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Response of Marjoram (*Majorana Hortensis* L.) Plant to Foliar Spraying by Some Antioxidants under Siwa Oasis Conditions

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Abstract: *This experiment was conducted in north western desert of Egypt in Siwa Oasis region to study the effect of foliar spraying by some antioxidants (ascorbic acid (AA) at 0.0,150 and 300ppm, salicylic acid (SA)at 0.0, 100 and 200ppm and their combination treatments) on growth and productivity of marjoram (Majorana hortensis L.) plants in two seasons of 2015/2016 and 2016/2017. The experiment was distributed in a randomized complete block design with three replicates. Results observed that, different applied treatments significantly increased the values of most recorded parameters compared to control. Meanwhile, the better plant height, higher yield of herb per feddan, essential oil yield per feddan total phenolic content as well as total antioxidant activity obtained by spraying AA at 300 ppm in combined with SA at 200 ppm. Increase in growth characteristics were associated with increase in antioxidant activity. Also, the major compounds of marjoram essential oil under study were sabinene, 4-Thajanol , Terpinen-4-ol , α-Terpineol , α-Terpinolene , C-Terpinene, D-Limonene and α-Terpinene. The essential oil possessed a strong antimicrobial activity. AA and SA application gave an increase in the antagonistic activity of marjoram oil against tested pathogenic microbes.*

Keywords: *Majorana hortensis, Chemical constituents, Antioxidants, Essential oil, Ascorbic acid, Salicylic acid*

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I. Introduction

High temperatures lead to the decrease of photosynthetic activity due to inhibiting chlorophyll biosynthesis and over-producing reactive oxygen species (ROS) of the cells**.** The elevated ROS poses great damage to nucleic acid, proteins, lipids and to the normal functioning of the cell **[1,2,3]** However, plants are able to cope with ROS by enzymatic and non enzymatic antioxidants. Salicylic acid (SA) and Ascorbic acid (AA) are the two of the most important non enzymatic antioxidants and exists in various cell organelles and in the apoplast **[4,5]**.

Salicylic acid (SA) is known as a signaling molecule that is involved in plant resistance and tolerance to biotic and abiotic stresses. It is a plant growth regulator known as an endogenous signaling molecule, which is involved in various physiological processes in plants, such as growth regulation, photosynthesis, stomatal conductance, nutrient uptake, plant water relations and mechanisms of plant resistance and tolerance to biotic and abiotic stresses **[6,7,8]**.

Ascorbic acid is a natural product in plants has an essential role in several physiological processes such as antioxidative defence, regulation of key enzymes and control cell division and expansion, growth, development and senescence **[9,10,11]**. Ascorbic acid is associated with chloroplasts to prevent the oxidative stress of photosynthesis. In addition, AA has a number of other roles in cell division and protein modification. One approach for inducing oxidative stress tolerance would to acts as a primary substrate in the cyclic pathway of enzymatic detoxification of hydrogen peroxide **[12]**.

Siwa Oasis features, a climate arid to semiarid with a negligible rainfall, the monthly mean maximum temperature range from 20 °C in January to 38 °C in July, with a yearly average of approximately 30 °C. The monthly mean minimum temperature ranges from 4 °C in January to 21 °C in July. Absolute maximum temperatures can reach 50 °C while the absolute minimum temperature measured was 4.5 °C. Mean monthly relative humidity ranges from 30 to 58% **[13,14].** Also, The Oasis is considered a new promising region in Egypt for cultivation and production of medicinal and aromatic plants with its environmental conditions favorable for the growth of these plants **[15,16,17]**.

One of the most important medicinal and aromatic plants is marjoram (*Majorana hortensis* L.). It is an important aromatic and medicinal plant. It has been cultivated in the Mediterranean countries and is still widely cultivated today. The active principles are found chiefly in the aerial parts (majorana herb). Also, it is considered as an important economic agricultural export crop. It grows well in Upper Egypt. Dried marjoram herb and the oil are used as spices in the food industry, as well as for their preservative and medicinal properties

[18]. It has been used not only to flavour food but also as a miraculous herb with the power to heal practically various diseases. Their essential oils have been known since antiquity to possess biological activity, notably antibacterial, antifungal as well as antioxidant properties **[19]**. The essential oil components of marjoram were terpinen-4-ol, gamma-terpinene, trans-sabinene hydrate, linalool, thujanol, terpinolene and thymol **[20,21]**.

This proposal aims to the enhance growth, yield and essential oil productivity of Marjoram (*Majorana hortensis* L.) by foliar application of ascorbic acid and salicylic acid under Siwa Oasis conditions.

II. Matrials And Methods

This work was implemented during the two successive seasons of 2015/2016 and 2016/2017 in semiarid region of the Agricultural Experimental Station of the Desert Research Center at Khamisa Village (29.21º N and 25.40º E), Siwa Oasis, Egypt. Seedlings of marjoram plants were obtained from Desert Research Center (D.R.C.) and sown on 4th of April during both seasons and spaced at 30cm between plants and 50cm between rows under drip irrigation system with the drippers of four liters/hr for half an hour every day. The experiment treatments were arranged in randomized complete block design with three replicates, which were three levels from Ascorbic acid (0, 150 and 300ppm) , Salicylic acid (0, 100 and 200 ppm) and their combinations . The nine treatments were applied as foliar spraying after 30 and 45 days from transplanting date and were carried out again after 15 and 30 days from first cut date. All agricultural practices of growing marjoram plants were done when ever needed. All treatments were fertilized with 200 kg/fed ammonium sulphate (20.5% N), 100 kg/fed calcium superphosphate (15.5% $P2O_5$) and 50 kg/fed potassium sulphate (48% K_2O). Calcium superphosphate was added before planting in each season in only one dose. Nitrogen and potassium fertilizers were applied in three equal doses in the season. The first one was added one month after transplanting, the second and third doses were added one week after every cut. Marjoram plants were harvested twice per season in September 23rd and December 24th in the two seasons respectively by cutting the vegetative parts of plants 10 cm above the soil surface.

