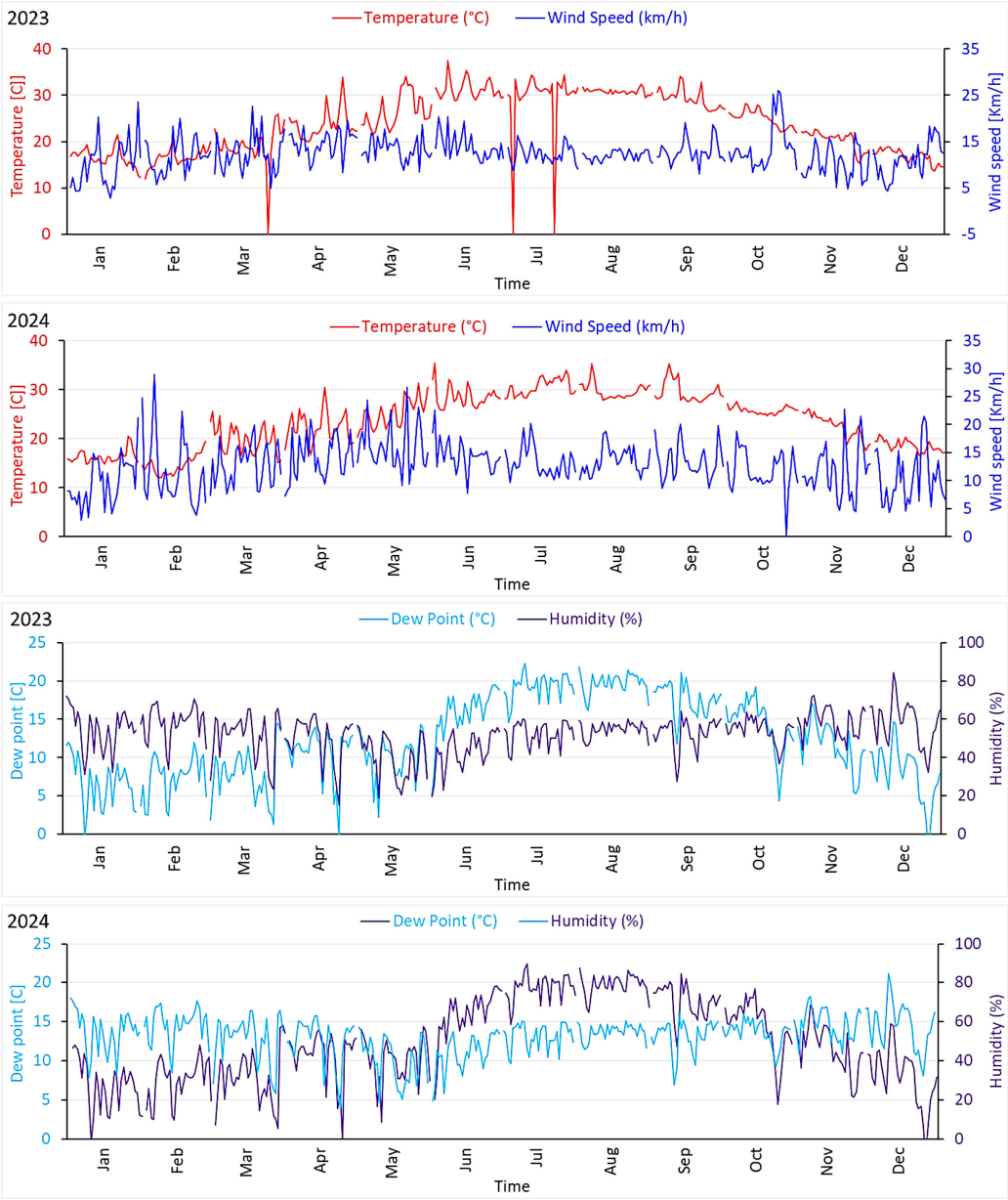


Figure 1: Monthly variations in maximum and minimum temperatures (˚C), relative humidity (%), wind speed (km/day), and dew point (˚C) during the 2022/2023-2023/2024 growing season in Bilbies, Egypt.

The irrigation water salinity concentrations were: control (fresh Nile River water, 340 ppm), 1500, 3000, and 4500 ppm of well water with and without glutathione (1, 2, and 3 mM) and hydrogen peroxide (5, 10, and 20 mM). The concentrations of H_2O_2 and GSH were chosen based on literature and preliminary experiments [33,34].

Each pot received 1.930 L (at hold capacity) of saline water every five days from November to January, and every three days until May 1st. To prevent excessive salt accumulation, irrigation with saline water was

alternated with farm water, following a ratio of three saline irrigations to one fresh water irrigation (3:1). The salinity treatments were introduced gradually to prevent osmotic shock in the plants [35]. Weekly measurements of the electrical conductivity (EC) of the water in the pots were taken to maintain a consistent salinity level. If the desired salinity levels were exceeded, leaching with water was conducted to remedy the situation [36]. Weekly monitoring of soil electrical conductivity (EC) confirmed that salinity levels remained within acceptable ranges around the targeted treatments, with minor fluctuations attributed to irrigation cycles. Detailed EC dynamics are presented in Supplementary Table S2. Starting 30 days after transplanting, foliar applications of glutathione (GSH) and hydrogen peroxide (H_2O_2) was performed at 30, 60, and 90 days after transplanting (DAT), corresponding to the vegetative stage and the initiation of flowering, resulting in a total of three applications. This schedule ensured sustained antioxidant activity, supporting plant growth, reducing stress, and facilitating nutrient uptake [37,38].

Foliar applications were conducted using a hand-held pressure sprayer that delivered a fine mist until runoff occurred, ensuring complete coverage of all aerial parts of the plants. The spray volume was approximately 200 mL plant^{-1}, with slight adjustments based on plant size and developmental stage, to achieve uniform coverage without excessive runoff. A non-ionic surfactant, Tween-80, was added at a concentration of 0.03% (v/v) to enhance adhesion and promote even distribution of the solution on the plants. Distilled water with the same surfactant concentration was used to treat the control plants. Spraying was carried out in the early morning under mild environmental conditions (moderate temperature and low wind) to ensure a uniform application and minimize evaporation losses.

All cultural techniques, such as weed control, fertilization, and irrigation, were conducted in accordance with the recommended of [39]. Ammonium sulfate (20.5% N), calcium superphosphate (15.5% P_2O_5), and potassium sulfate (48% K_2O) were applied as inorganic fertilizers at rates of 500 kg/fed (22.5 g/pot), 300 kg/fed (13.5 g/pot), and 100 kg/fed (4.5 g/pot), respectively. Calcium superphosphate was mixed with the soil before transplanting. Ammonium sulfate was divided into five equal doses (The first dose was applied one month after transplanting, while the second and third doses were applied one month after the first, at one-month intervals. The fourth and fifth doses were added after the second and fourth cuts, respectively). Potassium sulfate was applied in two equal doses, with the first dose in January and the second dose in March.

2.3 Data Recorded

2.3.1 Morphological Measurements

During the flowering stage, growth characteristics were recorded. The following parameters were measured for nine randomly selected plants from each experimental unit: plant height (cm), number of branches per plant, and fresh and dry weights (g) of chamomile plants.

2.3.2 Flower Production

Flower harvesting began 90 days after transplanting and continued at 10-day intervals, at the full bloom stage (when the petals were fully extended). The harvested flowers were allowed to air-dried in the shade under ambient conditions before further analysis, and the following data were recorded (number of flowers/plants, fresh and dry weights of flowers/plant (g)).

