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EFFECT OF SALINITY AND ASCORBIC ACID ON GERANIUM VOLATILE AND ANTIOXIDANT ACTIVITY

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Abstract

This study aimed to evaluate effect of salinity, ascorbic acid and their interaction on growth and chemical constituents of geranium plant. Increasing salinity levels up to 5000 ppm caused a gradual reduction in plant height, number of branches, plant fresh weight and carbohydrate contents. On the contrary, it caused gradually increased in proline content and 1000 ppm salinity application recorded increased in number of branches. Ascorbic acid application at 50 ppm was the most effective concentration compared with other treatments. It caused significantly increases on plant height, proline content and oil percentage. On the other hand, ascorbic application had no effective role on herb fresh weight. The interaction between salinity (1000 ppm and ascorbic 50 and/or 150 ppm showed additive effect on growth parameters. The total phenoilcs in essential oils were determined using folin–Ciocalteu method, while the total flavonoids were assayed by aluminum chloride method. While, phenolic contents varied from 0.473 to 1.048 mg GA/mL in the treatment of 5000 ppm salinity to the treatment of 5000 ppm interacted with 150 ppm ascorbic acid respectively, flavonoids content ranged from 0.043 to 0.107 mg QE/mL in the aforementioned treatments. The antioxidant activity was carried out using two spectrophotometric methods: 2,2'-diphenyl-picrlyhydrazyl (DPPH) and β-Carotene-linoleic assays. The chemical composition of essential oil were studied using GC and GC/MS with 30 identified volatile compounds. The major volatile compounds were citronellol, menthone, *trans*-Linalool oxide, linalool and α-thujene**.** The increase of salinity level exhibited decrease in the main volatile compounds.

***Keywords*:** Geranium, salinity, antioxidant, volatile.

Introduction

Geranium (*Pelargonium graveolones* L.) is one of the most important genus of the family geraniaceae, which known to be rich in tannins, several of pelargonium species are cultivated for production of volatile oil (geranium oil) which is of commercial value. Plant grown in Egypt formation and as ornamental plants, the oil equal in its economic value with rose oil producing highest yield with highest oil percentage usually depending on various environmental factors. Saline water stress is one of the main problems in arid and semi arid regain. Yang *et al*. (1990) reported that salinity could retard growth by damaging the growth of cells that they cannot perform their function and/or by limiting their supply of essential metabolites.

Sairam and Strivasta (2002) reported that salt stress caused inhibition of growth and development, reduction in photosynthesis, respiration and protein synthesis and disturbs nucleic acid metabolize. In this respect it could be reported that salinity affect adversely on growth as mentioned by several investigation, Hussein *et al*. (2006), Arafa *et al*. (2007) and Navarro *et al*. (2008).

Essential oil of *P. graveolens*is used as a fragrant component in perfumery, food and beverages industry, also as antidepressant and antiseptic remedy. The essential oil and extracts of *P. graveolens* showed antioxidant, antibacterial and antifungal activity which can be attributed to significant cytotoxic effect which this extracts provided and probably flavonoid derivatives have positive contribution to this biological activity (Fayed, 2009; Misra and Srivastava, 2010; Cavar and Maksimovic, 2012), as well as the main volatile constituents are (citronellol, geraniol and linalool).

Ascorbic acid one of the water soluble reductants which is very important antioxidant which protect plants by suppressing oxidative injury, by affecting many enzymes activities Conklin (2001).

This study aimed to investigate the role of antioxidant - ascorbic acid- to alleviate the harmful effect salinity on photochemical, volatile and antioxidant activity of geranium.

Materials and Methods

This work had been conducted at the green house of National Research Centre, at Dokki, Cairo. Plastic pots 30 cm in diameter were used for cultivation. Rooted terminal cutting of *P. graveolones* 6-8 cm height with 7 leaves were reported in December 2015-2016 in both seasons. All pots were supplied with soil fertilizers at usual level and proper dates.

The experiment design was factorial with 4 salinity levels 0, 1000, 3000 and 5000 ppm interacted with four levels of ascorbic acid 0, 50,100 and 150 ppm. Moisture soil was controlled by weighing pot and daily loss of water was supplemented. The pots were irrigated with various levels of salinity. Applications of ascorbic acid were carried out twice as foliar spray, the first was one month from transplanting and the second was 15 day from the first.

Tow cuts were taken, by about 4 and 8 month from transplanting. The growth parameters for the two cuts were recorded, plant height, number of branches, plant fresh weight. For photochemical evaluation determination of essential oil content of fresh herbs was carried out by the water distillation methods (Guenther, 1961) on herbage fresh weight.

