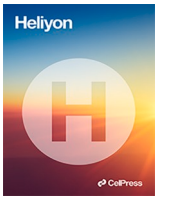
Heliyon 9 (2023) e18262

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon



Glutathione to ameliorate growth criterions and chemical constituents of geranium irrigated with salt water

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ARTICLE INFO

Keywords:
Saline irrigation water
Glutathione
Growth criterions
Essential oil
Chemical contents

ABSTRACT

Essential oil of geranium (*Pelargonium graveolens* **L**) has biological activities that make it used in food and pharmaceutical manufactures. High salinity is one of the factors that lead to lack of expansion in the production of medicinal and aromatic plants, especially in the new reclaimed soil located at arid and semi arid regions. Glutathione is a natural antioxidant that can help plants to withstand unfavorable environmental conditions such as the salinity of irrigation water. This trial aimed to diminish the undesirable effect of exposure to irrigation with salt water on geranium herbs through subjected them to exogenous application of glutathione. Geranium plants were irrigated with various concentrations of salt water with sodium chloride (0.0, 34.2, 51.3, and 68.4 mM) without (0 mg/L) or with glutathione (375 mg/L). Plants exposed to various rates of saline irrigation water with glutathione resulted in higher values of growth criterions (fresh and dry aerial parts), photosynthetic pigments, carbohydrates, protein, proline, essential oil (% or yield), antioxidant enzymes (peroxidase and superoxide dismutase), nitrogen, phosphorous, potassium, calcium, iron, zinc, manganese and copper than those subjected to saline irrigation water without glutathione. Higher amounts were found in sodium and chloride of plant treated with saline irrigation water than those treated saline irrigation water with glutathione. It may be summarized that productivity of geranium plants can be improved with adapting them under saline irrigation conditions by adding glutathione. This trial benefits the producers of geranium to alleviate the hurtful effects of salinity in reclaimed regions with adding glutathione.

1. Introduction

Cultivation and production of medicinal and aromatic plants has been economically advantageous for food, cosmetic, perfumes, pharmaceutical and drug manufactures [1]. They contain various secondary products with several biological activities; experiments have proven that these natural ingredients (secondary products) do not have any undesirable side effects on human health compared to those manufactured chemically [2]. Therefore, it is necessary to expand the production of medicinal and aromatic plants by increasing the cultivated areas of them to be used for various purposes [2]. Geranium plants (*Pelargonium graveolens* **L**) are perennial evergreen herbal crops; they are among plants of family Geraniaceae. They are native to Mediterranean regions and used in cosmetics and perfume manufactures [3]. Geranium essential oils are used in aromatherapy because it contains geraniol, citronellol and linalool as major constituents [4]. Previous investigations indicated that essential oils from geranium have various characters such as skin care,

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https://doi.org/10.1016/j.heliyon.2023.e18262
Received 13 October 2022; Received in revised form 11 July 2023; Accepted 12 July 2023
Available online 13 July 2023

decline pain, treating dysentery, hemorrhoids, inflammation, heavy menstrual flows, antioxidant, anticancer, diabetes, diarrhea, gallbladder troubles, gastric ulcers, jaundice, liver, sterility and urinary stones [5,6].

High rate of salt stress in soil or irrigation water is one of the basic restrictions of several aromatic crops reproduction all over the world [7]. Salinity stress raises sodium, reduce availability of water to plants and decrease nutrient uptakes, leading to nutrient imbalance, cell membrane harm, and lessening in chlorophylls which retard morphological characters [8]. In aromatic plants, the biosynthesis of osmolytes such as carbohydrates and proline is modulated under salinity stress conditions; while, the secondary product especially essential oil significantly modified for improving antioxidant defense [8]. Osmotic and oxidative stresses, ion toxicity, ionic disturbance and configure the reactive oxygen species (ROS) are among the most important processes that occur in plants when exposed to salt stress [9]. Plant tissues generate different antioxidant substances which assist in beating the ROS [10]. Antioxidant substances have proven to have an effective role in protecting cells from various oxidative stresses, as well as playing conclusive function in preserving redox-homeostasis, and offering a potential targets for ameliorative stress tolerance in plant cells [11]. Consequently, salinity stress affects plant growth and development as well as several metabolic processes and thus the chemical contents of plant cells such as active ingredients, photosynthesis products, protein, amino acids, enzymes, carbohydrates, sugars, lipids, antioxidant substances, phenols, flavonoids, hormones and nutrients [12,13]. Aromatic plants are varied in response to salt stress; in general it can be divided into two categories [14–16]. The first of them are halophytic plants, which tolerate high rates of salinity stress, the second one is plants sensitive to salinity stress (glycophytes), and the most plants are located under this category [17]. However, there may be genetic differences within the same group in the degree of salinity tolerance and also within the same species [17]. Knowing how and mechanics of how halophytic plants tolerate salt stress helps in finding ways to increase the tolerance sensitive plants to salt stress [17].

Different amino acids accumulate in plant cell as antioxidants substances when plants are exposed to salt stress conditions, where they play an important physiological roles in plant adaptation or improving plant growth and productivity; they act as osmolyte, arranging of ion relocate, modulating stomatal opening, detoxification of heavy metals, synthesis and activity of some enzymes, gene expression and redox homeostasis [18]. It was noted that proline is one of the most important amino acids that accumulate in plant tissues when plants are exposed to stress [19]. There is a positive relationship between proline accumulations and stress factors [19]. It helps plants to recover from stress rapidly because it has superior osmolytes and antioxidative defense [19]. Glutathione is a string of three amino acids (a polypeptide): glutamic, cysteine and glycine; it acts as a coenzyme and antioxidant materials to conserve plant tissues from free radical damage under salt stress agents [20].

Egyptian government tends to expand aromatic plants in reclaimed desert areas, the most important of which is geranium due to the importance of its essential oil in pharmaceutical industries. Unfortunately, these places are located at arid zones and characterized by high salt levels, whether in cultivation soil or irrigation water. Thus, this investigation is hold to minimize the hazard effectiveness of salinity stress on geranium plants by adapting them to salinity stress with exogenous application of glutathione to geranium plants.

2. Materials and methods

2.1. Description of experiment

During 2 sequential seasons, this study was conducted in the greenhouse of agricultural experiments of National Research Center. Young seedlings of geranium were brought from Medicinal and Aromatic Plants Research Institute (MAPRI), Ministry of Agriculture, Egypt. In plastic pots with 30 cm diameter and 50 cm height, seedlings were implanted on the first week of March of the 2 seasons; each pot was filled with ten kg of dry soil (Table 1). Atmospheric conditions surrounding the plants during their growth period were adjusted as follows: temperature (38/27 °C, maximum/minimum), relative humidity (93/64%, day/night), and light intensity ~ 3700

Table 1
Soil characteristics.

Textile	Sand
Sand (%)	93.1
Silt (%)	3.8
Clay (%)	3.1
pH	8.4
EC (dS/m)	3.3
Organic matter	0.2%
$CaCO_3$	5.9%
Total N	1.1%
P (mg)	1.2
Cations (mg/100 g Soil)	
K	0.7
Ca	5.8
Mg	4.2
Na	26.2
Anions (mg/100 g Soil)	
HCO_3	18.6
Cl	11.7
SO_4	6.6

μmol m^{-2} s^{-1}. Four weeks after transferring the pots to experimental greenhouse, weak plants were removed and the three healthiest plants in each pot were retained. All pots were split into two sections; the first section was irrigated by saline water with sodium chloride at different levels 0.0 (spout water) 34.2, 51.3, and 68.4 mM of sodium chloride. Different salinity levels of irrigation water were prepared by using highly soluble sodium chloride salt. Adding suitable amount of salt to water to produce various levels of salinity; then using EC meter instrument to adjust (to check the salt concentration). The second section was irrigated with salted water, as in the first section, with the addition of glutathione at a level of 375 mg per litter. It is worth noting that all pots were irrigated with spout water every week to avoid the accumulation of salt in them and thus change the concentrations of salts under study. Glutathione solution was prepared by dissolving pure glutathione (0.375 g) in few amounts of alcohol; then supplemented with distilled water to 1000 ml. Plants were sprayed with glutathione twice, the first one after 60 days from planting seedlings, and the second one after two weeks from the first one [control plants (without glutathione) sprayed as well with few amounts of alcohol and supplemented with distilled water to 1000 ml]. All agricultural operations were carried out in accordance with the instructions of Ministry of Agriculture. Note: the concentrations of salinity and glutathione were selected according to the literature and some preliminary experiments.

2.2. Harvesting

Plant aerial parts were cut 5 cm above soil surface twice in growth season. The first one was after 90 days and the second one was after 150 days. Fresh and dry aerial parts (g/plant) were listed as mean of each season.