2.3.3 Essential Oil Production and Constituents

The percentage of essential oil content in dried chamomile flowers was determined according to the British Pharmacopoeia method [40].

Additionally, the oil yield (mL per plant) was calculated in terms of the ratio between the mass of essential oils and the initial biomass of the flower (g).

$$\text{Volatile oil\%} = \frac{\text{Oil volume in the graduated tube} \times 100}{\text{Weight of dry matter (g)}} \quad (3)$$

$$\text{Essential oil yield (mL)/plant} = \frac{\text{Essential oil\%} \times \text{the biomass of flowers (g/plant)}}{100} \quad (4)$$

The composition analysis of the essential oil extracted during the second season was performed using gas-liquid chromatography (GLC). A BPX-5 capillary column (30 × 0.25 mm ID) was utilized for analyzing the essential oil samples at the Horticulture Research Institute. Nitrogen served as the carrier gas with a flow rate of 1 mL/min, while air and hydrogen flow rates were set at 330 mL/min and 30 mL/min, respectively. The split ratio was 1:10. The column temperature was increased at a rate of 10°C per minute, starting from 70°C and reaching 200°C, where it was held for 3 min. This maximum temperature was maintained for an additional 10 min before the column was cooled. The detector and injector temperatures were set at 300°C and 250°C, respectively. The analysis of the chromatograms allowed for the determination of the percentages of the main components in the essential oil. The essential oil constituents were identified by comparing their retention indices with those of authentic standards analyzed under identical operating conditions.

2.3.4 Standards Compounds

A set of standard compounds representing various chemical groups, each with a stated purity of 99% as verified by Gas Liquid Chromatography (GLC), was acquired from Sigma Chemical Co. in St. Louis, MO, USA. The chemical groups included are terpene hydrocarbons, ketones, alcohols, oxides, and esters.

2.3.5 Chemical Determinations

The protocol by Gao [41] was used to measure the chlorophyll and carotenoid content (mg/g fresh weight) of the terminal leaf, the third leaf at the tip of the plant. A 0.1 g sample of leaves was weighed, chopped, and placed in 20 mL of a 95:5 mixture of absolute ethanol and water. The leaf was incubated in the dark at room temperature (25°C) for 12 h until completely faded. The mixture was then filtered to retain the supernatant, which was used to measure absorbance at 665, 649, and 470 nm. The formats were as follows:

$$\text{Chlorophyll a} = A665 \times 13.95 - A649 \times 6.88$$

$$\text{Chlorophyll b} = A649 \times 24.96 - A665 \times 7.32$$

$$\text{Chl a + b} = \text{Chlorophyll b} + \text{Chlorophyll a}$$

$$\text{Carotenoids} = (A470 \times 1000 - 3.27Ca - 104Cb)/245$$

$$\text{Total chlorophyll content} = \text{Chl (a + b)} + \text{carotenoids}$$

2.3.6 Proline Percent

Samples: Dry leaves were collected for analysis, with purified proline used for quantification. Reagents: Prepare acid-ninhydrin by dissolving 1.25 g of ninhydrin in 30 mL of glacial acetic acid and 20 mL of 6 M phosphoric acid. This reagent is stable for 24 h at 4°C. Procedure:

1. Homogenize 0.5 g of plant material in 10 mL of 3% sulfosalicylic acid and filter through Whatman #2 paper.
2. Mix 2 mL of the filtrate, 2 mL of ninhydrin, and 2 mL of glacial acetic acid in a test tube. Incubate at 100°C for 1 h and then cool in an ice bath.
3. Extract with 4 mL of toluene, mixing vigorously for 15–20 s, then aspirate the toluene phase.
4. Measure the absorbance at 520 nm using toluene as a blank, and determine proline concentration from a standard curve [42].

2.3.7 Nitrogen Content

Nitrogen content was evaluated in the digested solution based on the guidelines provided by the Association of Official Analytical Chemists [43].

2.3.8 Sodium and Potassium Content

Sodium and potassium contents were measured using a flame photometer, following the method outlined by [44].

2.3.9 Total Protein Percentage

The total protein percentage was calculated according to [43].

2.4 Statistical Analysis

Two factors were used for a factorial arrangement in a Randomized Complete Block Design (RCBD) experiment. The first one was irrigation water salinity at four levels. The second factor was foliar application of different concentrations of glutathione (GSH) and hydrogen peroxide (H_2O_2), compared to an untreated control (farm water). Additionally, One-way analysis of variance (ANOVA) was used to evaluate the effect of different saline irrigation levels (340, 1500, and 4500 ppm), with or without a foliar application of 2 mM glutathione, on the essential oil composition of *Matricaria chamomilla* L. during the 2024 growing season. The treatments were replicated three times.

Before analysis, the normality of the data was assessed using the Shapiro–Wilk test, which confirmed that the data followed a normal distribution. Homogeneity of error variances across years was evaluated using [45], supporting the validity of analyzing each year separately for most traits. All data were analyzed using the COSTAT software package, and ANOVA (analysis of variance) for RCBD module was calculated. The use of Duncan's Multiple Range Test (DMRT) at the 0.05 probability level was used to compare treatment means, as described by [46].

3 Results

3.1 Effect of Salinity, Glutathione, and Hydrogen Peroxide on Growth Characteristics

Salinity stress induced by irrigation with saline well water at different levels adversely influenced all studied growth traits of *M. chamomilla* (Table 2). The reduction in plant height, number of branches/plants, herb fresh and dry weights induced by saline well water at 4500 ppm reached 38.89 and 37.38%, 32.38 and 30.38%, 43.34 and 42.04%, and 48.70 and 45.69%, respectively, in both seasons, compared with the control (340 ppm), which produced the highest values. Foliar application with GSH and H_2O_2 at different concentrations enhanced growth traits compared with control plants (Table 2). GSH at 2 mM surpassed other treatments with significant differences in plant height and herb fresh weight in both seasons. For the number of branches/plant and herb dry weight, no significant influence was observed between H_2O_2 at 5 mM and GSH at 2 mM. Regarding the combined effect of different levels of saline well water and foliar

application of GSH or H_2O_2, as protective agents against salinity stress, Table 2 demonstrated that GSH and H_2O_2 mitigated the adverse effects of salinity stress in M. chamomilla plants. The results indicated higher growth values for plants irrigated with different saline well water and treated with GSH and H_2O_2 compared with unstressed plants (control) and plants exposed to salinity without foliar application. The obtained values in all studied growth traits of plants irrigated with saline well water at 1500 ppm + foliar application with GSH at 2 mM were significantly higher than those of the control plants. Irrigation with saline water at 3000 ppm + foliar application with GSH at 2 mM increased growth characteristics compared with the control, but these increments were insignificant. In addition, H_2O_2 at a low concentration (5 mM) enhanced growth traits when combined with irrigation with saline well water at 1500 or 3000 ppm.

Table 2: Effect of salinity levels and foliar applications of glutathione (GSH) and hydrogen peroxide (H_2O_2) on growth characteristics of chamomile (*Matricaria chamomilla* L.) during the 2022/2023 and 2023/2024 seasons.