Chemical analysis

The photosynthetic pigments of fresh leaves, chlorophyll a and b as well as carotenoids were determined using for such purpose the 4th leaf from the growing point of the plant using the spectrophotometric method recommended by Metzener *et al*. (1965) according to the following equations:

For mg chlorophyll a/g tissue = $(12.7A_{663})$-$2.69(A_{645})$ v/Wx1000.

For mg chlorophyll b/g tissue=$22.9A_{665}$-$4.68A_{645}$ v/Wx1000

Total chlorophyll = chlorophyll a + chlorophyll b

Carotenoids content = (1000 A_{470}-1.82 chlorophyll a-85.02 chlorophyll b)/198

Samples were collected and dried for 48 h at 70 °C to determine soluble sugars which were extracted following the method of Highkin and Frankel (1962).

Total carbohydrates percentages were determined according to Dubois *et al*. (1956). Total proline content in leaves, was determined using dry material according to Bates *et al*. (1973). Relative water content determine according to Weatherly (1962).

Extraction of volatile oils

The leaves of geranium were subjected to hydro distillation using a Clevenger-type apparatus for 3 hrs. After decanting and drying the oil over sodium sulfate anhydrous. The oils in selected treatments were stored in sealed vial in a refrigerator (6°C) before being analyzed by GC and GC/MS.

Determination of total phenolic content (TPC)

The TPC of the essential oils (Eos) in the selected treatments was determined according to the method described by Taga et al., (1984). Briefly, 100 µl of each EO was dissolved in 10 ml of methanol, and 2 ml of this solution was made up with 0.3% HCl to 5 ml. A 100 µl aliquot of the resulting solution was added to 2 ml of 2% Na_2CO_3 and after 2 min, 100 µl of Folin-Ciocalteau reagent (Merck, Darmstadt, Germany) (diluted with methanol 1:1) was added and mixed well. After 30 min incubation, the absorbance of mixtures was recorded spectrophotometrically at 750 nm using UV-Vis Shimadzu (UV-1601, PC) spectrophotometer. The total phenolic contents were calculated as gallic acid equivalent (GAE) from a calibration curve of gallic acid standard solutions.

Determination of total flavonoid content (TFC)

Total flavonoid content (TFC) was determined using the aluminium chloride ($AlCl_3$) method according to a reliable approach using quercetin as the standard (Ordonez *et al*., 2006). In this regard, the methanol extract (0.1 mL) was added to 0.3 mL of distilled water followed by addition of 0.03 mL of $NaNO_3$ (5% w/v). After 5 min. at 25 ºC, $AlCl_3$ (0.03 mL, 10%) was added. After further 5 min., the reaction mixture was treated with 0.2 mL of NaOH (1 mM). Finally, the reaction mixture was diluted to 1 mL with water and the absorbance was measured at 510 nm using UV-Vis Shimadzu (UV-1601, PC) spectrophotometer. The results were expressed as quercetin (QE)/g.

The DPPH assay for evaluation of antioxidant activity

The antioxidant capacity of the essential oils was evaluated by the method of Wang *et al*. (1998). The essential oils at different concentrations (200–300 ug/ml) were mixed with the same volume of 0.2 mM methanol solution of DPPH. The disappearance of $DPPH^o$ by essential oils after 30 min of incubation at room temperature was determined spectrophotometrically at 515 nm. Methanol was used to zero spectrophotometer. The absorbance of the $DPPH^0$ radical without antioxidant, i.e. the control was measured daily using UV-Vis Shimadzu (UV-1601, PC) spectrophotometer at 515 nm against a blank, i.e. without $DPPH^o$.

Scavenging activity (%) was calculated using the following formula:

$$\% \text{ Inhibition} = [(A_{control} - A_{treatment}/A_{control})] \text{ X } 100$$

where: $A_{control}$: is the absorbance of the control; $A_{treatment}$: is the absorbance of the treatments. Butylated hydroxyl anisol (BHA) and *tert*-Butylated hydroxyl qunione (TBHQ) were used as positive controls. All tests were run in triplicate and an average was used.

β-Carotene-linoleic assay

Te assay was carried out according to the method of Koleva *et al*. (2002), β-carotene (2 mg) was dissolved in 20 mL chloroform, then, linoleic acid (40 mg) and Tween 40 (400 mg) were added to 4 mL of this mixture. After, the chloroform was evaporated at 40°C under vacuum. The mixture was supplemented with 100 mL of oxygenated water and then shaked vigorously. Samples (10 µL) with different concentrations (200-300 ug mL^{-1}) of oils extracts in methanol/water (90:10, v/v) were added to an aliquot (150 µL) of the β-carotene/linoleic acid emulsion. The mixture was stored for 120 minutes at 50°C, and the absorbance was measured at 470 nm by UV-Vis Shimadzu (UV-1601 PC) spectrophotometer. Readings of all samples were performed immediately (t= 0 min) and after 120 minutes of incubation. The antioxidant activity (%) of oils methanolic extracts was evaluated in terms of β-carotene bleaching inhibition using the following formula:

$$\% \text{ Inhibition} = [(A_t - C_t)/(C_0 - C_t)] \times 100$$

Where, A_t and C_t are the absorbance values measured for the test sample and control, respectively, after incubation for 120 minutes, and C_0 is the absorbance values for the control measured at zero time during the incubation. All experiments were carried out in triplicate.