2.3. Essential oil extraction

Essential oil was extracted from the aerial parts of geranium plants by hydro distillation [21]. Hydro - distillation process was done as follows: Plant sample was mixed with a liter of water in a 2-L round bottomed flask, then the boiling point was set at 100 °C to extract the essential oil. Obtained essential oil was treated with anhydrous sodium sulphate to get rid of the traces of water present with it; then it was placed in the refrigerator (4 °C) until it is analyzed. Essential oil percentage was calculated as w/w as follows: [weight of essential oil (g)/sample dry weight (g)] × 100; while essential oil yield (g/plant) was calculated by reference to dry weight of the aerial parts.

2.4. Gas chromatography (GC) and gas chromatography/mass spectrometry (GC/MS) analysis

Essential oil was analyzed with Shimadzu GC-9 gas chromatograph equipment which contains dimethylsiloxane, 5% phenyl (DB5) fused silica column. The specifications of DB5 column were as follows: 60 m × 0.25 mm i. d., film thickness 0.25 μm. Oven temperature was stabilized for 5 min at 50 °C; then it was set to rise to 240 °C at a level of 3 °C/minute. Essential oil samples were diluted with n-pentane at the rate of 1/100 (v/v). The amount of sample injected was 1 μl. Temperatures of the flame ionization detector (FID) and injectors were 265 °C and 250 °C, respectively. Helium was used as a gas carrier with a rate of 32 cm/s. External standard process made with calibration curves that were obtained from the GC analyses of representative constituents to make the quantification as previously.

A Varian 3400 GC-MS system has been used to perform the GC/MS analysis, which contains the column of DB5 (60 m × 0.25 mm i. d., film thickness 0.25 μm). Fifty to 240 °C at a level of 4 °C/minute were the oven temperatures, 260 °C was the transmit line temperature. Helium was used as a gas carrier with a level of 31.5 cm/s 1:60 was the spit ratio. Ionization energy 70 eV, scan time 1s, and mass range 45–600 amu.

2.5. Identification of volatile components

Computer library (NIST/NBS and Wiley 275.l), mass spectra of constituents (or from published data) and authentic constituents (emphasized by their retention indexes or published data; [22] were used to identify the essential oil constituents. The individual essential oil components were determined by the retention times, standard materials and mass spectral data with NIST/NBS and Wiley 275.l libraries or using published data of literature [22]. An external standard method was used for quantification of various constituents using calibration curves produced from GC permission of representative constituents. For various components, retention indices were specified with squirt a uniform concatenation of n-alkanes (C_8–C_{22}) into chromatographic column; then, they compared with the rates presented in the previous published data to assert correspondence [22]. On the other hand, co-injection with obtainable authentic materials for affirmation of assignment made is desired. Computer was suitable versus mercantile (Wiley GC/MS and Mass Finder 3 libraries).

2.6. Photosynthetic pigments evaluation

Photosynthetic pigments (Chlorophyll *a*, Chlorophyll *b* and total carotenoids) were specified in geranium fresh leaves by following the method of AOAC [23]. Fresh leaves tissues were ground in a mortar and pestles using 80% acetone. The optical density of the solution was recorded at 662 and 645 nm (for chlorophyll *a* and *b*, respectively) and 470 nm (for carotenoids) using a spectrophotometer (Shimadzu UV - 1700, Tokyo, Japan). The values of photosynthetic pigments were expressed in mg/100 g fresh weight.

2.7. Determination of total carbohydrates

Colorimetric method of Dubois [24] was followed to specify the total carbohydrates in geranium dry leaves. The extract was prepared from the homogenization of foliar tissues with 1 N H_2SO_4 and quantified by absorbance readings at 490 nm, using a D-glucose solution as standard.

2.8. Crude protein evaluation

Crude protein values were specified in geranium leaves according to micro kjeldahl method [23]. Approximately 1 g of dried plant material was hydrolyzed with 15 mL concentrated sulfuric acid (H_2SO_4) containing two copper catalyst tablets in a heat block (Kjeltec system 2020 digestor, Tecator Inc., Herndon, VA, USA) at 420 °C for 2 h. After cooling, H_2O was added to the hydrolysis's before neutralization and titration. The amount of total nitrogen in the raw materials was multiplied with both the traditional conversion factor of 6.25.

2.9. Proline determination

Bates [25] method was used for the determination of proline in geranium fresh leaves. 2 ml of proline extract, 2 ml of acid ninhydrin and 2 ml of glacial acetic acid were added and incubated for 1 h in a boiling water bath followed by an ice bath. The absorbance was measured at 520 nm using Spekol Spectrocololourimeter VEB Carl Zeiss. A standard curve was obtained using a known concentration of authentic proline.

2.10. Evaluation of antioxidant enzymes

Assay of superoxide dismutase activity (SOD) EC 1.15.1.1: SOD activity was determined by measuring the inhibition of the auto-oxidation of pyrogallol using a method described by Mukherjee [26]. Ten mls of the reaction mixture comprised: 3.6 ml of distilled water, 0.1 ml of the enzyme extract, 5.5 ml of 50 mM phosphate buffer (pH 7.8) and 0.8 ml of 3 mM pyrogallol (dissolved in 10 mM HCl). The rate of pyrogallol reduction was measured at 325 nm using UV-spectrophotometer (Spectronic 601). The enzyme activity was calculated as unit g^{-1} FW.

Assay of peroxidase activity (POX) EC 1.11.1.7: POX activity was assayed following the method of Kar [27] with slight modifications. Five ml of the assay mixture contained 300 μM of phosphate buffer (pH 6.8), 50 μM pyrogallol, 50 μM H_2O_2 and 1 ml of crude enzyme extract were prepared. After incubation at 25 °C for 5 min, the reaction was stopped by the addition of 1 ml of 10% H_2SO_4. The optical density was recorded at 340 nm by using spectrophotometer (Spectronic 601, Milton Roy Company), and the activity was expressed as unit g^{-1} FW.

2.11. Determination of elements

For the digestion of plant samples, 0.5 g sample of powdered leaves of the plants under study were weighed into the Teflon PFA

Table 2
Response of geranium growth and essential oil content to irrigation with salted water and/or glutathione.

Saline irrigation water (mM)		Weight of aerial parts (g/pant)				Essential oil			
		Fresh		Dry		%		g/100 plant	
		Seasons							
		1st	2nd	1st	2nd	1st	2nd	1st	2nd
Without glutathione	0.0	258.4 ± 13.5	360.9 ± 12.4	69.3 ± 3.7	84.3 ± 3.9	0.1 ± 0.0	0.2 ± 0.0	13.8 ± 0.9	16.9 ± 1.1
	34.2	252.3 ± 10.6	326.6 ± 11.8	66.1 ± 3.3	73.7 ± 3.6	0.2 ± 0.0	0.3 ± 0.1	13.2 ± 0.9	22.1 ± 1.4
	51.3	233.9 ± 10.1	179.8 ± 9.6	51.2 ± 3.1	58.1 ± 2.9	0.2 ± 0.0	0.3 ± 0.1	10.2 ± 0.9	17.4 ± 1.2
	68.4	140.9 ± 7.9	124.6 ± 9.6	39.3 ± 2.2	43.9 ± 2.5	0.3 ± 0.1	0.3 ± 0.1	11.8 ± 0.7	13.2 ± 0.7
Overall without glutathione		221.4 ± 9.3	248.0 ± 10.3	56.5 ± 2.9	65.0 ± 3.1	0.2 ± 0.0	0.3 ± 0.1	12.3 ± 0.8	17.4 ± 0.9
With glutathione	0.0	281.2 ± 11.2	424.0 ± 13.6	75.1 ± 3.5	96.6 ± 4.1	0.2 ± 0.0	0.2 ± 0.0	15.0 ± 1.1	19.3 ± 1.4
	34.2	264.3 ± 11.4	354.8 ± 19.3	69.8 ± 3.3	82.8 ± 2.6	0.3 ± 0.1	0.4 ± 0.1	20.9 ± 0.2	33.1 ± 2.3
	51.3	250.6 ± 9.8	195.1 ± 7.4	55.8 ± 3.1	66.5 ± 2.5	0.3 ± 0.1	0.4 ± 0.1	16.7 ± 2.9	26.2 ± 2.1
	68.4	162.8 ± 9.9	141.7 ± 5.9	47.9 ± 2.7	55.5 ± 2.2	0.4 ± 0.1	0.4 ± 0.1	19.2 ± 3.1	22.2 ± 1.7
Overall with glutathione		239.7 ± 10.2	278.9 ± 7.8	62.2 ± 2.9	75.4 ± 3.8	0.3 ± 0.1	0.4 ± 0.1	18.0 ± 2.9	25.2 ± 2.1
Overall saline irrigation water	0.0	269.8 ± 8.7	392.5 ± 11.5	72.2 ± 2.6	90.5 ± 2.7	0.2 ± 0.0	0.2 ± 0.0	14.4 ± 2.1	18.1 ± 0.9
	34.2	258.3 ± 7.9	340.7 ± 11.1	68.0 ± 2.8	78.3 ± 3.1	0.3 ± 0.1	0.4 ± 0.1	17.1 ± 2.2	27.6 ± 1.1
	51.3	242.3 ± 7.8	187.5 ± 7.7	53.5 ± 2.4	62.3 ± 2.3	0.3 ± 0.1	0.4 ± 0.1	13.5 ± 0.8	21.8 ± 1.1
	68.4	151.9 ± 8.1	133.2 ± 7.5	43.6 ± 2.1	49.7 ± 1.9	0.4 ± 0.2	0.4 ± 0.1	15.5 ± 2.2	17.7 ± 0.9
F values									
Saline irrigation water		172824.10***	464213.2***	11336.3***	18255.1***	2.4^{ns}	3.4 ^{ns}	88.7***	3277.7***
Glutathione		20203.4***	29331.9***	22455.4***	6071.4***	3.4 ^{ns}	3.4 ^{ns}	1203.6***	9360.1***
Salinity X glutathione		377.3***	3782.4***	8707.0***	45.7***	0.4 ^{ns}	0.4 ^{ns}	83.4***	539.6***