Foliar Application (B)	First Season					Second Season				
	Salinity Levels (A)									
	340 ppm	1500 ppm	3000 ppm	4500 ppm	Mean (B)	340 ppm	1500 ppm	3000 ppm	4500 ppm	Mean (B)
					Plant Height (cm)					
Control	44.67ef	42.33fg	35.67h	26.00k	37.17D	47.00f	43.33gh	36.67jk	28.67m	38.92E
GSH (1 mM)	54.00b–d	51.67cd	40.67g	32.00ij	44.58C	54.00b–e	52.00e	41.3hi	33.67kl	45.24D
GSH (2 mM)	57.67a	56.00ab	46.33e	36.33h	49.08A	58.00a	57.33ab	46.00fg	38.67ij	50.00A
GSH (3 mM)	55.33ab	54.67a–c	44.33ef	34.67hi	47.25B	57.00a–c	55.67a–d	44.33f–h	35.33k	48.08BC
H_2O_2 (5 mM)	56.33ab	54.33bc	43.67e–g	34.67hi	47.25B	57.33ab	55.67a–d	44.67f–h	36.00jk	48.42B
H_2O_2 (10 mM)	54.00b–d	53.67b–d	43.00fg	33.67h–j	46.09B	55.67a–d	53.67c–e	43.00gh	34.67kl	46.75C
H_2O_2 (20 mM)	51.67cd	51.00d	40.67g	31.00j	43.58C	52.67de	52.67de	41.3hi	32.00l	44.66D
Mean (A)	53.38A	51.95B	42.05C	32.62D		54.52A	52.91B	42.47C	34.14D	
					Number of Branches/Plants					
Control	10.33f–j	9.67g–j	7.00kl	5.67l	8.17D	11.00e–i	10.33g–k	8.00k–m	6.67m	9.00E
GSH (1 mM)	12.00b–g	11.33d–h	8.33i–k	6.67kl	9.58C	12.33b–h	11.67d–i	9.00i–m	7.33lm	10.08DE
GSH (2 mM)	14.67a	14.00a–c	12.33a–f	10.67e–i	12.92A	15.00a	14.33a–c	12.67a–g	11.33e–i	13.33A
GSH (3 mM)	13.67a–d	13.00a–e	11.67c–g	9.50g–j	11.96A	14.00a–d	13.33a–f	11.00e–i	10.00g–k	12.08BC
H_2O_2 (5 mM)	14.33ab	13.33a–d	12.00b–g	10.33f–j	12.50A	14.67ab	13.67a–e	12.00c–h	10.67f–j	12.74AB
H_2O_2 (10 mM)	12.33a–f	11.67c–g	10.67e–i	8.67i–k	10.84B	12.67a–g	12.00c–h	11.00e–i	9.00i–m	11.17CD
H_2O_2 (20 mM)	10.67e–i	10.33f–j	9.00h–k	8.00jk	9.50C	11.33e–i	11.00d–h	9.67h–l	8.33j–m	10.08DE
Mean (A)	12.57A	11.90A	10.14B	8.50C		13.00A	12.33A	10.48B	9.05C	
					Herb Fresh Weight (g/plant)					
Control	113.18e	98.67h	79.33h	54.33m	86.38E	116.33ij	109.91j	84.68k	66.18m	94.28E
GSH (1 mM)	125.67a–d	124.67a–d	83.33i	70.33l	101.00D	137.93a–e	125.93f–h	111.91j	78.97kl	113.68D
GSH (2 mM)	131.00a	128.33ab	119.00c–f	80.33ij	114.66A	144.48a	139.69a–d	121.92g–i	85.16k	122.81A
GSH (3 mM)	128.33ab	126.00a–c	117.33d–g	75.00j–l	111.67AB	132.66d–f	129.75e–h	116.83ij	81.77kl	115.25CD
H_2O_2 (5 mM)	128.33ab	124.00e–g	114.33de	75.33j–l	110.50BC	142.30ab	133.51c–f	129.25e–h	82.61kl	121.92AB
H_2O_2 (10 mM)	126.33a–c	122.00b–e	110.33g	72.00kl	107.67C	141.57a–c	130.38e–g	121.13hi	80.25kl	118.33BC
H_2O_2 (20 mM)	123.67a–d	120.00c–f	101.67h	69.33lm	103.67D	135.38b–e	125.81d–g	111.18j	76.00l	112.09D
Mean (A)	125.22A	120.52B	103.62C	70.95D		135.81A	127.85B	113.84C	78.71D	
					Herb Dry Weight (g/plant)					
Control	25.67i	23.80i–k	19.30lm	14.27o	20.76D	26.37hi	25.53hi	21.30kl	14.87o	22.02E
GSH (1 mM)	31.87a–f	28.83f–h	22.67jk	15.90no	24.82B	32.79a	29.80ef	24.67ef	17.80ij	26.26C
GSH (2 mM)	34.73a	33.27a–c	26.77g–i	17.00m–o	27.94A	35.60a	34.23ab	27.37gh	19.77lm	29.24A
GSH (3 mM)	32.70a–d	30.73c–f	24.53ij	15.47o	25.86B	33.67a–c	32.20b–d	25.50hi	18.83m	27.55B
H_2O_2 (5 mM)	34.00ab	31.10b–f	25.80i	18.77l–n	27.42A	34.47ab	32.93b–d	26.93g–i	19.47lm	28.45AB
H_2O_2 (10 mM)	32.10a–e	29.20e–g	23.8 3e–g	16.37m–o	25.38B	32.60b–d	31.80c–e	24.77ij	16.27no	26.36C
H_2O_2 (20 mM)	30.13d–f	26.13hi	21.50kl	15.73o	23.37C	30.83de	28.63fg	23.10gh	15.90no	24.62D
Mean (A)	31.60A	29.01B	23.49C	16.21D		32.33A	30.73B	24.81C	17.56D	

Means followed by the same letter(s) are not significantly different, whereas means followed by different letters differ significantly. The highest mean value is denoted by the first letter of the English alphabet. Capital letters indicate main effects, while lowercase letters represent interaction effects according to Duncan's New Multiple Range Test at $p \leq 0.05$.

3.2 Effect of Salinity, Glutathione, and Hydrogen Peroxide on Flower Parameters

A marked reduction in flower parameters was observed with increasing levels of saline well water (Table 3). Irrigation with saline well water at 4500 ppm reduced the number of flowers/plants by 60.15 and 59.32%, the fresh weight of flowers/plant by 56.14 and 56.13%, and the dry weight of flowers/plant by 59.76 and 58.70%, compared with the control in both seasons, respectively. Table 3 showed that both GSH and H_2O_2 enhanced the flower parameters of M. chamomilla compared with the control. Foliar application with 2 mM GSH was superior to other treatments and resulted in the highest significant values for all the studied flower parameters. A significant enhancement was achieved when foliar application with GSH or H_2O_2 was combined with saline well water irrigation at different levels (Table 3). No significant impact was observed between GSH at 2 mM and H_2O_2 at 5 mM when applied with saline well water at 1500 ppm in the case of the number of flowers/plant and fresh weight of flowers/plant, as the obtained values were higher than the control and plants treated with saline well water only. Dry weight of flowers/plant enhanced by GSH at 2 mM under irrigation with saline well water compared with the control and non-foliar application plants.

Table 3: Effect of salinity levels and foliar applications of glutathione (GSH) and hydrogen peroxide (H_2O_2) on flower parameters of chamomile (*Matricaria chamomilla* L.) during the 2022/2023 and 2023/2024 seasons.