Gas chromatography–mass spectrometry analysis

Gas chromatography–mass spectrometry (GC–MS) analysis were performed with an Agilent 7890 GC coupled to a 5977 MS detector and equipped with a DB-5 column (a length of 60 m × internal diameter of 0.25 mm, and 0.25 mm film thickness). The column oven temperature was set at 60 ^oC for 8 min, and then increased to 250 ^oC at rate of 2^oC/min. The injector and detector temperatures were kept respectively at 230 and 240 ^oC. Carrier gas was helium, the flow through the column was1ml/min, and the split ratio was set to 1:50 with injection of 2 µl of oil sample. The GC/mass spectrometry (MS) analysis was performed with a Quadrupole mass spectrometer that operated at 70 eV. Constituent's identification was based on comparison of retention times with those of corresponding reference standards using the NIST and WILEY libraries. Percentage compositions of essential oils were calculated according to the area of the chromatographic peaks.

Compound identification

Identification of EO constituents was made by matching their recorded mass spectra with those stored in the Wiley/NBS mass spectral library of the GC-MS data system and other published mass spectra. Retention index was calculated for each compound using the retention times of a homologous series of C_6-C_{22} *n*-alkanes (Adams, 2007).

Statistical analysis

All experiments were carried out in triplicate and the results are expressed as mean± s.d. the means were compared using the Least Significant Difference (LSD) tests to compare the difference among mean values of treatments at the level of 0.05 and SAS software (version 9.1; statistical analysis system institute Inc., Cary, NC, USA) was used for analysis (SAS, 2006).The graphs were plotted using Excel (2003) software.

Results and Discussion

Effect of salinity and ascorbic acid on growth

At relatively low salinity level 1000 ppm, plant height, number of branches in both cutting showed slight increments compared with the control plants. On the other hand, Plant fresh weight in both cutting decreased in the first and second cut by about 5.85, 21.01, 32.72, and 4.25, 45.16, 62.16 % respectively less than the control plants.

Increasing salinity level up to 5000 ppm, significantly caused a gradual reduction in plant height, number of branches and plant fresh weight in both cuttings compared with unsalinyed plants Table (1).

Irrespective of salinity foliar spray of ascorbic acid significantly increased plant height, the highest increments were 19.35 and 45.90 in the first and second cut respectively by 50 ppm ascorbic acid. On the contrary, ascorbic acid had no effective role on herb fresh weight Table (2).

The interaction between different salinity levels and different concentrations of ascorbic acid show that 50 ppm ascorbic acid interacted with 1000 ppm salinity proved to be the most effective treatment to increase plant height especially in the first cut. Furthermore the highest numbers of branches and fresh weight/plant were recorded by 150 ppm ascorbic acid interacted with the same 1000 ppm salinity level. These results hold true in both cuttings. The lowest interaction recorded by increasing salinity levels up to 5000 ppm Table (3).

Concerning the interaction effect between salinity and ascorbic acid data in Table (3) show that salinity level 1000 ppm interacted with 50 ppm and/or 100 ppm and 150 ppm achieved the highest number of branches and fresh weight, that means ascorbic acid alleviated the harmful effect of salinity on plant growth, and may be due to the activities of antioxidant enzyme as well as the increase in proline content (Batoal *et al*., 2012).

Total carbohydrate

High salt stress 5000 ppm in the irrigation solution is bound to create reduction in carbohydrate content. Increasing salinity levels from 0 up to 5000 decreased carbohydrate gradually and reached the lowest values 0.1864 mg/g compared with 0.687 mg/g for plants irrigated with fresh water. The interaction between low 50 and/or 100 ppm and low salinity levels gave high values Table (4). Our results also showed that total carbohydrate content under salinity condition decreased gradually. While ascorbic acid treatment had appositive effect. This results hold true with finding of Merval *et al*. (2010). Thus, it means that the interaction between Ascorbic acid and salinity metegate the adverse effect of salinity these may be due increasing physiological availability of nutrient (Bassouny *et al*., 2008).

Photosynsetic pigments

The results in Table (4) show that, chlorophyll a, b and carotenoids were the highest by spraying 50 ppm ascorbic acid interacted with zero salinity levels. Higher values for chlorophyll b and carotenoids compared with the control recorded by interaction between lower level 1000 ppm and ascorbic acid concentrations.