vessels and digested for 3 h at 85 °C with conc. HNO_3: HCl (3:1) mixture. Then conc. $HClO_4$ (1.0 ml) was added to enhance the oxidation process in the digestion. The resulting solutions were filtered and diluted to 50 ml with distilled water. The blank solution was taken as the same procedure without addition of the sample [28]. Nitrogen (N) was determined using micro Kjeldahl method as described by AOAC [23], phosphorous (P) was determined by spectrophotometer method as described by Snell [29], potassium (K), calcium (Ca), iron (Fe), zinc (Zn), manganese (Mn), copper (Cu), and sodium (Na) were estimated using flame photometer method described by Chapman [30], chloride (Cl) was estimated according the method described by Adriano [31].

2.12. Experimental design and statistical analysis

In this study, a completely randomized experimental design was followed with four replicates for each treatment. One replicate included eight pots, one pot contained three individual plants. This experiment included two main factors; the first one was four rates of saline irrigation water, while the second one was 2 levels of glutathione; thus, the number of experimental units was 288 pots. Averages data of both seasons were statistically analyzed using 2-way analysis of variance (ANOVA) allowed Snedecor [32]. Data of the effect of glutathione, salinity and their interactions were analyzed (as mean or overall). Significant values determined according to P values ($P < 0.05$ = significant (*), $P < 0.01$ = moderate significant (**) and $P < 0.001$ = highly significant (***). Data were given as mean ± SD (standard divination) and that allowed to the STAT-ITCF program [33].

3. Results

3.1. Response of growth criterions to irrigation with salted water and glutathione

Salted water and/or glutathione influenced various growth criterions (fresh and dry aerial parts) in both the first and second seasons (Table 2). The increase in salt levels of irrigation water led to a deterioration in growth criterions especially at 68.4 mM, but

Table 3
Response of geranium essential oil constituents t to irrigation with salted water x glutathione.

No.	Constituents (%)	RI	Saline irrigation water (mM)								
			Without glutathione				With glutathione				F values
			0.0	34.2	51.3	68.4	0.0	34.2	51.3	68.4	
1	α-Pinene	939	0.8 ± 0.2	1.1 ± 0.3	1.6 ± 0.4	0.7 ± 0.2	0.7 ± 0.1	0.8 ± 0.2	1.1 ± 0.3	1.2 ± 0.3	5.9**
2	Myrcene	991	1.1 ± 0.2	0.9 ± 0.2	0.6 ± 0.2	1.6 ± 0.4	2.5 ± 0.4	1.9 ± 0.4	1.7 ± 0.4	0.7 ± 0.2	20.8***
3	Linalool	1098	10.1 ± 1.8	8.9 ± 1.8	11.6 ± 2.2	7.1 ± 1.2	11.7 ± 2.9	12.4 ± 2.8	11.5 ± 2.9	14.4 ± 2.1	142.5***
4	*cis*-Rose oxide	1111	0.6 ± 0.2	1.7 ± 0.7	2.1 ± 0.4	0.5 ± 0.1	2.1 ± 0.7	1.9 ± 0.4	2.5 ± 0.5	1.8 ± 0.4	11.6***
5	*trans*-Rose oxide	1115	1.5 ± 0.3	1.7 ± 0.5	0.7 ± 0.2	1.1 ± 0.3	1.1 ± 0.4	1.9 ± 0.3	1.1 ± 0.3	0.5 ± 0.1	5.6**
6	*Citronellal*	1153	13.4 ± 2.2	13.1 ± 2.2	14.2 ± 2.2	12.9 ± 1.9	7.9 ± 2.2	7.2 ± 1.9	7.1 ± 2.1	7.8 ± 3.1	28.6***
7	Menthone	1154	1.8 ± 0.4	1.8 ± 0.3	0.7 ± 0.2	1.9 ± 0.3	2.7 ± 0.7	0.8 ± 0.1	0.5 ± 0.1	1.7 ± 0.4	15.8***
8	Citronellol	1228	43.3 ± 3.8	43.5 ± 3.8	44.9 ± 4.2	44.1 ± 4.8	42.9 ± 3.8	49.1 ± 4.8	47.7 ± 3.9	49.9 ± 4.7	198.2***
9	Citral	1240	0.7 ± 0.2	1.3 ± 0.3	1.8 ± 0.4	0.9 ± 0.2	1.2 ± 0.3	1.5 ± 0.4	1.3 ± 0.3	1.5 ± 0.3	3.7*
10	Geraniol	1255	11.6 ± 2.2	12.5 ± 2.8	7.4 ± 2.2	17.8 ± 2.6	13.4 ± 2.3	7.3 ± 1.5	8.6 ± 2.1	10.9 ± 3.1	207.1***
11	Geranyl formate	1300	1.2 ± 0.3	0.9 ± 0.2	1.7 ± 0.3	2.6 ± 0.5	1.1 ± 0.3	0.9 ± 0.2	1.1 ± 0.3	0.9 ± 0.3	25.1***
12	Citronellyl acetate	1354	2.1 ± 0.3	1.8 ± 0.4	1.8 ± 0.4	0.5 ± 0.2	1.3 ± 0.3	1.3 ± 0.3	0.9 ± 0.2	1.1 ± 0.3	18.9***
13	β-Caryophyllene	1428	0.6 ± 0.2	0.8 ± 0.2	0.9 ± 0.3	0.5 ± 0.2	0.6 ± 0.2	0.5 ± 0.1	1.7 ± 0.4	0.6 ± 0.2	18.6***
14	Geranyl propionate	1475	0.5 ± 0.1	2.8 ± 0.6	0.6 ± 0.2	0.5 ± 0.2	0.5 ± 0.1	0.6 ± 0.2	1.4 ± 0.4	0.6 ± 0.2	77.9***
15	Germacrene D	1480	1.1 ± 0.3	0.9 ± 0.2	1.1 ± 0.3	1.2 ± 0.3	1.2 ± 0.3	1.2 ± 0.2	0.5 ± 0.1	1.2 ± 0.3	9.0***
16	γ-Cadinene	1513	1.1 ± 0.3	1.1 ± 0.3	1.1 ± 0.4	1.2 ± 0.3	0.5 ± 0.1	1.8 ± 0.4	1.1 ± 0.3	0.5 ± 0.1	35.7***
17	Citronellyl butyrate	1529	2.9 ± 0.5	1.1 ± 0.2	1.1 ± 0.3	0.6 ± 0.2	1.7 ± 0.4	1.9 ± 0.3	1.9 ± 0.4	1.3 ± 0.3	61.2***
18	.Elemol	1547	2.5 ± 0.4	1.3 ± 0.3	2.5 ± 0.5	0.6 ± 0.2	2.3 ± 0.4	2.1 ± 0.4	2.2 ± 0.4	0.7 ± 0.2	3.8*
19	Phenylethyl tiglate	1584	0.5 ± 0.1	1.1 ± 0.3	1.1 ± 0.3	0.6 ± 0.2	1.7 ± 0.1	1.8 ± 0.3	1.9 ± 0.3	1.3 ± 0.2	12.9***
20	γ-Eudesmol	1630	1.3 ± 0.3	0.6 ± 0.1	0.9 ± 0.2	2.1 ± 0.3	1.4 ± 0.3	0.5 ± 0.1	2.5 ± 0.4	0.9 ± 0.3	28.9***
21	α-Bisabolol	1683	1.1 ± 0.3	0.8 ± 0.2	0.9 ± 0.2	0.5 ± 0.1	2.4 ± 0.4	1.8 ± 0.4	2.1 ± 0.3	0.7 ± 0.2	10.3***
Monoterpene hydrocarbons (1,2)			1.9 ± 0.4	2.0 ± 0.3	2.2 ± 0.4	2.3 ± 0.4	3.2 ± 0.5	2.7 ± 0.5	2.8 ± 0.6	1.9 ± 0.3	16.5***
Oxygenated monoterpenes (3–10)			83.0 ± 3.7	84.5 ± 4.2	83.4 ± 4.7	86.3 ± 4.9	83.0 ± 4.2	82.1 ± 5.1	80.3 ± 4.6	88.5 ± 4.1	24.5***
Sesquiterpene hydrocarbons (13, 15, 16)			2.8 ± 0.4	2.8 ± 0.3	3.1 ± 0.4	2.9 ± 0.4	2.3 ± 0.3	3.5 ± 0.5	3.3 ± 0.5	2.3 ± 0.3	7.2**
Oxygenated sesquiterpenes (18, 20, 21)			4.9 ± 0.9	2.7 ± 0.4	4.3 ± 0.8	3.2 ± 0.7	6.1 ± 0.8	4.4 ± 0.4	6.8 ± 0.5	2.3 ± 0.4	52.7***
Fatty alcohol esters (11, 12, 14, 17)			6.7 ± 0.9	6.6 ± 0.7	5.6 ± 0.7	4.5 ± 0.8	3.4 ± 0.9	5.1 ± 0.8	4.7 ± 0.6	3.1 ± 0.2	25.0***
Phenols (19)			0.5 ± 0.1	1.1 ± 0.4	1.1 ± 0.3	0.6 ± 0.2	1.7 ± 0.4	1.8 ± 0.5	1.9 ± 0.3	1.3 ± 0.3	3.7*
Total identified			99.8	99.7	99.7	99.8	99.7	99.6	99.8	99.4	