Foliar Application (B)	First Season					Second Season				
	Salinity Levels (A)									
	340 ppm	1500 ppm	3000 ppm	4500 ppm	Mean (B)	340 ppm	1500 ppm	3000 ppm	4500 ppm	Mean (B)
	Number of Flowers/Plants									
Control	521.33g	502.33gh	317.00m	218.00o	389.67E	524.00f	512.33fg	323.00k	231.67n	397.75E
GSH (1 mM)	646.67de	614.67f	448.67jk	237.33no	486.84C	667.00bc	621.33de	450.67ij	243.33mn	495.58C
GSH (2 mM)	707.33a	670.33b–d	481.67hi	291.00m	537.58A	713.33a	683.33ab	499.33fg	308.00k	551.00A
GSH (3 mM)	684.33a–c	650.67de	471.00i–k	278.33m	521.08B	688.67ab	661.67bc	481.33gh	282.00kl	528.42B
H_2O_2 (5 mM)	690.33ab	657.33c–e	475.67hi-	282.00m	526.33AB	692.33ab	672.00bc	489.00gh	286.00kl	534.83B
H_2O_2 (10 mM)	650.00de	630.00d–f	455.00i–k	260.67mn	498.92C	661.67bc	642.33cd	459.67hi	266.67l m	507.58C
H_2O_2 (20 mM)	614.33f	602.00g	440.33kl	231.67no	472.08D	616.67de	610.33e	444.00i	238.67mn	477.42D
Mean (A)	644.90A	618.19B	441.33C	257.00D		651.95A	629.05B	449.57C	265.19D	
	Fresh Weight of Flowers/Plant (g)									
Control	120.90i	115.84j	74.43o p	51.38t	90.64F	122.55f	116.87g	76.38lm	52.15q	91.99F
GSH (1 mM)	141.36ef	143.47ef	87.60kl	68.39qr	110.21C	153.76bc	149.42cd	88.22hi	71.37n	115.69B
GSH (2 mM)	158.00a	151.69bc	90.61k	71.80pq	118.02A	160.06a	154.28b	91.49h	73.33lm	119.79A
GSH (3 mM)	142.81ef	139.91fg	80.44mn	60.74s	105.98D	154.79b	140.02e	81.86jk	61.84p	109.63D
H_2O_2 (5 mM)	153.66b	148.08cd	87.25kl	67.80qr	114.20B	155.41b	151.28bc	88.49hi	70.95n	116.53B
H_2O_2 (10 mM)	149.73b–d	145.49de	83.71lm	64.91r	110.96C	151.18bc	146.32d	85.39ij	66.44o	112.33C
H_2O_2 (20 mM)	136.92gh	133.92h	76.38no	55.04t	100.57E	138.47e	135.99e	78.59kl	58.53p	102.90E
Mean (A)	143.34A	139.77B	82.92C	62.87D		148.03A	142.03B	84.35C	64.94D	
	Dry Weight of Flowers/Plant (g)									
Control	26.54h	24.10i	14.94m	9.35p	18.73F	30.19fg	23.66h	15.84k–n	11.65q	20.34F
GSH (1 mM)	30.17d	28.5ef	17.97kl	13.26n	22.48C	32.67c–e	29.03fg	17.56j–l	13.73op	23.25D
GSH (2 mM)	37.70a	33.63b	19.32j	14.66m	26.33A	40.40a	33.46cd	22.55h	16.11k–n	28.13A
GSH (3 mM)	29.11d–f	26.86gh	16.73l	11.63o	21.08D	34.18c	29.59g	17.71jk	15.10m–o	24.15C
H_2O_2 (5 mM)	33.13bc	31.99c	18.41jk	13.74mn	24.32B	38.18b	31.07ef	19.95i	15.46m–o	26.16B
H_2O_2 (10 mM)	29.47de	28.95d–f	17.56kl	12.90n	22.22C	33.22cd	32.03de	18.98ij	14.60no	24.71C
H_2O_2 (20 mM)	28.02fg	27.16gh	14.93m	10.61o	20.18E	31.31ef	28.58g	16.51k–m	12.51pq	22.23E
Mean (A)	30.59A	28.74B	17.12C	12.31D		34.31A	29.63B	18.44C	14.17D	

Means followed by the same letter(s) are not significantly different, whereas means followed by different letters differ significantly. The highest mean value is denoted by the first letter of the English alphabet. Capital letters indicate main effects, while lowercase letters represent interaction effects according to Duncan's New Multiple Range Test at $p \leq 0.05$.

3.3 Effect of Salinity, Glutathione, and Hydrogen Peroxide on Chemical Composition

Data in Tables 4–6 revealed that the lowest values of total chlorophyll (mg/g FW), carotenoid content (mg/g FW), carbohydrates (%), nitrogen (%), protein (%), potassium (%) and K^+/Na^+ ratio were found in the leaves when the plants were irrigated with the highest level of saline well water (4500 ppm). Moreover, both sodium (%) and proline (mg/g DW) recorded the highest values. All chemical constituents under study showed statistically significant responses to different salinity protective agent levels. GSH at 2 mM was superior, resulting in the highest significant values for all studied chemical constituents, except sodium, which had the lowest values. Proline accumulation in plant tissues was enhanced by H_2O_2 (20 mM) with no significant differences relative to other treatments. At 1500 ppm saline well water, GSH at 2 mM results in higher values than the control on total chlorophyll, carotenoid content, carbohydrates, nitrogen, protein, potassium and K^+/Na^+. This was associated with a reduction in sodium % as the lowest values were recorded in both seasons. Under all levels of saline well water, both GSH and H_2O_2 enhanced the accumulation of the foliar-applied plants compared to control and stressed plants only (without foliar application). H_2O_2 at 20 mM produced the highest non-significant values of proline under the highest levels of salinity (4500 ppm) in both seasons.

Table 4: Effect of salinity levels and foliar applications of glutathione (GSH) and hydrogen peroxide (H_2O_2) on total chlorophyll (mg/g FW), carotenoid content (mg/g FW) and carbohydrates (%) of chamomile (*Matricaria chamomilla* L.) during the 2022/2023 and 2023/2024 seasons.