Relative water contents responded positively to ascorbic acid treatments. Ascorbic acid at 100 ppm interacted with relatively low salinity level 1000 ppm, gave the highest value followed in descending order by 150 ppm ascorbic acid interacted with 5000 ppm salinity in both cuts Table (5).

The results showed also that, the interaction between ascorbic acid and salinity levels showed appositive effect on chlorophyll a, b and carotenoids and the most effective concentrations was 50 ppm ascorbic acid. The results in a good agreement with those obtained by (Alqurainy, 2007 and Zann *et al*., 2015).

Proline content

Irrespective of salinity treatments Table (5) shows that ascorbic acid in absents of salinity 50 ppm recorded the highest proline content. Also increasing salinity increased gradually proline content. The interaction show increase in proline content and the maximum value 132/mg/l recorded by the interaction 50 ppm ascorbic acid and 1000 ppm salinity level.

Ascorbic acid considered one of those antioxidant has also been to shown to play multiple role in plant growth, such as cell division, cell wall expansion and other developmental processes (Poured *et al*., 2007). Also ascorbic acid acts as antioxidant detoxifies H_2O_2 the dismutation of o^{-2} which is confirmed by Shalata and Neumann (2001).

Ascorbic acid interacted with salinity increased proline content, the increments were more apparent by 50 ppm ascorbic acid, the promative effect of ascorbic on proline was reported by other investigators Meneguzza *et al*. (1999).

Oil percentage

In the absent of salinity, 50 ppm ascorbic acid showed apromotive effect on oil%. Also high salinity level 5000 ppm created the higher 0.66% oil percentage. The interaction recorded high value of oil by 100 ppm ascorbic acid interacted with 3000 ppm salinity Table (5).

From the fomentation results, it could be concluded that increasing salinity levels up to 5000 ppm showed a gradual decrease in growth of geranium plants compared with the unsalinyed plants Jamil *et al*., (2007) recorded that under high salinity condition water uptake by plants were reduced due to soil osmotic potential. This results may be attributed to specific ion toxicity, disturbance in homeostasis of Na^+ and Cl^- ions, stomatelclosure, and the increase production of Rose in chloroplast (Meneguzzo *et al*., 1999; Stedute *et al*., 2000; Gunes *et al*., 2008). In this study data showed that

salinity treatments decreased fresh weight, number of leaves and plant height in both cuttings. On the other hand, approximately low level caused promotive and stimulated effect on growth.

Our results also showed that sprays 50 ppm ascorbic acid alleviated the effect of salinity on number of branches and plant growth. In this respect Buettner and Schafer (2004) reported that ascorbic acid has some roles in plant growth aspects such as cell division and cell enlargement, developed of cellular wall and other physiological processes.

It could be concluded the increase in proline could help the plant to maintain their growth under stress condition and may regard as indicator of salinity tolerance.

These results hold true with the finding of Zana *et al*. (2015). The increase in proline content in response to 50 ppm ascorbic and different salinity levels may be due to increase in proline synthesis and inhibition of proline utilization by both oxidative and protine synthesis. Similar results were obtained by Mervat *et al*. (2010).

Salinity and ascorbic acid effect on phytochemicals and antioxidant activity

The selected samples were subjected to evaluation of phenolic and flavonoid contents which determined by folin–Ciocalteu and aluminum chloride, respectively and the obtained results are given in Table (6).

The data showed that phenolic contents varied from 0.473 to 1.048 mg GA/mL in the treatment of 5000 ppm salinity to the treatment of 5000 ppm interacted with 150 ppm ascorbic acid respectively. The same trend was observed for total flavonoid contents which ranged from 0.43 to 1.07 mg QE/mL in the aforementioned treatments.

A significant decrease in both total phenolics and flavonoids had occurred with increase the salinity level as compared with control or interaction with ascorbic acid (Table 6). The present results was similar to obtained by Wu et al., (2010) who found increase in total phenolic contents after treatment with ascorbic acid.

The antioxidant activity of the essential oil extracted from the selected treatments under investigation was determined through the two spectrophotometry methods (DPPH^0 and β-carotene assays), the results are shown in Fig. 1. The data showed that the increase in salinity level showed a significant decrease in antioxidant activity which correlated with the phenolic content (Table 6). The interaction of ascorbic acid with 5000 ppm salinity showed significant increase in antioxidant activity as compared to the control and all salinity levels alone as well as BHA however, less than TBHQ (Fig. 1).

The chemical composition of essential oil from the selected samples was analyzed by GC-MS, and 30 compounds were identified and the obtained results are given in Table (7). The data showed that the selected oil samples contain a significant amount of citronellol (41.07%), menthone (8.34%), trans- linalool oxide (6.09%), geraniol (6.02%), linalool (5.23%) and α-thujene (4.25%) in control sample. Our results in good agreement with (Virendra et al., 2002; Verma et al., 2010; Ben-Hsouna and Hamdi, 2012).