there was an improvement when plants were treated with glutathione. Compared with salinity treatments, glutathione x salinity applications resulted in increases by a percentage of 8.8, 17.5%; 4.8, 8.6%; 7.1, 7.7%; 15.5, 13.7% of fresh weights during both seasons, respectively; also, dry weights increased by a percentage of 8.4, 14.6%; 5.6, 12.4%; 9, 14.5%; 21.9, 26.2%. The maximum values of fresh (281.2 and 424 g/plant) and dry (75.1 and 96.6 g/plant) weights were recorded under control x glutathione treatment during the first and second seasons, respectively. The effects on both fresh and dry weights of herbs were highly significant ($P < 0.001$) in response to saline irrigation water, glutathione and their interactions.

3.2. Response of essential oil contents (% or yield) to irrigation with salted water and glutathione

Table 2 indicates that essential oil (%) was raised under various saline irrigation water rates with or without glutathione through the two seasons. The greatest amount of essential oil (%) was obtained at the highest level of salted water (68.4 mM) x glutathione. The increases in essential oil (%) were non significant due to the rates of saline irrigation water, glutathione and their interactions. Different changes were observed in essential oil yield (g/100 plants) in response to saline irrigation water levels; but it is clear that there has been an improvement in the essential oil yield by adding glutathione. The interaction between salinity treatments and glutathione produced different increments by a percentage of 7.8, 14.2%; 58.3, 49.8%; 63.7, 50.6%; 62.7, 68.2% compared with salinity doses during the first and second seasons, respectively. The highest value of essential oil percentage (0.4%) was recorded with the plants exposed to 68.4 mM salinity; while the greatest amounts of essential oil yield (20 and 33.1 g/100 plants) were obtained from the plants subjected to 34.2 mM NaCl X glutathione of both seasons. The variations in essential oil yield were much considerable ($P < 0.001$) for saline irrigation water, glutathione and their interactions.

3.3. Response of essential oil constituents to irrigation with salted water and glutathione

Twenty one constituents were detected in geranium aerial parts essential oil under saline irrigation water levels, glutathione and their interactions (Tables 3 and 4). Citronellol, *citronellal*, geraniol and linalool were the major compounds. Various identified components belonged to six chemical fractions; oxygenated monoterpenes formed as a major one, while monoterpene hydrocarbons, sesquiterpene hydrocarbons, oxygenated sesquiterpenes, fatty alcohol esters and phenols created as minor fractions. Several variations were observed in the major constituents and all chemical sections due to saline irrigation water, glutathione and their interactions. The greatest value of fatty alcohol esters (6.7%) was recorded with control treatment without glutathione, while the highest amount of *citronellal* (14.2%) was obtained from plants treated with 51.3 mM saline irrigation water without glutathione (Table 3). The maximum value of geraniol was obtained from plants exposed to 68.4 mM saline irrigation water without glutathione. Untreated plants (control) with glutathione resulted in the greatest value of monoterpene hydrocarbons (3.2%). Plants subjected to 34.2 mM saline irrigation

Table 4
Response of geranium essential oil constituents to irrigation with salted water or glutathione separately.

No.	Constituents (%)	RI	Saline irrigation water (mM)				Glutathione		F values	
			0.0	34.2	51.3	68.4	Without	With	Salinity	Glutathione
1	α-Pinene	939	0.8 ± 0.2	0.9 ± 0.2	1.3 ± 0.3	0.9 ± 0.2	1.1 ± 0.3	0.9 ± 0.2	8.0**	1.26^{ns}
2	Myrcene	991	1.8 ± 0.4	1.4 ± 0.3	1.2 ± 0.2	1.2 ± 0.3	1.1 ± 0.3	1.7 ± 0.4	7.2**	32.2***
3	Linalool	1098	10.9 ± 2.6	10.7 ± 21	11.5 ± 2.3	10.5 ± 2.5	9.4 ± 1.7	12.5 ± 2.6	9.2***	533.9***
4	*cis*-Rose oxide	1111	1.4 ± 0.3	1.8 ± 0.4	2.3 ± 0.4	1.7 ± 0.3	1.2 ± 0.3	2.1 ± 0.8	29.2***	80.7***
5	*trans*-Rose oxide	1115	1.3 ± 0.3	1.8 ± 0.3	0.9 ± 0.2	0.8 ± 0.2	1.3 ± 0.3	1.2 ± 0.3	20.7***	1.0^{ns}
6	*Citronellal*	1153	10.7 ± 1.9	10.2 ± 2.1	10.7 ± 2.5	10.2 ± 2.6	13.4 ± 2.9	7.6 ± 1.7	10.3***	5302.5***
7	Menthone	1154	2.2 ± 0.4	1.3 ± 0.3	0.6 ± 0.2	1.8 ± 0.3	1.6 ± 0.3	1.4 ± 0.3	53.3***	1.9^{ns}
8	Citronellol	1228	43.1 ± 4.6	46.3 ± 5.1	46.3 ± 6.1	47.0 ± 5.1	43.9 ± 5.8	47.4 ± 6.8	285.1***	1107.6***
9	Citral	1240	0.9 ± 0.2	1.4 ± 0.3	1.6 ± 0.3	1.2 ± 0.3	1.2 ± 0.3	1.4 ± 0.3	4.0*	2.4^{ns}
10	Geraniol	1255	12.5 ± 1.7	9.9 ± 1.1	8.0 ± 1.1	14.4 ± 2.1	12.3 ± 2.8	10.1 ± 1.7	338.4***	224.4***
11	Geranyl formate	1300	1.1 ± 0.3	0.9 ± 0.2	1.4 ± 0.3	1.7 ± 0.4	1.6 ± 0.3	1.1 ± 0.3	21.8***	59.6***
12	Citronellyl acetate	1354	1.7 ± 0.4	1.5 ± 0.3	1.3 ± 0.3	0.8 ± 0.2	1.5 ± 0.3	1.1 ± 0.4	24.8***	25.6***
13	β-Caryophyllene	1428	0.6 ± 0.2	0.7 ± 0.2	1.3 ± 0.2	0.6 ± 0.2	0.6 ± 0.2	0.9 ± 0.2	42.6***	7.7*
14	Geranyl propionate	1475	0.5 ± 0.1	1.7 ± 0.4	1.0 ± 0.3	0.5 ± 0.1	1.1 ± 0.3	0.7 ± 0.1	57.0***	19.5***
15	Germacrene D	1480	1.1 ± 0.3	1.1 ± 0.3	0.8 ± 0.2	1.2 ± 0.3	1.1 ± 0.4	1.1 ± 0.3	7.6**	0.6^{ns}
16	γ-Cadinene	1513	0.8 ± 0.2	1.6 ± 0.4	1.1 ± 0.3	0.9 ± 0.2	1.1 ± 0.3	0.9 ± 0.1	30.2***	7.7*
17	Citronellyl butyrate	1529	2.3 ± 0.4	1.5 ± 0.3	1.5 ± 0.4	0.9 ± 0.3	1.4 ± 0.4	1.7 ± 0.3	78.1***	19.1***
18	Elemol	1547	2.4 ± 0.4	1.7 ± 0.4	2.4 ± 0.7	0.7 ± 0.2	1.7 ± 0.3	1.8 ± 0.4	40.9***	0.6^{ns}
19	Phenylethyl tiglate	1584	1.1 ± 0.1	1.5 ± 0.3	1.5 ± 0.3	0.9 ± 0.2	0.8 ± 0.3	1.7 ± 0.3	32.3***	2.3^{ns}
20	γ-Eudesmol	1630	1.4 ± 0.4	0.6 ± 0.1	1.7 ± 0.4	1.5 ± 0.3	1.2 ± 0.3	1.3 ± 0.3	22.2***	0.9^{ns}
21	α-Bisabolol	1683	1.7 ± 0.5	1.3 ± 0.3	1.5 ± 0.3	0.6 ± 0.2	0.8 ± 0.2	1.8 ± 0.4	40.4***	141.6***
Monoterpene hydrocarbons (1,2)			2.5 ± 0.4	2.4 ± 0.3	2.5 ± 0.3	2.1 ± 0.4	2.1 ± 0.5	2.7 ± 0.4	5.4**	40.3***
Oxygenated monoterpenes (3–10)			83.0 ± 4.5	83.3 ± 5.1	81.8 ± 4.8	87.4 ± 5.3	84.3 ± 5.7	83.5 ± 6.8	98.9***	11.4**
Sesquiterpene hydrocarbons (13, 15, 16)			2.5 ± 0.4	3.2 ± 0.5	3.2 ± 0.7	2.7 ± 0.5	2.8 ± 0.4	2.9 ± 0.4	9.3***	0.2^{ns}
Oxygenated sesquiterpenes (18, 20, 21)			5.5 ± 1.1	3.6 ± 0.6	5.6 ± 0.9	2.8 ± 0.3	3.8 ± 0.6	4.9 ± 0.5	198.7***	126.5***
Fatty alcohol esters (11, 12, 14, 17)			5.1 ± 0.9	5.8 ± 0.9	5.2 ± 1.1	3.9 ± 0.4	5.9 ± 0.7	4.1 ± 0.5	65.9***	285.3***
Phenols (19)			1.1 ± 0.3	1.5 ± 0.3	1.5 ± 0.3	0.9 ± 0.2	0.8 ± 0.2	1.7 ± 0.4	21.2***	204.2***
Total identified			99.7	99.9	99.8	99.8	99.7	99.8		