Foliar Application (B)	First Season					Second Season				
	Salinity Levels (A)									
	340 ppm	1500 ppm	3000 ppm	4500 ppm	Mean (B)	340 ppm	1500 ppm	3000 ppm	4500 ppm	Mean (B)
	Total Chlorophyll (mg/g FW)									
Control	0.73d–g	0.66e–i	0.54h–l	0.42l	0.59D	0.79b–d	0.65cd	0.57cd	0.44d	0.61D
GSH (1 mM)	0.99b	0.73d–g	0.62f–k	0.53h–l	0.72B	1.07bc	0.75b–d	0.66cd	0.54cd	0.76BC
GSH (2 mM)	1.26a	0.78c–f	0.66e–i	0.68d–i	0.85A	1.60a	0.79b–d	0.68cd	0.59cd	0.92A
GSH (3 mM)	0.85b–d	0.70d–h	0.59g–l	0.48j–l	0.66B–D	0.92b–d	0.72b–d	0.61cd	0.53cd	0.70CD
H_2O_2 (5 mM)	1.01b	0.73d–g	0.63e–j	0.54h–l	0.73B	1.26ab	0.76b–d	0.66cd	0.56cd	0.81B
H_2O_2 (10 mM)	0.94bc	0.73d–g	0.60f–l	0.51h–l	0.70BC	1.01b–d	0.74b–d	0.63cd	0.53cd	0.73BC
H_2O_2 (20 mM)	0.82c–e	0.69d–i	0.57g–l	0.44kl	0.63CD	0.90bcd	0.70b–d	0.60cd	0.47d	0.67CD
Mean (A)	0.94A	0.72B	0.60C	0.51D		1.08A	0.73B	0.63C	0.52D	
	Carotenoid Content (mg/g FW)									
Control	0.33c–g	0.31c–i	0.24h–k	0.16k	0.26d	0.34c–g	0.32c–g	0.25e–i	0.17i	0.27E
GSH (1 mM)	0.51b	0.35c–e	0.29c–i	0.28d–j	0.36b	0.52b	0.37c–e	0.30c–g	0.29c–g	0.37BC
GSH (2 mM)	0.64a	0.39c	0.32c–h	0.31c–i	0.42a	0.70a	0.39c	0.33c–g	0.32c–g	0.44A
GSH (3 mM)	0.35c–e	0.30c–i	0.26f–j	0.23i–k	0.29cd	0.38cd	0.33c–g	0.27d–i	0.24f–i	0.31DE
H_2O_2 (5 mM)	0.53b	0.37cd	0.31c–i	0.29c–i	0.38b	0.63a	0.38cd	0.31c–g	0.30c–g	0.41AB
H_2O_2 (10 mM)	0.39c	0.33c–g	0.29c–i	0.25g–j	0.31c	0.40c	0.34c–g	0.29c–g	0.26d–i	0.32CD
H_2O_2 (20 mM)	0.34c–f	0.32c–h	0.26f–j	0.20jk	0.28cd	0.36c–f	0.32c–h	0.26e–i	0.21hi	0.29DE
Mean (A)	0.44A	0.34B	0.28C	0.25D		0.48A	0.35B	0.29C	0.26D	
	Carbohydrates (%)									
Control	26.85de	24.41gh	23.17hi	20.50j	23.73E	26.91d–f	24.46g–j	23.47h–l	20.53m	23.84F
GSH (1 mM)	31.70a–c	26.79de	25.82d–g	23.30hi	26.90B	31.83a–c	26.90d–f	25.87d–g	23.47h–l	27.02BC
GSH (2 mM)	32.89a	27.41d	26.92de	24.72f–h	27.99A	32.94a	27.45d	27.04d–f	24.83gh	28.07A
GSH (3 mM)	30.78bc	25.65d–g	24.45gh	22.22i	25.78CD	30.98c	25.82e–g	24.64g–i	22.84kl	26.07D
H_2O_2 (5 mM)	31.81ab	27.06de	25.37e–g	23.40hi	26.91B	32.57ab	27.14de	25.45fg	23.83h–k	27.25B
H_2O_2 (10 mM)	31.23a–c	26.39d–f	24.33gh	22.88hi	26.21BC	31.72a–c	26.43d–f	24.78gh	22.90j–l	26.46CD
H_2O_2 (20 mM)	30.01d–g	25.57d–g	23.01hi	21.97ij	25.14D	31.29bc	26.08d–g	23.16i–l	21.98l	25.63E
Mean (A)	30.75A	26.18B	24.72C	22.71D		31.18A	26.33B	24.92C	22.91D	

Means followed by the same letter(s) are not significantly different, whereas means followed by different letters differ significantly. The highest mean value is denoted by the first letter of the English alphabet. Capital letters indicate main effects, while lowercase letters represent interaction effects according to Duncan's New Multiple Range Test at $p \leq 0.05$.

Table 5: Effect of salinity levels and foliar applications of glutathione (GSH) and hydrogen peroxide (H_2O_2) on nitrogen (%), protein (%) and potassium (%) of chamomile (*Matricaria chamomilla* L.) during the 2022/2023 and 2023/2024 seasons.

Foliar Application (B)	First Season					Second Season				
	Salinity Levels (A)									
	340 ppm	1500 ppm	3000 ppm	4500 ppm	Mean (B)	340 ppm	1500 ppm	3000 ppm	4500 ppm	Mean (B)
					Nitrogen (%)					
Control	2.12jk	2.03l	1.59n	1.21q	1.74F	2.24h–j	2.12cd	1.71o	1.38q	1.86F
GSH (1 mM)	2.43bc	2.32d–f	2.16i–k	1.64n	2.14C	2.51g–i	2.38o	2.22i–k	1.76no	2.22C
GSH (2 mM)	2.52a	2.38cd	2.29e–g	1.81m	2.25A	2.73a	2.54bc	2.33f–h	1.95m	2.39A
GSH (3 mM)	2.38cd	2.25f–h	2.11k	1.51o	2.06D	2.47g–i	2.29g–i	2.14j–l	1.56p	2.12D
H_2O_2 (5 mM)	2.46ab	2.32ef	2.20h–j	1.76m	2.18B	2.64ab	2.42bc	2.29g–i	1.83n	2.30B
H_2O_2 (10 mM)	2.37cd	2.27e–h	2.15i–k	1.57n	2.09D	2.40j–l	2.33f–h	2.20no	1.67o	2.15D
H_2O_2 (20 mM)	2.34de	2.23g–i	2.08kl	1.39p	2.01E	2.36def	2.27g–i	2.07l	1.42q	2.03E
Mean (A)	2.38A	2.26B	2.08C	1.56D		2.48A	2.34B	2.14C	1.65D	
					Protein (%)					
Control	13.25ij	12.66k	9.94m	7.54p	10.85F	13.99g–k	13.23kl	10.70op	8.65r	11.64F
GSH (1 mM)	15.21bc	14.49de	13.53h–j	10.24m	13.37C	15.68b–d	14.89c–h	13.87h–l	10.97o	13.85C
GSH (2 mM)	15.76a	14.86cd	14.31ef	11.33l	14.06A	17.04a	15.86bc	14.59e–i	12.16mn	14.91A
GSH (3 mM)	14.89cd	14.07e–g	13.20j	9.41n	12.89D	15.42c–e	14.29f–j	13.38j–l	9.76pq	13.21D
H_2O_2 (5 mM)	15.39ab	14.47de	13.76g–i	11.01l	13.66B	16.48ab	15.11c–f	14.33f–j	11.42no	14.34B
H_2O_2 (10 mM)	14.84cd	14.21e–g	13.43h–j	9.81mn	13.07D	15.01c–g	14.59e–i	13.73i–l	10.46op	13.45D
H_2O_2 (20 mM)	14.59de	13.92f–h	13.03jk	8.72o	12.56E	14.73d–i	14.21f–k	12.93lm	8.87qr	12.69E
Mean (A)	14.85A	14.10B	13.03C	9.72D		15.48A	14.60B	13.36C	10.33D	
					Potassium (%)					
Control	2.13e–g	1.87h–k	1.71k–m	1.53m	1.81E	2.15f–h	1.96h–j	1.74kl	1.54m	1.85F
GSH (1 mM)	2.47bc	2.36b–d	1.99g–i	1.81i–l	2.16C	2.50cd	2.44c–e	2.04g–i	1.90i–k	2.22C
GSH (2 mM)	2.78a	2.54b	2.21d–f	2.03f–h	2.39A	2.81a	2.57bc	2.31d–f	2.13f–h	2.46A
GSH (3 mM)	2.28c–e	2.24de	1.95g–j	1.76j–l	2.06D	2.32d–f	2.25e–g	1.97h–j	1.81jk	2.09D
H_2O_2 (5 mM)	2.52b	2.46b–d	2.12e–g	1.97g–i	2.27B	2.71ab	2.53bc	2.15f–h	2.04g–i	2.36B
H_2O_2 (10 mM)	2.37b–d	2.26de	1.77j–l	1.61lm	2.00D	2.40c–e	2.32d–f	1.85i–k	1.82jk	2.10D
H_2O_2 (20 mM)	2.25de	1.98g–i	1.65lm	1.53m	1.85E	2.27ef	2.19fg	1.75kl	1.56lm	1.94E
Mean (A)	2.40A	2.24B	1.91C	1.75D		2.45A	2.32B	1.97C	1.83D	

Means followed by the same letter(s) are not significantly different, whereas means followed by different letters differ significantly. The highest mean value is denoted by the first letter of the English alphabet. Capital letters indicate main effects, while lowercase letters represent interaction effects according to Duncan's New Multiple Range Test at $p \leq 0.05$.