As shown in (Table 7), the increase of salinity level exhibited decrease in the main volatile compounds. On the other hand, ascorbic acid at 150 ppm interaction at 5000 ppm level of salinity keep and increase the characteristic volatile compounds of geranium especially citronellol which increase from 39.16% to 43.07% after interaction with 5000 ppm of salinity. The previous studies revealed that the antioxidant activity of the essential oil could be attributed in part to the presence of compounds such as β -citronellol and geraniol and its ability to decompose free radicals by quenching reactive oxygen species and trapping radicals before reaching their cellular targets (Boukhris *et al*., 2012).

Conclusion

In conclusion salinity at high concentration of 3000 and 5000 ppm had adversely effect on plant growth, photosysthetic pigments, acid proline accumulated. Application of ascorbic acid 50 ppm mitigated the adverse effect of salinity through increasing proline content. The total phenolics, flavonoids and antioxidant activity showed a remarkable decrease with increase the salinity level as compared with control or interaction with ascorbic acid. The increase in salinity cause a significant decrease in the characteristic volatile compounds of geranium essential oil.

Conflict of Interest

The authors declared that present study was performed in absence of any conflict of interest.

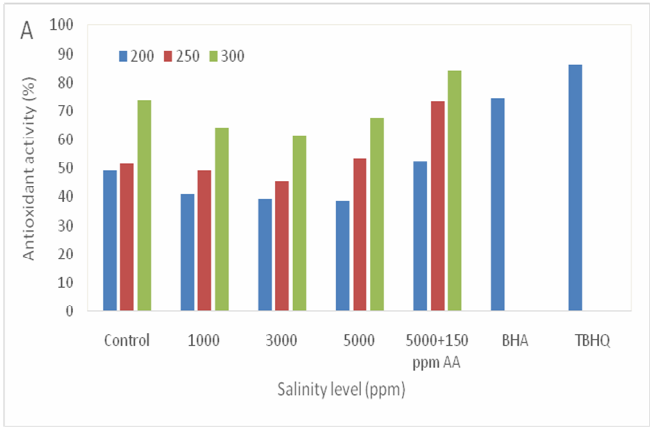

Fig. 1 : Effect of salinity and ascorbic acid (AA) on antioxidant activity of geranium essential oil determined by (A) DPPH^0 and (B) β-carotene assays

Table 1 : Effect of Salinity on plant growth of geranium plant (mean of two seasons)

Chrac.	Plant height (cm)		No. of branches		Plant fresh weight	
Treat.	**1 cut**	**2 cut**	**1 cut**	**2 cut**	**1 cut**	**2 cut**
Cont.(0)	33.33	15.0	4.41	8.08	72.58	76.41
1000	35.50	17.0	4.58	8.91	68.33	73.16
3000	29.91	7.58	4.08	5.75	57.33	41.90
5000	24.91	5.91	3.50	9.75	48.83	28.91
LSD at 0.5%	5.39	2.25	0.70	1.64	13.77	6.68

Table 2 : Effect of ascorbic acid on plant growth of geranium plant (mean of two seasons)

Chrac.	Plant height (cm)		No. of branches		Plant fresh weight	
Treat.	**1 cut**	**2 cut**	**1 cut**	**2 cut**	**1 cut**	**2 cut**
Cont.(0)	29.6	8.91	4.91	7.5	67.75	59.00
50	35.33	13.0	3.75	6.16	68.66	49.91
100	31.66	12.58	7.58	8.00	58.83	63.33
150	30.50	11.00	4.33	10.85	51.83	48.16
LSD at 0.5%	5.9	1.05	1.28	1.81	20.33	12.00

Table 3 : Effect of Salinity and Ascorbic acid on plant growth of geranium plant (mean of two seasons)

Salinity	Ascorbic acid ppm	Plant height (cm)		No. of branches		Plant fresh weight	
		1 cut	**2 cut**	**1 cut**	**2 cut**	**1 cut**	**2 cut**
Cont.(0)	**0**	31.66	34.66	5.33	9.33	82.33	100.33
	50	36.33	35.66	4.00	8.00	92.33	54.00
	100	34.33	40.00	4.00	8.33	86.66	72.33
	150	31.00	41.00	4.33	6.66	49.00	54.00
1000 ppm	**0**	30.66	41.00	5.66	8.66	70.00	72.66
	50	40.66	43.30	4.00	8.33	79.00	68.35
	100	38.00	36.00	3.66	7.66	59.35	80.66
	150	32.66	35.66	5.00	11.00	65.00	71.00
3000 ppm	**0**	31.00	28.16	4.66	6.00	66.37	37.00
	50	33.00	32.00	2.66	4.33	45.33	40.66
	100	30.33	28.66	4.00	7.00	64.00	39.00
	150	25.33	28.66	5.00	5.66	53.66	51.00
5000 ppm	**0**	23.33	22.oo	4.00	6.00	52.33	26.00
	50	31.33	28.00	4.33	6.00	58.00	31.66
	100	24.00	31.30	2.66	4.00	45.33	41.33
	150	**21.00**	**23.60**	**3.00**	**9.00**	36.66	**16.66**
LSD at 0.5%		2.52	1.47	1.92	2.09	6.12	6.56