water with glutathione produced the highest value of sesquiterpene hydrocarbons (3.5%). The maximum value of oxygenated sesquiterpenes (6.8%) was recorded with the plants treated with 51.3 mM saline irrigation water with glutathione. The ultimate rates of linalool (14.4%), citronellol (49.9%) and oxygenated monoterpenes (88.5%) were recorded with the plants treated with 68.4 mM saline irrigation water with glutathione (Table 3). Higher values were recorded in citronellol, linalool, monoterpene hydrocarbons, sesquiterpene hydrocarbons, oxygenated sesquiterpenes and phenols of essential oil extracted from plants exposed to saline irrigation water x glutathione than those subjected to saline irrigation water (Table 4). Lower amounts were recorded in *citronellal*, geraniol, oxygenated monoterpenes and fatty alcohol esters of essential oils isolated from geranium plants treated with saline irrigation water x glutathione than those treated with salted water without glutathione (Table 4).

3.4. Response of photosynthetic pigments to irrigation with salted water and glutathione

Plants irrigated salted water produced deterioration in the contents of photosynthetic pigments (chlorophyll *a,* chlorophyll *b* and carotenoids), but they were improved by spraying them with glutathione during both seasons (Table 5). Application of glutathione under saline irrigation water treatments gave increases in chlorophyll *a* by a percentage of 29.5, 13.9%; 31.1, 8.4%; 26.6, 7.8%; 14.1, 6.9% of both seasons; while chlorophyll *b* increased by a percentage of 46.3, 20.5%; 79.6, 53.7%; 36.6, 64.3%; 39.3, 58.3% in both seasons; then carotenoids increased by a percentage of 24.4, 20.4%; 34.8, 17.4%; 56.3, 60.3%; 54.8, 34.9% during both seasons. Plants untreated with salinity (control) x glutathione with produced the highest amounts of chlorophyll *a* (19.3, 19.7 mg/g), chlorophyll *b* (9.8, 9.4 mg/g) and carotenoids (10.7, 11.8 mg/g) of both seasons. All variations of different photosynthetic pigments were highly significant ($P < 0.001$) for saline irrigation water, glutathione and their interactions.

3.5. Response of total carbohydrates to irrigation with salted water and glutathione

Treating plants with saline water and/or glutathione led to an accumulation of carbohydrates in plant tissues where the increase was gradual by increasing the level of salt in irrigation water with or without glutathione (Table 5). Plants exposed to 68.4 mM of saline irrigation water x glutathione gave the greatest values of carbohydrates (27.5, 27.4 g/100 g) of both seasons. Various elevates in carbohydrates were much considerable ($P < 0.001$) for saline irrigation water and glutathione; while, they were moderate significant ($P < 0.001$) for saline irrigation water x glutathione.

3.6. Response of crude protein to irrigation with salted water and glutathione

Data in Table 6 reveal that different variations were detected in crude protein content in response to saline irrigation water and glutathione. Exposure of plants to irrigation with saline water led to a decrease in the level of crude protein in plant tissues, but it improved when adding glutathione. Plants exposed to control treatment x glutathione gave the maximum accumulations of crude protein with the values of 10.6 and 10% at first and second seasons, respectively. The differences of crude protein were much considerable ($P < 0.001$) for saline irrigation water or glutathione; while, they were non significant for saline irrigation water with glutathione.

Table 5
Response of geranium photosynthetic pigments and carbohydrates to irrigation with salted water and/or glutathione.

Saline irrigation water (mM)		Photosynthetic pigments						Carbohydrates (g/100 g)	
		Chlorophyll *a*		Chlorophyll *b*		Total carotenoids			
		.mg/g							
		Seasons							
		1st	2nd	1st	2nd	1st	2nd	1st	2nd
Without glutathione	0.0	14.9 ± 2.4	17.3 ± 3.1	6.7 ± 1.6	7.8 ± 1.9	8.6 ± 1.7	9.8 ± 2.1	19.8 ± 3.6	20.7 ± 3.5
	34.2	13.2 ± 2.2	15.5 ± 2.8	5.4 ± 1.3	5.4 ± 1.7	6.9 ± 1.4	8.6 ± 1.3	21.6 ± 3.5	22.8 ± 3.6
	51.3	10.5 ± 1.8	11.5 ± 1.7	4.1 ± 1.2	4.2 ± 1.1	4.8 ± 1.1	5.8 ± 1.1	23.7 ± 3.8	24.6 ± 3.8
	68.4	8.5 ± 1.3	10.2 ± 1.5	2.8 ± 0.6	2.4 ± 0.9	3.1 ± 0.6	4.3 ± 0.8	25.7 ± 3.9	26.7 ± 4.1
Overall without glutathione		11.8 ± 2.2	13.6 ± 2.7	4.8 ± 1.1	5.0 ± 1.4	5.9 ± 1.1	7.2 ± 1.7	22.7 ± 3.3	23.7 ± 3.2
With glutathione	0.0	19.3 ± 3.7	19.7 ± 3.9	9.8 ± 1.9	9.4 ± 2.2	10.7 ± 2.1	11.8 ± 2.4	20.7 ± 2.9	21.9 ± 2.4
	34.2	17.3 ± 2.8	16.8 ± 3.1	7.9 ± 1.4	8.3 ± 2.1	9.3 ± 1.7	10.1 ± 2.4	23.8 ± 3.9	24.8 ± 2.7
	51.3	13.3 ± 1.8	12.4 ± 1.9	5.6 ± 1.1	6.9 ± 1.3	7.5 ± 1.4	9.3 ± 1.9	26.4 ± 3.9	25.9 ± 2.8
	68.4	9.7 ± 1.4	10.9 ± 1.3	3.9 ± 0.9	3.8 ± 1.0	4.8 ± 1.1	5.8 ± 1.1	27.5 ± 4.1	27.4 ± 2.8
Overall with glutathione		14.9 ± 2.6	15.0 ± 2.6	6.8 ± 1.6	7.1 ± 1.3	8.1 ± 1.9	9.3 ± 1.9	24.6 ± 3.3	25.0 ± 2.1
Overall saline irrigation water	0.0	17.1 ± 3.2	18.5 ± 3.7	8.3 ± 1.9	8.6 ± 1.7	9.7 ± 2.1	10.8 ± 2.2	20.3 ± 3.3	21.3 ± 2.6
	34.2	15.3 ± 2.9	16.2 ± 2.2	6.7 ± 1.2	6.9 ± 1.4	8.1 ± 1.8	9.4 ± 1.6	22.7 ± 3.7	23.8 ± 2.8
	51.3	11.9 ± 2.2	12.0 ± 1.9	4.9 ± 1.1	5.6 ± 0.9	6.2 ± 1.4	7.6 ± 1.7	25.1 ± 3.8	25.3 ± 2.4
	68.4	9.1 ± 1.2	10.6 ± 1.8	3.4 ± 0.8	3.1 ± 0.8	4.0 ± 0.9	5.1 ± 0.8	26.6 ± 2.6	27.3 ± 3.3
F values									
Saline irrigation water		665.2***	699.3***	589.3***	367.8***	423.2***	578.1***	425.3***	472.1***
Glutathione		515.1***	10.5***	545.1***	316.9***	344.3***	425.0***	199.2***	135.2***
Salinity X glutathione		28.1***	0.9***	27.1***	9.9***	3.2***	21.1***	8.0**	5.7**

Table 6
Response of crude protein, proline and antioxidant enzymes to irrigation with salted water and/or glutathione.