Table 6: Effect of salinity levels and foliar applications of glutathione (GSH) and hydrogen peroxide (H_2O_2) on sodium %, K^+/Na^+ ratio and proline (mg/g DW) of chamomile (*Matricaria chamomilla* L.) during the 2022/2023 and 2023/2024 seasons.

Foliar Application (B)	First Season					Second Season				
	Salinity Levels (A)									
	340 ppm	1500 ppm	3000 ppm	4500 ppm	Mean (B)	340 ppm	1500 ppm	3000 ppm	4500 ppm	Mean (B)
					Sodium (%)					
Control	2.75d–l	3.07a–j	3.21a–f	3.63a	3.17A	2.89c–h	3.11b-	3.22b–e	4.12a	3.34A
GSH (1 mM)	2.49i–m	2.81c–l	2.87c–l	3.34a–e	2.88AB	2.54e–h	2.89c–h	2.94c–h	3.38b–d	2.94C
GSH (2 mM)	1.92m	2.30lm	2.66f–l	2.99b–k	2.47C	2.34h	2.39gh	2.64e–h	3.18b–f	2.64D
GSH (3 mM)	2.52h–m	2.87c–l	2.96b–k	3.37a–d	2.93AB	2.61e–h	2.91c–h	3.07c–g	3.50bc	3.02BC
H_2O_2 (5 mM)	2.43j–m	2.37k–m	2.77d–l	3.21a–f	2.70BC	2.49f–h	2.79d–h	2.88c–h	3.36b–d	2.88CD
H_2O_2 (10 mM)	2.56g–l	2.92c–l	3.10a–i	3.44a–c	3.01A	2.63e–h	2.94c–h	3.11b–f	3.55a–c	3.06B
H_2O_2 (20 mM)	2.72e–l	2.97b–k	3.16a–h	3.57ab	3.11A	2.77d–h	3.00c–h	3.18b–f	3.75ab	3.17AB
Mean (A)	2.48D	2.76C	2.96B	3.36A		2.61C	2.86B	3.01B	3.55A	
					K^+/Na^+ Ratio					
Control	0.78e–i	0.62i–l	0.54k–m	0.42m	0.59E	0.76n–p	0.64j–o	0.54n–p	0.38q	0.58E
GSH (1 mM)	1.00e–i	0.85d–g	0.70f–k	0.54j–m	0.77C	0.99bc	0.85c–g	0.70h–n	0.57m–p	0.78C
GSH (2 mM)	1.47a	1.11b	0.84e–h	0.69g–k	1.03A	1.20a	1.07ab	0.87c–e	0.67h–n	0.96A
GSH (3 mM)	0.91b–d	0.78e–i	0.66h–k	0.53k–m	0.72CD	0.89c–e	0.78e–i	0.65j–o	0.52op	0.71D
H_2O_2 (5 mM)	1.04bc	1.04bc	0.78e–i	0.62i–l	0.87B	1.10ab	0.92cd	0.75f–l	0.61k–o	0.84B
H_2O_2 (10 mM)	0.93c–e	0.78e–i	0.57j–m	0.47lm	0.69D	0.92cd	0.80d–i	0.60m–o	0.52op	0.71D
H_2O_2 (20 mM)	0.83e–h	0.67g–k	0.53k–m	0.43m	0.62E	0.83d–h	0.92f–l	0.55m–p	0.42pq	0.63E
Mean (A)	0.99A	0.84B	0.66C	0.53D		0.96A	0.83B	0.66C	0.53D	

Table 6: *Cont.*

Foliar Application (B)	First Season					Second Season				
	Salinity Levels (A)									
	340 ppm	1500 ppm	3000 ppm	4500 ppm	Mean (B)	340 ppm	1500 ppm	3000 ppm	4500 ppm	Mean (B)
	Proline (mg/g DW)									
Control	4.43i	4.59hi	5.06c–i	5.65a–h	4.93B	4.57j	4.73h–j	5.32b–j	5.82a–i	5.11B
GSH (1 mM)	4.54hi	4.83d–i	5.75a–g	5.86a–f	5.25AB	4.68ij	4.97f–j	5.88a–h	6.20a–e	5.43AB
GSH (2 mM)	4.67g–i	4.98c–i	5.78a–g	6.04a–c	5.37AB	4.79h–j	5.10e–j	5.98a–g	6.32a–d	5.55AB
GSH (3 mM)	4.51hi	4.72f–i	5.45b–i	5.74a–g	5.11AB	4.63ij	4.85g–j	5.60a–j	6.00a–g	5.27AB
H_2O_2 (5 mM)	4.74f–i	5.01c–i	5.89a–e	6.29ab	5.48A	4.86g–j	5.15d–j	6.03a–g	6.33a–c	5.59AB
H_2O_2 (10 mM)	4.76e–i	5.05c–i	5.94a–d	6.48ab	5.56A	4.87g–j	5.16c–j	6.04a–g	6.37ab	5.61AB
H_2O_2 (20 mM)	4.85d–i	5.09c–i	5.96a–d	6.67a	5.64A	5.02f–j	5.26b–j	6.14a–f	6.60a	5.75A
Mean (A)	4.64C	4.90C	5.69B	6.10A		4.77C	5.03C	5.86B	6.23A	

Means followed by the same letter(s) are not significantly different, whereas means followed by different letters differ significantly. The highest mean value is denoted by the first letter of the English alphabet. Capital letters indicate main effects, while lowercase letters represent interaction effects according to Duncan's New Multiple Range Test at $p \leq 0.05$.

3.4 Effect of Salinity, Glutathione, and Hydrogen Peroxide on Essential Oil Content and Yield

When the salinity levels were increased (up to 4500 ppm), the percentage of essential oil increased, whereas the yield reduced (Table 7). As shown in Table 7, all concentrations of GSH and H_2O_2 enhanced essential oil percentage and yield/plant compared with the control. GSH at 2 mM produced the highest values, which were significant compared with control in both seasons. The combined treatment of GSH at 2 mM + irrigation with saline well water at 4500 ppm resulted in the highest essential oil percentage in both seasons compared with the control and stressed plants without foliar application of GSH or H_2O_2. Under other saline well water levels, GSH at 2 mM also enhanced the essential oil percentage, but the values were less than those of the outstanding treatment. On the other hand, saline well water at 1500 ppm in addition to foliar application with GSH at 2 mM produced the highest essential oil yield/plant.

Table 7: Effect of salinity levels and foliar applications of glutathione (GSH) and hydrogen peroxide (H_2O_2) on the essential oil % and yield (mL/plant) of chamomile (*Matricaria chamomilla* L.) during the 2022/2023 and 2023/2024 seasons.