Table 4 : Effect of Salinity and Ascorbic acid on Relative water content, proline and oil percentage of geranium plant (mean of two seasons)

Salinity	Ascorbic acid ppm	Mean of the two cuts		
		R.W.C. %	**Proline content (µ mg-1 F.W.)**	**Oil %**
Cont.(0)	**0**	31.94	12.57	0.19
	50	64.62	15.37	0.43
	100	64.42	11.37	0.34
	150	58.95	6.99	0.33
1000 ppm	**0**	66.04	7.79	0.22
	50	55.37	13.12	0.25
	100	89.84	9.71	0.29
	150	50.31	3.80	0.22
3000 ppm	**0**	52.25	8.12	0.33
	50	59.80	11.63	0.39
	100	74.50	10.19	0.49
	150	69.15	6.12	0.20
5000 ppm	**0**	60.40	8.67	0.42
	50	66.67	10.58	0.40
	100	60.53	9.00	0.23
	150	85.90	10.24	0.22

Table 5 : Effect of Salinity and Ascorbic acid on photosynthetic pigments and carotenoids of geranium plant (mean of two seasons)

Salinity	Ascorbic acid ppm	Mean of the two cuts			
		Carbohydrate(mg/g)	Chlorophyll (mg/g)		Carotenoids (mg/g)
			a	b	
Cont.(0)	0	0.2687	4.02	1.09	1.21
	50	0.2354	8.56	20.89	3.02
	100	0.2154	1.06	3.04	2.85
	150	0.2299	1.05	2.85	2.92
1000 ppm	0	0.2160	1.09	2.73	1.09
	50	0.2212	1.93	2.82	2.63
	100	0.2195	0.46	1.19	1.12
	150	0.2220	0.41	11.84	3.28
3000 ppm	0	0.2120	0.49	1.37	1.26
	50	0.1967	0.48	1.39	1.35
	100	0.1940	0.59	1.68	1.55
	150	0.2040	0.68	2.06	1.89
5000 ppm	0	0.1864	0.54	1.55	1.43
	50	0.1827	0.39	0.91	1.65
	100	0.1730	0.43	0.98	0.94
	150	0.1699	0.51	1.22	1.17

Table 6 : Effect of salinity and ascorbic acid (AA) on total phenolic and total flavonoids contents of geranium essential oil.

Salinity (ppm)	Phenolic content (mg GAE/mL)	Flavonoids content (mg QE)/mL)
Control	0.835±0.19*	0.096±0.12
1000	0.741±0.37	0.086±0.17
3000	0.619±0.28	0.072±0.18
5000	0.473±0.13	0.043±0.09
5000+150 ppm AA	1.048±0.25	0.107±0.26

*: Values are expressed as mean ± SD

Table 7 : Effect of salinity and ascorbic acid (AA) on oil chemical compositions of *P. graveolones*

Volatile compound	RI[a]	Salinity level (ppm)				
		Zero	1000	3000	5000	5000+150 ppm AA
(*Z*)-Hexenol	852	0.73[b]	0.52	0.48	1.27	0.95
α-Thujene	927	4.25	2.16	3.09	3.13	4.01
α-Pinene	939	0.19	1.24	0.15	0.06	0.09
Myrcene	995	0.43	0.07	1.28	2.03	0.48
p-Cymene	1026	0.65	1.92	0.43	1.18	0.57
Limonene	1029	0.09	0.18	1.25	0.07	0.14
γ-Terpinene	1059	0.18	1.28	0.04	0.02	0.02
cis-Linalool oxide	1071	4.14	3.15	2.92	3.83	4.06
trans-Linalool oxide	1086	6.09	5.74	5.86	4.95	5.18
Linalool	1099	5.23	4.32	4.18	4.51	2.93
cis-Rose oxide	1107	0.68	0.01	0.02	0.75	1.18
Menthone	1152	8.34	7.19	7.92	5.83	9.25
α-Terpineol	1186	0.16	1.35	1.26	0.78	0.19
Citronellol	1225	41.07	39.16	38.81	38.32	43.07
Neral	1239	1.15	0.42	0.39	0.54	0.63
Geraniol	1250	6.02	6.75	5.83	5.29	7.91
Nerylformate	1276	0.93	1.18	1.75	1.34	2.77
Geranylformate	1303	4.14	3.97	3.63	3.08	4.53
Citronellic acid	1312	0.17	1.34	1.19	0.94	0.09
Methyl geranate	1325	0.36	0.53	0.78	1.06	0.52
Citronellyl acetate	1351	3.49	3.46	2.29	3.82	2.35
α-Copaene	1375	0.82	0.58	2.07	1.03	0.78
β-Bourbonene	1382	1.58	1.25	1.34	1.18	2.04
(*Z*)-Caryophyllene	1405	0.62	1.08	2.22	2.93	0.08
(*E*)-Caryophyllene	1419	0.19	0.01	0.03	1.06	0.34
β-Copaene	1432	0.75	1.24	1.18	0.79	0.09
Citronellyl propionate	1442	0.03	1.62	2.47	4.36	1.18
γ-Cadinene	1513	0.29	1.06	0.02	0.23	0.26
Caryophyllene oxide	1587	3.12	2.17	3.95	1.77	2.94
Geranyl tiglate	1693	0.83	1.06	1.15	2.84	0.68