Saline irrigation water (mM)		Crude protein (%)		Proline (μmoles/g)		Antioxidant enzymes			
						POX		SOD	
						Unit/g fresh weight. mint			
		Seasons							
		1st	2nd	1st	2nd	1st	2nd	1st	2nd
Without glutathione	0.0	9.4 ± 1.6	8.8 ± 1.5	5.6 ± 0.9	5.9 ± 1.4	2.3 ± 0.4	1.9 ± 0.5	0.1 ± 0.0	0.1 ± 0.0
	34.2	8.8 ± 1.2	7.5 ± 1.2	6.6 ± 0.7	7.2 ± 1.6	3.1 ± 0.4	2.9 ± 0.7	0.2 ± 0.1	0.2 ± 0.1
	51.3	8.1 ± 1.1	5.6 ± 0.9	11.1 ± 2.8	11.3 ± 2.6	3.5 ± 0.3	3.2 ± 0.7	0.2 ± 0.1	0.2 ± 0.1
	68.4	5.0 ± 0.9	3.8 ± 0.5	11.9 ± 2.6	12.2 ± 2.9	3.9 ± 0.4	3.8 ± 0.9	0.3 ± 0.1	0.3 ± 0.1
Overall without glutathione		7.8 ± 1.3	6.4 ± 1.2	8.8 ± 1.4	9.2 ± 2.6	3.2 ± 0.4	3.0 ± 0.5	0.2 ± 0.1	0.2 ± 0.1
With glutathione	0.0	10.6 ± 2.3	10.0 ± 1.7	6.7 ± 1.3	6.8 ± 1.4	2.8 ± 0.5	2.9 ± 0.6	0.2 ± 0.1	0.2 ± 0.1
	34.2	9.4 ± 1.6	8.1 ± 1.3	7.1 ± 2.4	8.1 ± 2.1	3.2 ± 0.6	3.4 ± 0.8	0.3 ± 0.1	0.3 ± 0.1
	51.3	8.8 ± 1.2	6.9 ± 1.1	12.1 ± 2.6	12.4 ± 2.9	3.9 ± 0.7	4.2 ± 0.9	0.4 ± 0.1	0.4 ± 0.1
	68.4	5.6 ± 0.8	4.4 ± 0.9	13.1 ± 2.8	13.4 ± 3.1	4.5 ± 0.7	4.9 ± 0.9	0.5 ± 0.2	0.5 ± 0.1
Overall with glutathione		8.6 ± 1.1	7.4 ± 1.1	9.8 ± 1.8	10.2 ± 2.6	3.6 ± 0.7	3.9 ± 0.8	0.4 ± 0.1	0.4 ± 0.1
Overall saline irrigation water	0.0	10.0 ± 1.9	9.4 ± 1.9	6.2 ± 1.9	6.4 ± 1.7	2.6 ± 0.3	2.4 ± 0.4	0.2 ± 0.0	0.2 ± 0.1
	34.2	9.1 ± 1.4	7.8 ± 1.6	6.8 ± 1.2	7.7 ± 1.5	3.2 ± 0.4	3.2 ± 0.6	0.3 ± 0.1	0.3 ± 0.1
	51.3	8.5 ± 1.2	6.4 ± 1.1	11.6 ± 2.7	11.7 ± 2.9	3.7 ± 0.5	3.7 ± 0.7	0.3 ± 0.1	0.3 ± 0.1
	68.4	5.3 ± 0.9	4.1 ± 0.9	12.5 ± 2.9	12.8 ± 3.2	4.2 ± 0.5	4.4 ± 0.9	0.4 ± 0.1	0.4 ± 0.1
F values									
Saline irrigation water		241.3***	193.4***	4574.9***	862.6***	220.4***	102.8***	7.4***	7.4***
Glutathione		34.7***	35.0***	393.8***	91.7***	69.8***	121.5***	15.4***	15.4***
Salinity X glutathione		1.2^{ns}	1.5^{ns}	10.5***	12.9***	5.1*	2.8*	0.6^{ns}	0.6^{ns}

3.7. Response of proline to irrigation with salted water and glutathione

Changes in proline gathering as a result of exposure to saline irrigation water with or without glutathione are shown in Table 6. The accumulation of proline in plant tissues was increased by increasing salinity of irrigation water. Plants subjected to saline irrigation water with glutathione gave higher values of proline than those exposed to saline irrigation water without glutathione. The highest accumulations of proline (13.1 and 13.4 μmol/g) were obtained from plants treated with 68.4 mM of saline irrigation water x glutathione. The increments of proline contents were much considerable ($P < 0.001$) for saline irrigation water, glutathione and their interactions.

3.8. Response of antioxidant enzymes to irrigation with salted water and glutathione

The activity of antioxidant enzymes (POX and SOD) in plant cells increased when plants were exposed to saline irrigation water and

Table 7
Response of macro elements to irrigation with salted water and/or glutathione.

Saline irrigation water (mM)		Nitrogen		Phosphorous		Potassium		Calcium	
		(g/kg)							
		seasons							
		1st	2nd	1st	2nd	1st	2nd	1st	2nd
Without glutathione	0.0	15.1 ± 1.9	14.6 ± 2.3	5.1 ± 0.7	7.2 ± 0.8	13.1 ± 2.6	14.2 ± 3.3	23.1 ± 3.4	22.2 ± 3.5
	34.2	14.2 ± 1.1	12.4 ± 2.1	4.2 ± 0.5	5.3 ± 0.5	12.1 ± 2.3	11.1 ± 2.4	21.2 ± 3.3	18.3 ± 3.4
	51.3	13.1 ± 1.6	9.3 ± 1.9	2.1 ± 0.3	3.1 ± 0.2	9.2 ± 1.7	7.2 ± 1.7	19.3 ± 3.1	17.2 ± 3.5
	68.4	8.3 ± 0.8	6.5 ± 0.8	1.1 ± 0.2	2.2 ± 0.3	5.1 ± 0.7	3.3 ± 0.5	13.1 ± 2.5	11.1 ± 1.9
Overall without glutathione		12.7 ± 0.9	10.7 ± 1.1	3.1 ± 0.4	4.5 ± 0.4	9.9 ± 1.5	9.0 ± 2.5	19.2 ± 2.4	17.2 ± 2.4
With glutathione	0.0	17.0 ± 2.3	16.2 ± 2.4	7.2 ± 1.1	9.8 ± 1.7	15.2 ± 2.3	17.2 ± 3.5	26.3 ± 3.4	25.2 ± 3.7
	34.2	15.3 ± 2.1	13.3 ± 2.1	6.3 ± 0.9	6.4 ± 1.2	14.3 ± 2.4	13.4 ± 2.4	22.1 ± 2.3	21.2 ± 3.6
	51.3	14.3 ± 2.2	11.4 ± 1.3	4.2 ± 0.7	4.2 ± 0.9	11.2 ± 1.9	9.3 ± 1.7	21.4 ± 1.9	19.4 ± 2.4
	68.4	9.7 ± 1.3	7.8 ± 0.8	2.1 ± 0.6	3.5 ± 0.9	9.1 ± 1.7	5.4 ± 0.6	14.2 ± 2.3	13.1 ± 1.8
Overall with glutathione		14.1 ± 2.2	12.2 ± 2.2	5.0 ± 0.7	6.0 ± 1.2	12.5 ± 2.3	11.3 ± 1.3	21.0 ± 3.4	19.7 ± 3.1
Overall saline irrigation water	0.0	16.1 ± 2.4	15.4 ± 2.2	6.2 ± 0.5	8.5 ± 1.5	14.2 ± 2.5	15.7 ± 2.5	24.7 ± 3.8	23.7 ± 2.7
	34.2	14.8 ± 2.6	12.9 ± 2.3	5.3 ± 0.8	5.9 ± 0.8	13.2 ± 2.4	12.3 ± 2.4	21.7 ± 3.3	19.8 ± 2.4
	51.3	13.7 ± 1.9	10.4 ± 1.5	3.2 ± 0.6	3.7 ± 0.5	10.2 ± 2.3	8.3 ± 2.1	20.4 ± 3.6	18.3 ± 2.4
	68.4	9.2 ± 1.1	7.2 ± 0.8	1.6 ± 0.3	2.9 ± 0.3	7.1 ± 1.9	4.4 ± 1.3	13.7 ± 1.8	12.1 ± 2.3
F values									
Saline irrigation water		36.2***	57.4***	27.9***	35.3***	34.3***	92.6***	24.6***	22.2***
Glutathione		5.3*	9.8*	21.0***	9.4***	21.4***	13.4***	3.5*	6.1*
Salinity X glutathione		0.2^{ns}	0.4^{ns}	0.4^{ns}	0.8^{ns}	0.9^{ns}	0.3^{ns}	0.3^{ns}	0.9^{ns}

Table 8
Response of micro elements to irrigation with salted water and/or glutathione.