Foliar Application (B)	First Season					Second Season				
	Salinity Levels (A)									
	340 ppm	1500 ppm	3000 ppm	4500 ppm	Mean (B)	340 ppm	1500 ppm	3000 ppm	4500 ppm	Mean (B)
	Essential Oil %									
Control	0.51j	0.54h–j	0.58f–j	0.61a–f	0.56D	0.53n	0.56mn	0.60lm	0.64j–l	0.58E
GSH (1 mM)	0.65b–i	0.67a–h	0.71a–e	0.74a–d	0.69AB	0.68g–k	0.70e–i	0.74b–f	0.77a–c	0.72B
GSH (2 mM)	0.68a–g	0.73a–d	0.75a–c	0.79a	0.74A	0.71d–h	0.76b–d	0.78a–c	0.83a	0.77A
GSH (3 mM)	0.62c–j	0.65a–e	0.68a–g	0.70a–e	0.66BC	0.65i–l	0.68g–k	0.71d–h	0.73c–g	0.69C
H_2O_2 (5 mM)	0.66a–i	0.71a–e	0.72a–h	0.76ab	0.70AB	0.60lm	0.69f–j	0.74b–f	0.80ab	0.71AB
H_2O_2 (10 mM)	0.58f–j	0.60e–j	0.62c–j	0.67a–h	0.62CD	0.60lm	0.63kl	0.65i–l	0.70e–i	0.65D
H_2O_2 (20 mM)	0.53ij	0.57g–j	0.60e–j	0.64b–j	0.59D	0.55mn	0.59lm	0.63kl	0.67h–k	0.61E
Mean (A)	0.60D	0.64C	0.67B	0.70A		0.62D	0.66C	0.69B	0.73A	
	Essential Oil Yield (mL/plant)									
Control	0.14ij	0.13i–k	0.09mn	0.06o	0.10F	0.16a–f	0.13b–f	0.10b–f	0.07f	0.12E
GSH (1 mM)	0.20de	0.19ef	0.13jk	0.10lm	0.15C	0.22a–e	0.20a–f	0.13a–f	0.11d–f	0.18C
GSH (2 mM)	0.26a	0.24a	0.14kl	0.12k–m	0.19A	0.29a	0.24a–c	0.18a–c	0.13b–f	0.21A
GSH (3 mM)	0.18e–g	0.17f–h	0.11k–m	0.08mn	0.14D	0.22a–e	0.20a–f	0.13a–f	0.11d–f	0.17C
H_2O_2 (5 mM)	0.22cd	0.23bc	0.13jk	0.10lm	0.17B	0.26a–e	0.25b–e	0.15b–e	0.12c–f	0.18C
H_2O_2 (10 mM)	0.17f–h	0.17f–h	0.11k–m	0.09mn	0.13D	0.20a–f	0.20a–f	0.12a–f	0.10ef	0.16C
H_2O_2 (20 mM)	0.15mn	0.15h–j	0.09mn	0.07no	0.11E	0.17a–f	0.17a–f	0.10a–f	0.08f	0.13D
Mean (A)	0.19A	0.18A	0.11B	0.09C		0.21A	0.19B	0.13B	0.10C	

Means followed by the same letter(s) are not significantly different, whereas means followed by different letters differ significantly. The highest mean value is denoted by the first letter of the English alphabet. Capital letters indicate main effects, while lowercase letters represent interaction effects according to Duncan's New Multiple Range Test at $p \leq 0.05$.

3.5 Effect of Salinity, Glutathione, and Hydrogen Peroxide on Essential Oil Composition

Table 8 presents the active constituents of chamomile essential oil extracted from dried chamomile flowers using gas-liquid chromatography (GLC). It was significantly affected by saline irrigation levels (340 as control, 1500, 3000 and 4500 ppm), foliar application of Glutathione (GSH) at 2 mM, and their interactions. Some of these components were identified, and the others were absent due to different treatments. The highest number of volatile components (20) was identified in the chamomile oil under irrigation with saline well water at 1500 ppm. In control plants (340 ppm), only 18 components were identified, with the absence of menthone and caryophyllene oxide. The main constituents in control plants were α-bisabolol oxide A (61.49%), trans-β-farnesene (7.96%), α-bisabolol (5.78%), bisabolone oxide B (5.13%), chamazulene (2.19%), bisabolol oxide B (0.98%).

Table 8: Effect of saline irrigation levels (ppm), with or without Glutathione foliar application (2 mM), on the essential oil composition of chamomile (*Matricaria chamomilla* L.) during the 2024 season.

	Components	340 ppm	1500 ppm	4500 ppm	340 ppm + Glutathione	1500 ppm + Glutathione
1	**cis-Ocimene**	0.85 ± 0.15	0.81 ± 0.14	0.51 ± 0.10	0.86 ± 0.09	0.07 ± 0.02
2	**1,8-Cineole**	0.93 ± 0.13	3.60 ± 0.42	5.18 ± 0.80	1.16 ± 0.09	-
3	**Artemisia ketone**	1.88 ± 0.29	1.49 ± 0.06	1.25 ± 0.29	1.94 ± 0.09	1.58 ± 0.32
4	**Menthone**	-	1.01 ± 0.12	1.58 ± 0.28	-	-
5	**trans-β-Farnesene**	7.96 ± 1.37	6.20 ± 0.61	4.79 ± 0.40	9.19 ± 1.54	8.42 ± 1.00
6	**Germacrene D**	1.26 ± 0.33	0.72 ± 0.03	0.67 ± 0.05	0.12 ± 0.07	0.87 ± 0.30
7	**δ-Cadinene**	0.48 ± 0.15	0.55 ± 0.07	-	0.50 ± 0.11	-
8	**Bisabolol oxide B**	0.98 ± 0.10	1.16 ± 0.14	1.29 ± 0.21	1.59 ± 0.06	1.80 ± 0.15
9	**Caryophyllene oxide**	-	0.65 ± 0.09	-	0.12 ± 0.06	-
10	**Bisabolone oxide B**	5.13 ± 0.08	7.60 ± 2.66	6.13 ± 0.48	6.13 ± 0.48	7.49 ± 0.57
11	**cis-α-Santalol**	0.96 ± 0.044	0.86 ± 0.046	0.81 ± 0.125	0.98 ± 0.046	0.96 ± 0.121
12	**Ageratochromene**	0.87 ± 0.036	1.17 ± 0.053	1.87 ± 0.437	0.91 ± 0.822	-
13	**α-Bisabolol**	5.78 ± 0.34	4.88 ± 0.20	4.51 ± 0.21	6.25 ± 0.29	4.15 ± 0.20
14	**Chamazulene**	2.19 ± 0.07	2.20 ± 0.29	2.40 ± 0.10	2.50 ± 0.27	2.74 ± 0.83
15	**α-Bisabolol oxide A**	61.49 ± 0.76	62.39 ± 0.52	62.42 ± 0.50	61.91 ± 1.09	65.83 ± 0.86
16	**2-Pentylfuran**	0.78 ± 0.046	1.26 ± 0.23	2.01 ± 0.052	0.80 ± 0.12	-
17	**Z-Spiroether**	3.82 ± 1.37	3.11 ± 0.26	2.34 ± 0.15	4.00 ± 0.31	4.02 ± 0.54
18	**cis-Bicycloether**	0.79 ± 0.072	0.83 ± 0.10	1.03 ± 0.10	0.81 ± 0.082	1.63 ± 0.24
19	**E-Spiroether**	1.87 ± 0.06	0.86 ± 0.05	0.78 ± 0.036	-	0.42 ± 0.079
20	**Methyl hexadecanoate**	1.99 ± 0.11	0.43 ± 0.026	0.41 ± 0.05	-	-

Each essential oil component was analyzed individually. Values are presented as mean ± standard deviation (n = 3).