[a]: RI retention indices determined on DB-5 capillary column; [b]:Values are expressed as relative area percentage to the total identified volatile compounds.

References

Adams, R. (2007). Identification of essential oil components by gas chromatography/mass spectrometry, 4th Ed. Allured Publishing Corporation, Carol Stream, Illinois, USA.

Algurainy, F. (2007). Response of bean and pea to vitamin C under salinity stress. Research journal of agriculture and biological sciences, 6: 714-722.

Arafa, H.; Ather, H.R. and Ashraf, M. (2007). Does exogen application of salicylic acid through the rooting medium modulate growth and photosynthetic capacity in two differently adapted spring wheat cultivars under salt stress. J. Plant Phys., 164: 685-690.

Bassouny, F.M.; Hassamein, R.A.; Baraka, D.M. and Khalil, R.R. (2009). Physiological effect of nicotine-amid and ascorbic acid on Zea mays plant grown under salinity stress. Austr. J. Basic & Applied Sci., 2: 350-359.

Bates, L.; Waldrewn, R. and Dteare, I. (1973). Rapid determination free proline for water stress studies. Plant and Soil., 39: 205-207.

Batoal, Z. and Faheem, A. (2012). Effect of exogenous application of ascorbic acid on antiocidant enzyme activities, proline contents and growth parameters of Saccharum Spp. hybrid cv. HSF-240 under Salt Stress. Turkish J. Biol., 36: 630-640.

Ben-Hsouna, A. and Hamdi, N. (2012). Phytochemical composition and antimicrobial activities of the essential oils and organic extracts from pelargonium graveolens growing in Tunisia. Lipids Health Dis., 11: 167-174.

Boukhris, M.; Monique, S.J.; Simmonds, S. and Bouaziz, M. (2012). Chemical composition and biological activities of polar extracts and essential oil of rose-scented geranium, Pelargonium graveolens. Phytothe. Res., 27: 1206-1213.

Buettner, C.R. and Schafer, F.Q. (2004). Ascorbic acid an antioxidant in vitamin C. functions and biochemistry in animals and plants. Bios Scientific Publishers, Oxford. PP. 173-183.

Cavar, S. and Maksimovic, M. (2012). Antioxidant activity of essential oil and aqueous extract of Pelargonium graveolens. L'Her. Food Cont., 23: 263-267.

Conklin, P. (2001). Recent advances in the role and biosynthesis of ascorbic acid in plants. Plant, Cell & Envir., 24: 383-394.

Dubios, M.K.; Gilles, J.; Hamilton, P. and Smith, F. (1956). Colorimetric method for determination of sugars and related substances. Analy. Chem., 28: 350-356.

Fayed, S. (2009). Antioxidant and anticancer activities of Citrus reticulatae (*Petitgrain mandarin*) and Pelargonium graveolens (Geranium) essential oil. Research J. agric. Biol. Sci., 5: 740-747.

Guenther, E. (1969). The essentail oils. D-Van Wastrond Comp. Ine. New York, Vol. 1.

Gunes, A., Kadioglu, Y.K., Pilbeam, D.J., Inal, A., Coban, S. and Aksu, A. (2008). Influence of silicon on sunflower cultivars under drought stress, II: essential and non essential element uptake determined by polarized energy dispersive X-ray fluorescence. Comm Soil Sci Plant Anal. 39: 1904–1927.

Highkin, H.R. and Frankel, F. (1962). Studies on growth and metabolism of barley mutant lacking chlorophyll b. Plant Physiol. 31: 814-820.