Saline irrigation water (mM)		Sodium		Chloride		Iron		Zinc		Manganese		Copper	
		(g/kg)				.mg/kg							
		Season											
		1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd
Without glutathione	0.0	3.1 ± 0.6	4.3 ± 0.6	2.1 ± 0.5	3.2 ± 0.4	290.1 ± 6.8	285.4 ± 6.3	191.4 ± 3.1	185.7 ± 2.6	204.4 ± 3.4	211.6 ± 3.8	91.3 ± 0.6	76.5 ± 0.9
	34.2	4.2 ± 0.4	6.3 ± 0.7	3.2 ± 0.6	5.2 ± 0.5	280.2 ± 6.1	271.6 ± 5.4	172.5 ± 2.5	167.5 ± 2.4	177.3 ± 3.3	186.4 ± 2.6	66.4 ± 0.5	52.4 ± 0.6
	51.3	6.4 ± 0.5	7.1 ± 0.8	5.3 ± 0.9	7.1 ± 0.7	160.4 ± 3.8	140.4 ± 3.7	95.8 ± 2.5	88.3 ± 1.9	120.3 ± 2.5	111.6 ± 2.1	23.9 ± 0.4	19.7 ± 0.5
	68.4	8.2 ± 0.7	9.3 ± 0.9	7.3 ± 1.1	9.2 ± 1.2	140.6 ± 3.7	120.6 ± 2.4	77.3 ± 2.1	67.4 ± 0.8	76.2 ± 1.1	74.3 ± 0.7	11.7 ± 0.3	9.7 ± 0.1
Overall without glutathione		5.5 ± 0.4	6.8 ± 0.5	4.5 ± 0.7	6.2 ± 0.7	217.8 ± 4.4	204.5 ± 5.6	134.3 ± 3.6	127.2 ± 2.4	144.6 ± 2.1	146.0 ± 1.8	48.3 ± 0.7	39.6 ± 0.5
With glutathione	0.0	2.1 ± 0.4	3.2 ± 0.4	1.5 ± 0.4	1.2 ± 0.4	440.1 ± 7.8	397.3 ± 4.2	201.5 ± 3.1	199.5 ± 2.7	221.4 ± 2.6	245.3 ± 2.6	112.6 ± 0.9	98.2 ± 0.8
	34.2	3.2 ± 0.5	5.3 ± 0.6	2.2 ± 0.5	3.4 ± 0.5	325.6 ± 4.5	311.3 ± 3.6	188.5 ± 2.7	180.5 ± 2.2	185.5 ± 2.3	196.5 ± 1.4	77.6 ± 0.6	67.4 ± 0.5
	51.3	5.2 ± 0.4	6.1 ± 0.7	4.1 ± 0.7	5.2 ± 0.5	179.3 ± 3.7	151.7 ± 2.4	110.3 ± 2.4	90.3 ± 2.4	127.5 ± 1.4	118.4 ± 2.5	34.6 ± 0.3	26.5 ± 0.4
	68.4	6.1 ± 0.5	7.2 ± 0.9	5.1 ± 0.4	6.3 ± 0.6	150.1 ± 2.9	147.6 ± 2.1	84.3 ± 2.7	71.5 ± 0.8	81.6 ± 0.9	89.5 ± 0.8	23.7 ± 0.2	13.7 ± 0.3
Overall with glutathione		4.2 ± 0.4	5.5 ± 0.5	3.2 ± 0.5	4.0 ± 0.6	273.8 ± 4.9	252.0 ± 4.5	146.2 ± 2.6	135.5 ± 0.9	154.0 ± 2.5	162.4 ± 2.1	62.1 ± 0.2	51.5 ± 0.6
Overall saline irrigation water	0.0	2.6 ± 0.3	3.8 ± 0.4	1.8 ± 0.4	2.2 ± 0.4	365.1 ± 3.6	341.4 ± 5.4	196.5 ± 2.8	192.6 ± 2.1	212.9 ± 2.4	228.5 ± 2.6	102.0 ± 0.8	87.4 ± 0.5
	34.2	3.7 ± 0.3	5.8 ± 0.6	2.7 ± 0.4	4.3 ± 0.6	302.9 ± 3.3	291.5 ± 3.5	180.5 ± 2.7	174.0 ± 1.8	181.4 ± 1.6	191.5 ± 2.2	72.0 ± 0.4	59.9 ± 0.5
	51.3	5.8 ± 0.6	6.6 ± 0.9	4.7 ± 0.5	6.2 ± 0.7	169.9 ± 2.6	146.1 ± 2.8	103.1 ± 1.1	89.3 ± 0.9	123.9 ± 1.5	115 ± 1.1	29.3 ± 0.3	23.1 ± 0.4
	68.4	7.2 ± 0.6	8.3 ± 1.1	6.2 ± 0.7	7.8 ± 0.0	145.4 ± 2.3	134.1 ± 2.7	80.8 ± 0.9	69.5 ± 0.7	78.9 ± 0.8	81.9 ± 0.9	17.7 ± 0.3	11.7 ± 0.3
F values													
Saline irrigation water		29.5***	15.5***	19.5***	27.5***	833787.6***	484589.3***	4299.5***	124983.5***	145719.6***	165630.9***	79086.1***	51015.7***
Glutathione		13.5*	6.8*	7.5*	24.3*	234780.2***	101108.4***	212.9***	2270.8***	539.6***	9840.1***	9936.0***	5990.0***
Salinity X glutathione		1.5^{ns}	0.8^{ns}	0.3^{ns}	0.3^{ns}	78043.8***	22201.4***	8.4***	306.7***	39.5***	1310.8***	329.8***	686.9***

glutathione (Table 6). Higher amounts of antioxidant enzymes were recorded in plants irrigated with saline water with glutathione than those exposed to saline irrigation water without glutathione. Plants subjected to saline irrigation water with glutathione gave excesses of POX by a percentage of 21.7, 52.6%; 3.2, 17.2%; 11.4, 31.3%; 15.3, 28.9% of both seasons; and it produced increases in SOD by percentage of 100%; 50%; 100%; 66.7% in both seasons. The maximum accumulations of POX (4.5 and 4.9 unit/g fresh weight. min) and SOD (0.5 unit/g fresh weight. min) were obtained from plants treated with 68.4 mM of saline irrigation water x glutathione of both seasons. The increase in both antioxidant enzymes were highly significant ($P < 0.001$) for saline irrigation water or glutathione. The increases in POX were significant ($P < 0.05$) for the interactions between saline irrigation water x glutathione; while the increase in SOD were non significant for saline irrigation water x glutathione.

3.9. Response of elemental contents to irrigation with salted water and glutathione

Different variations were observed in the contents of macro and micro elements in response to saline irrigation water and/or glutathione (Tables 7 and 8). Irrigation with saline water led to a decrease in the content of macro elements (N, P, K and Ca) and some micro elements such as Fe, Zn, Mn and Cu; but it was increased by adding glutathione. The opposite trend was found in other micro elements such as Na and Cl. When compared with salinity treatments, Addition of glutathione x saline irrigation water treatments increased the absorption of N (7.3–20%), P (20.4–100%), K (16–78.4%), Ca (4.2–18%), Fe (8–51.7%), Zn (2.3–19.1%), Mn (4.6–20.5%) and Cu (16.9–102.6%); while Na and Cl decreased by 14.1–32.3% and 22.6–62.5%, respectively. The variations in N, Ca, Na and Cl were highly significant ($P < 0.001$) for saline irrigation water, significant ($P < 0.05$) for glutathione and non significant for the interactions. The differences in phosphorous and potassium were much considerable ($P < 0.001$) for saline irrigation water or glutathione; while, they were non significant for the interactions. The differences in Fe, Zn, Mn and Cu were highly significant ($P < 0.001$) for saline irrigation water, glutathione and their interactions.