Among the identified sesquiterpene constituents, α-bisabolol oxide A consistently represented the highest proportion in both control and treated plants. Farnesene was the second most abundant compound in most treatments, followed by bisabolone oxide B. Salinity stress, applied alone or in combination with glutathione (GSH), did not result in qualitative changes in the essential oil composition; however, it significantly affected the quantitative distribution of individual components. Under severe salinity stress (4500 ppm without GSH), α-bisabolol oxide A reached its maximum value of 62.42%, along with increased levels of bisabolone oxide B (6.13%) and chamazulene (2.40%), while the farnesene content declined. Additionally, caryophyllene oxide and δ-cadinene were not detected under this treatment. In contrast, monoterpene content peaked at 0.86% in control plants treated with GSH. The combination of moderate salinity (1500 ppm) and GSH led to the highest accumulation of oxygenated sesquiterpenes (79.26%), which included peak values for α-bisabolol oxide A (65.83%), bisabolol oxide B (1.80%), bisabolone oxide B (7.49%), and chamazulene (2.74%). Conversely, the lowest diversity of volatile compounds was

observed under 1500 ppm salinity combined with 2 mM GSH, where several constituents, such as menthone, caryophyllene oxide, δ-cadinene, 2-pentylfuran, ageratochromene, 1,8-cineole, and methyl hexadecanoate, were not detected. These findings suggest that salinity stress and exogenous GSH primarily modulate the biosynthesis and accumulation of specific sesquiterpene fractions, rather than altering the overall qualitative composition of chamomile essential oil.

From the authors' perspective, two relatively underreported oxygenated components Z-spiroether and cis-bicycloether deserve attention due to their consistent presence and dynamic response to treatments. Z-spiroether increased notably to 4.02% under 1500 ppm plus GSH, while cis-bicycloether rose to 1.63% in the same treatment. Although present in low amounts, their changes reflect the plant's metabolic adaptation to combined oxidative and salt stress. The authors suggest that these minor constituents, often overlooked, may play a significant role in the aromatic complexity and possibly in the bioactivity of the essential oil with stress conditions.

4 Discussion

The present study demonstrates that salinity stress constrains the growth and productivity of *Matricaria chamomilla* in a concentration-dependent manner, revealing a physiological threshold beyond which adaptive mechanisms become insufficient. The use of diluted saline well water, rather than artificially prepared salt solutions, enhances the ecological relevance of the findings by simulating realistic field conditions. At 1500 ppm, plants exhibited relatively minor reductions compared to the control (340 ppm), indicating a capacity to tolerate mild osmotic stress. However, at ≥3000 ppm, a marked decline in growth and productivity was observed, reflecting a transition from osmotic stress to ion toxicity. This shift is primarily associated with excessive Na^+ accumulation and disruption of the K^+/Na^+ balance, leading to impaired enzymatic activity, membrane destabilization, and reduced photosynthetic efficiency. These findings highlight that salinity tolerance in chamomile is governed not only by osmotic adjustment but also by the plant's ability to maintain ionic and redox homeostasis [1,23,24,47,48].

The observed growth inhibition under high salinity conditions can be largely attributed to oxidative stress. Salinity disrupts the electron transport chain in chloroplasts, resulting in the overproduction of reactive oxygen species (ROS), which damage proteins, lipids, and cellular membranes. Consequently, plant tolerance depends on the efficiency of antioxidant defense systems that regulate redox balance. This shift suggests a strategic reallocation of carbon toward secondary metabolism as part of the plant's adaptive response. The enhanced accumulation of oxygenated sesquiterpenes is likely linked to the stimulation of the mevalonate (MVA) pathway, which produces farnesyl diphosphate as a key precursor [49,50]. Salinity stress also induced significant metabolic reprogramming. While vegetative growth parameters declined, the accumulation of protective metabolites such as proline and essential oil constituents increased [4,51]. The methylerythritol phosphate (MEP) pathway in plastids generates monoterpenes and related antioxidants. Additionally, carbohydrate metabolism is also affected by saline conditions, with soluble sugars temporarily increasing to act as osmotic regulators [52,53].

Consistent with previous studies on medicinal plants, salinity altered essential oil composition without necessarily inducing qualitative changes. Instead, it modulated the relative abundance of specific compounds, potentially through its effects on enzymatic activity and glandular secretion [53]. This explains the observed increase in certain bioactive constituents despite reductions in overall biomass and oil yield. In this regard, Khalid and Ahmed [54] showed an enhancement of the essential oil constituents in Nigella sativa under saline irrigation. Razmjoo et al. [55] found similar responses in chamomile, and Cordovilla et al. [56] found that salinity levels above 4 $ds{\cdot}m^{-1}$ increased essential oil percentages due to reduced leaf surface area and

altered oil gland secretion. Despite the reduced oil yield, a high content of active compounds has been found in Mentha spp. under salt stress [57]. Moreover, changes in plant dry matter during saline irrigation are correlated with variations in the essential oil yield [58].

Exogenous application of glutathione (GSH) played a crucial role in alleviating salinity-induced stress. As a key component of the AsA–GSH cycle, GSH contributes to ROS 1detoxification, maintains cellular redox balance, and stabilizes membrane structures [58,59]. Additionally, it promotes the accumulation of Osmo-protectants like proline and soluble sugars, which help with osmotic adjustment and sustain metabolic activity under saline conditions. Our research indicates that a foliar application of GSH at 2 mM 2 mM significantly enhances growth performance, flower yield, and essential oil production under salinity stress. These improvements are likely associated with its role in regulating antioxidant enzyme systems and reducing oxidative damage, thereby preserving cellular functions under stress [7,20,60–62]. Furthermore, glutathione can enhance oil quality and improve nitrogen assimilation in saline environments. Thus, applying GSH foliarly can be an effective strategy for enhancing chamomile tolerance to salinity by improving metabolic stability, oil quality, and nutrient assimilation.

Hydrogen peroxide (H_2O_2) exhibits a concentration-dependent dual behavior. At a concentration of 5 mM, H_2O_2 functions as a signaling molecule, which primes antioxidant defenses, activates MAPK cascades, and enhances potassium uptake, nitrogen assimilation, and photosynthetic efficiency. However, at higher concentrations, it can act as a damaging oxidant, overwhelming detoxification systems and accelerating lipid peroxidation. This hermetic response emphasizes the importance of carefully regulating reactive oxygen species (ROS) signaling to optimize stress tolerance [56,57].

Exogenous application of glutathione (GSH) and optimal hydrogen peroxide (H_2O_2) enhances essential oil composition by redirecting carbon flux toward protective secondary metabolites, even under compromised plant growth [63]. This highlights the importance of managing oxidative stress and redox signaling to maintain plant quality under saline conditions. The treatments promote the accumulation of oxygenated sesquiterpenes, linking redox regulation with secondary metabolism [64]. Additionally, chamomile's tolerance to salinity appears to depend more on maintaining cellular redox homeostasis than on osmotic adjustment, emphasizing oxidative signaling as a key mechanism in stress resilience [52,56]. Although enzymatic antioxidant activities were not directly assessed, the observed physiological responses strongly suggest the activation of redox-regulating pathways. The results indicate that chamomile's salinity tolerance is primarily linked to its ability to maintain redox homeostasis rather than to osmotic adjustment.

5 Conclusions

The foliar spraying of glutathione, particularly at 2 mM, represents an effective physiological strategy for enhancing the tolerance of *Matricaria chamomilla* L. plants to salinity stress up to 3000 ppm well water. It significantly reduced sodium accumulation, promoted potassium uptake, and improved the K^+/Na^+ ratio. Moreover, it enhanced the accumulation of proline and carbohydrates. Accordingly, it is recommended to foliar apply glutathione at a concentration of 2 mM to enhance the productivity, resilience, and growth of chamomile plants grown in environments affected by salt, particularly in marginal soils or areas irrigated with saline water. For these reasons, it is recommended to irrigate chamomile plants with saline water up to 3000 ppm and apply foliar treatments with glutathione (GSH) at 2 mM. This approach is expected to enhance growth characteristics and increase productivity.

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Abbreviations

The following abbreviations are used in this manuscript:

GSH	Glutathione
H_2O_2	Hydrogen peroxide
GLC	Gas liquid chromatography
EC	Electrical conductivity

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