Hussein, M.; Abd-El-Kader, A.; Aboe-El-Keir, M. and Kassab, O. (2006). Effect of diluted sea water and benzle adenine on macro and micro nutrient content of barley sheets. Egy. J. Agron., 28: 47-56.

Jamil, M.; Rehmas, L.; Kim, J. and Rha, E. (2007). Salinity reduced growth P511 photochemistry and chlorophyll content in radish. Sci. Agric., 64:111-118.

Koleva, I.; Teris, A.; Jozef, P.; Linssen, A. and Lyuba, N. (2002). Screening of Plant Extracts for Antioxidant Activity: A comparative Study on Three Testing Methods. Phytochem. Anal., 13: 8–17.

Meneguzza, S.; Navarri-Izzo, F. and Izzo, R. (1999). Antioxidative response of shoot and root of wheat to increasing NaCl concentrations. J. Plant Physi., 155: 274-280.

Mervet, S.; Magdi, T. and Abd-Elkarim, M. (2010). Physiological response of Faba been plants to ascorbic acid grown under salinity stress. Egy. J. Agron., 32: 89-106.

Metzener, H.; Ran, H. and Senger, H. (1965). Unler, Suchunger Zur. Synchronizer Barkiteinsieher-Pignentmangel. Mutanten Chlorella Plants., 65:180-185.

Misra, A. and Srivastava, N. (2010). Value addition of essential monoterpene oil (s) in Geranium (*Pelargonium graveolens*) on leaf positions for commercial exploitation. African J. Agric. Res., 5: 2077-2079.

Navarro, A.; Banon, S.; Conejero, W. and Sanchez-Blance, M. (2008). Ornamental characters ion accumulation and water status in Arbutus undo seedling irrigated with saline water and subsequent relief and transplanting and experimental. Bota., 62: 364-370.

Ordonez, A.; Gomez, J. and Vattuone, M. (2006). Antioxidant activities of Sechiumedule (Jacq) Swart extracts. Food Chem., 97: 452-458.

Poured, L.; Routaboul, J. and Cheynier, V. (2007). Flavonoid oxidation in plants from biochemical properties to physiological functions. Trends in Plant Sci., 12: 23-36.

Sairam, R. and Srivastava, C. (2002). Change in antioxidant activity in subcelluose fraction of tolerant and susceptible wheat genotypes in response to long term salt stress. Plant Sci., 162: 897-904.

SAS. (2006). SAS/STAT User's Guide Release 6.12 edition. Cary, NC, USA: SAS Inst. Inc.

Shalata, A. and Neumann, P. (2001). Exogenous ascorbic acid (Vitamin C) increases resistance to stress and reduce lipid peroxidation. J. Exper. Bota., 52: 2207-2211.

Steduto, P.; Albrizia, R.; Giorio, P. and Sorrentino, G. (2000). Gas exchange response and stomatal and non stomatal limitation to carbon assimilation of sunflower under salinity. Envir. Experimental Bota., 44: 243-255.

Taga, M.; Miller, E. and Pratt, D. (1984). Chia seeds as a source of natural lipid antioxidants. J. the Amer. Oil Chemists' Soc., 61: 928-931.

Verma, R.; Verma, K.; Yadav, A. and Chauhan, A. (2010). Changes in the essential oil composition of rose–scented geranium (*Pelargonium graveolens* L'Herit. ex Ait) due to date of transplanting under hill conditions of Uttarakhand, Indian. J. Natural Products and Res., 1: 367-370.

Virendra, S.; Rana, J.; Juyal, M. and Amparo, B. (2002). Chemical constituents of essential oil of pelargonium graveolens leaves. Inter. J. Aroma., 12: 216-218.

Wang, M.; Li, J.; Rangarajan, M.; Shao, Y.; La-Voie, E. and Huang, T. *et al*. (1998). Antioxidative phenolic compounds from sage (Salvia officinalis). J. of Agric. Food Chem., 46: 4869–4873.

Weatherly, P. (1962). Examination of moisture stress on carbohydrate development and growth in plants. American J. Bot., 41: 316-320.

Wu, N.; Zu, Y.; Fu, Y.; Kong, Y.; Zhao, J.; Li, X. *et al*. (2010). Antioxidant Activities and Xanthine oxidase inhibitory effects of extracts and main polyphenolic compounds obtained from geranium sibiricum L. J. Agric. Food Chem., 58: 4737–4743.

Yang, Y.; Newton, R. and Miler, F. (1990). Salinity tolerance in Sorghum. Corp Sci., 30: 781-785.

Zann, A.; Saran, S. and Saman, Y. (2015). Investigation of the effects of the pretreatment of seed with ascorbic acid on some physiological and biochemical characteristics of wheat seedlings under salinity stress condition. Biological Forum–An Inter, J., 7: 1389 - 1394.