4. Discussion

In this attempt, treated geranium plants with saline irrigation water and/or glutathione led to multiple changes in their morphological characters, essential oils and chemical contents. The occurrence of a decline in both fresh and dry weights of plant aerial parts when exposed to irrigation with salt water can be due to high osmotic pressure of soil solution which leads to a decrease in plant water content, absorption of important elements, volume of plant cells, photosynthetic enzymes, capacity of total photosynthesis, CO_2 exchange rate, total assimilatory area and consequently a lack of dry matter, which reduces the size and weight of plant [23,34–36]. Plant exposure to salt stress leads to accumulation of toxic ions in its cells, which leads to a deterioration in growth and plant weights [36]. Various changes that occur to geranium essentials oil and their components resulting from exposure of plants to saline irrigation water can be traced back to changes in the enzymatic activity of essential oil formations; essential oil is considered a means of plant resistance to unfavorable conditions such as salinity stress [37]. Rising in essential oil (%) in response to saline irrigation water can be attributed to an increase in glandular hair number as well as the increase in their densities [38]; while the changes in essential oil yield can be attributed to the variations of the plant dry matter when exposed saline irrigation water [39]. Glutathione as an amino acid can provide the plant with high energy that benefit in building plant tissues essentially under different stress conditions [40]. Glutathione is considered as one of effective substances in cellular redox homeostasis [41]. In this survey, essential oil of geranium could be modulated with glutathione addition. It is clear that foliar spray of glutathione drive to enhance the morphological characters, photosynthetic pigments and carbohydrates [42]. It impacts positively the internal hormones such as indole acetic acid so cell division and/or cell enlargement be activated then ameliorated morphological features [43]. For that, glutathione has fundamental roles in metabolism, production, components and fractions of essential oil [44,45]. *Glutathione* also share many physiological processes such as plant morphology, pH control, metabolic energy obstetrics or redox power, and tolerance of stress [46]. Glutathione structure contains nitrogen and sulfur, both of them is essential in different stages of plant growth. Nitrogen is a major component involved in formation of protein, chlorophyll and several enzymes which are responsible for essential oil production [47]. Nitrogen has a basic effect metabolism of essential oils out of intake of carbon and forming of acetyl-CoA by mevalonic acid [48]. Nitrogen increased the production of essential oils and their main components of aromatic plants [47]. Sulfur ion resulted in highly significant variations of essential oil yields and compositions [49]. Deterioration in photosynthetic pigments when plants irrigated with saline water is due to an overabundance of chloroplasts, which leads to decay of chlorophyll *a,* chlorophyll *b* and carotenoids [50]. The rising in carbohydrates in response to saline irrigation water may due to storage of carbohydrates for sustained metabolism, prolonged energy supply, and for better recovery under salinity factor [51]. Accumulation of proline in plant tissues during exposure to irrigation with saline water is due to the fact that proline is an amino acid; it is as a source of energy, carbon (C), and N for recovering plant tissues under salinity stress conditions [51,52]. Reduction in crude protein in response to saline irrigation water may be due to salinity stress cause oxidative damages by reactive oxygen species (ROS), which can attack protein [53]. High levels of antioxidant enzymes (POX and SOD) was observed under saline irrigation conditions; this is due to the release of harmful substances (ROS), which leads to an increase in enzymatic antioxidant activity to raise the efficiency of plant to resist the harmful effects of exposure to saline irrigation water [54]. POX and SOD are enzymes that can convert the O_2 to H_2O_2; so, they can be defense lines to protect plants against ROS [54]. Deficiency of essential elements (N, P, potassium, Ca, Fe, Zn, Mn and Cu) under saline irrigation water conditions is due to two reasons, first of which is the lack of availability of these elements, the second one is the lack of plant dry matter [55]. An increase in Na and chloride uptake and a decline in the absorption of other elements such as N, P, K, Ca, Fe, Zn, Mn and Cu as a result of a competition between Na, Cl ions and other ions under saline irrigation water condition [56]. Also, osmotic modification is done by the uptake of Na and Cl from soil solution and plant produces some dissolved substances which are not hurtful to leaves to preserve osmotic balance [57]. Under

salinity stress, to modulate osmotic balance, production of organic solutes as a source of energy and carbon is better than inorganic one [58]. The best way for plant to afford stress is increasing the organic solutes [59]. Responses of geranium growth, development and various chemical fractions to salinity stress are in accordance with obtained by some previous investigators. Significant decreases were recorded in growth characters, essential oil yield of damsesea and mint pants due to salinity stress treatments, while opposite trend was observed in essential oil yield and essential oil main constituents [60,61]. Growth criterions, essential oil (%), major compounds of essential oil, total carbohydrates and proline were significantly increased under saline irrigation water treatments, but nutrients and protein contents were reduced [62].Under salt stress conditions, different decreases were recorded in growth criterions, essential oil yield, photosynthetic pigments, protein, N, P, K and Ca of geranium, pot marigold, lemon balm, black cumin, Artemisia and sunflower crops [13,39,53,63–65]; on the other antioxidant enzymes activities, essential oil (%) and its main compounds, carbohydrates, proline, sodium and chloride were increased. Different studies were carried out previously and showed the effect of salinity on growth and chemical composition of medicinal and aromatic plants. Plants treated with different salinity levels gave different decreases in growth characters, essential oil yield, photosynthetic pigments, crude protein and elemental contents; on the other hand various increments were recorded in the percentage of essential oils and their major constituents, total carbohydrates, proline, Na, Cl and antioxidant enzymes activities [60–64]. Application of glutathione resulted in various improvements of geranium morphological and chemical characters. It has been proven from some previous studies that addition of glutathione to plants growing in a saline medium leads to an increase in the speed of their growth [66]. Glutathione has an antioxidant property, so it reduces oxidative stress, lipid peroxidation, conserves plasma membrane and sodium flow, maintains cellular redox balance and performs signaling functions; thus weakening the negative effects of saline irrigation water [67]. It was observed that glutathione stimulates the formation of methylglyoxal detoxifier, which helps the plant tolerate salt water irrigation [68]. Glutathione leads to a high activity of antioxidant enzymes such as SOD, catalase and POX, and thus the resistance of plant to irrigation with salt water [69]. Roles of glutathione as antioxidant constituents (enzymatic, non enzymatic or and compatible organic solutes or osmoprotectants) and functioned as a downstream component of signal transduction pathways of signal transduction pathways under salinity stress conditions were confirmed by some previous [67,70,71]. It was observed that glutathione improves the growth of plants exposed to salinity by improving their photosynthetic pigment content [70]. Glutathione reduces both stomata opening and respiration rate under saline conditions, which leads to good growth of roots and aerial parts [72]. Under salinity, plant performance can be enhanced by adding of glutathione due to regulation of the glutathione pool [73]. Glutathione decreases oxidative stress and prohibit peroxidation of lipids. Also, it can save plasma membrane by reducing passive Na^+ influx which improves plant tolerance. Glutathione aids preserve cellular redox balance and proceeds signaling functions in plants [67]. Antioxidant function of glutathione has been proven in many studies. Exogenous glutathione organizes antioxidants substances, antioxidant enzymes and osmoprotectants [67,70,71]. Higher levels of glutathione and the improved actions of antioxidant enzymes were related with the decrease of damage which caused by salt stress in pokkali cultivars. That is clear in reduced Na/K ratio, ROS status and oxidative DNA damage range [69]. Brassica seedlings can tolerate the stress of salinity when the seeds treated with exogenous glutathione. Furthermore, SOD and POX antioxidant enzymes activities were increased [70]. The main scavenger of $\hat{O}_2$ and the first defense factor with ROS is known as SOD [74]. In the extra cellular space, there is POX enzyme which is H_2O_2 scavenger. POX is implicated in different plant stages such as secondary cell wall formation [75], heal of wounds [76], seed germination [77], pollination [78], fruit ripening [79], senescence [80], auxin and anthocyanin catabolism [81]. Glutathione stimulates hormones production such as IAA which works to increase growth and size of cells, which is reflected on plant growth and chemical contents [40–45]. Glutathione is a source of high energy, which works to rebuild plant tissues, especially when the plant is exposed to salt stress [46]. Glutathione is exporter of N and S [82,83], which are paramount elements for plant development under salt stress conditions [13]. Exogenous application of glutathione produced significant improvements in chloroplast and therefore photosynthetic pigments growth characters of sunflower and chickpea plants under saline irrigation water treatments [65]. The improvements in protein content by different glutathione x saline irrigation water treatments may be due to the translocation of amino acids from shoots to other plant parts and hence increase protein synthesis [65]. The effect of glutathione on plant growth and its chemical composition are confirmed by very few previous investigations. Sunflower plants treated with glutathione alleviated saline irrigation water hazard effects through improving growth criterions, chlorophyll *a*, chlorophyll *b* carotenoids, osmolytes (proline and carbohydrates), antioxidant enzyme and minerals contents (N, P, K, Ca, Fe, Zn, Mn and Cu); as well as decreasing hydrogen peroxide, lipid peroxidation and sodium and chloride [65]. This investigation supports several farmers and pharmaceutical companies who will be able to produce geranium (as a source of natural products) in arid or semi arid regions of Egypt that are characterized by high salinity in irrigation water with adding glutathione to alleviate the hurtful effects of saline irrigation water.

5. Conclusions

This trial indicated that geranium plants can be improved with adapting them under saline irrigation conditions by adding glutathione; where glutathione helps in raising the geranium productivity and their essential oils that have high medicinal and biological properties. Glutathione adapted geranium plants to various rates of saline irrigation water by ameliorate the uptake of essential elements (N, P, K, Ca, Fe, Zn, Mn and Cu), photosynthetic pigments, protein, carbohydrates and antioxidants enzymes, with decreasing the absorption of harmful ions (Na and Cl).

Author contribution statement

Aisha M. A. Ahmed: Conceived and designed the experiments; Performed the experiments and Contributed reagents, materials, analysis tools or data. Khalid A. Khalid: Analyzed and interpreted the data and Wrote the paper.

Data availability statement

Data included in article/supp. material/referenced in article.

Additional information

No additional information is available for this paper.

Declaration of competing interest

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

Acknowledgements

Authors gratefully thank the National Research Centre for its efforts to provide available equipments, greenhouse materials, chemicals and plant analysis through internal project no. E120205 titled as: Elicitors to modify the growth, yield and secondary metabolism of aromatic plants as natural source of pharmaceutical and food industries.

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