The following data were recorded

1. Growth and yield characters

Plant height (cm), herb fresh yield g/plant, herb fresh yield kg/feddan, herb dry yield g/plant and herb dry yield kg/feddan .

2. Chemical analysis

2.1. Determination of essential oil percentage **:**Essential oil percentage was determined in the air dried herb by hydrodistillation for 3 hours using a Clevenger type apparatus **[22]**.

2.2. Determination of essential oil yield per plant (ml)

2.3. Determination of essential oil yield per feddan (L)

2.4.Determination of essential oil components: The essential oil samples of the second season were analyzed by using Gas Chromatography-Mass Spectrometry Gc Ms instrument stands at the Laboratory of Medicinal and Aromatic Plants, National Research Center, Egypt with the following specifications. Instrument: a TRACE GC Ultra Gas Chromatographs (THERMO Scientific Corp., USA), coupled with a THERMO mass spectrometer detector (ISQ Single Quadrupole Mass Spectrometer). The GC-MS system was equipped with a TR-5MS column (30 m x 0.32 mm i.d., 0.25 μm film thickness). Analyses were carried out using helium as carrier gas at a flow rate of 1.3 ml/min at a split ratio of 1:10 and the following temperature program: 80ºC for 1 min; rising at 4ºC/min to 300ºC and held for 1 min. The injector and detector were held at 220 and 200ºC, respectively. Diluted samples (1:10 hexane, v/v) of 1 μL of the mixtures were always injected. Mass spectra were obtained by electron ionization (EI) at 70 eV, using a spectral range of m/z 40-450. The separated components of the essential oil were identified by matching with the National Institute of Standards and Technology (NIST) published.

2.5. Determination of total antioxidant activity and total phenolic compounds in dry herb of plant.

2.5.1. Plant material extraction

For extraction of antioxidant and phenolic acids compounds, the leaves of Lavender plants were air dried at room temperature for 48 h. Air dried ground leaf tissue (0.5 g) was soaked in 50% methanol (50 mL). Then; the extract was transferred to 100 ml volumetric flask and made up the volume with 50% Methanol. The mixture was shaken in the orbital shaker for 20 min and centrifuged then filtered by muslin cloth. After that, the filtrate was used for the following experiments **[23].**

2.5.2. Measurement total antioxidant activity

The extract (0.1 ml) was mixed with 3 ml of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes were incubated at 95°C for 90 min. The mixture was

cooled to room temperature, and then the absorbance of the solution was measured at 695 nm against blank. The total antioxidant activity was expressed as ascorbic acid equivalents (AAE) in milligrams per gram of extract **[24].**

2.5.3. Measurement of total phenolic compounds

Total phenolic constituents of plant extracts were performed employing the literature methods involving Folin-Ciocalteu reagent and gallic acid as standard (Slinkard and Singleton, 1977). Extract solution (0.1 ml) containing 1000 ug extract was taken in a volumetric flask, 46 ml distilled water and 1 ml Folin-Ciocalteu reagent were added and flask was shaken thoroughly. After 3 min, 3 ml of solution 2% Na2CO3 was added and the mixture was allowed to stand for 2 h with intermittent shaking. Absorbance was measured at 760 nm. The same procedure was repeated to all standard gallic acid solutions (0-1000 mg, 0.1 ml-1) and standard curve was obtained. The obtained data were statistically analyzed according to **[25].**

3. Antimicrobial activity of marjoram essential oil: Antimicrobial activity of essential oils was detected against some pathogenic microorganisms namely: *Gram negative bacteria Escherchia coli, Entero bacter, Gram positive bacteria: Bacillus subtilis and staphylococcus aureus and Fungi : Verticillium dahlia and Fusarium solani.* The antimicrobial activity was determined by agar diffusion technique and measured according to **[26].**

The differences between means were assessed using the least significance difference (LSD) test at 5% by using computer program of Statistix version 9 **[27].** Soil and irrigation water analysis are shown in Tables (A,B and C) according to **[28,29].**

Table (A): The mechanical analysis of the experimental soil area.

Depth (cm)	Sand (%)	Silt (%)	Clay (%)	Soil texture
0-30	92.91	5.21	1.88	Sandy

Table (B): The chemical analysis of the experimental soil area.

pH	E.C.	O.M.	Soluble anions (meq/l)				Soluble cations (meq/l)			
	(ds/m)	(%)	CO_3^{--}	HCO_3^-	Cl^-	SO_4^{--}	Ca^{++}	Mg^{++}	Na^+	K^+
7.5	4.1	0.5	-	3.6	31.3	6.1	8.6	7.5	0.2	24.7

Table (C): The chemical analysis of irrigation water.

pH	E.C.	Soluble anions (meq/l)				Soluble cations (meq/l)			
	ppm	CO_3^{--}	HCO_3^-	Cl^-	SO_4^{--}	Ca^{++}	Mg^{++}	Na^+	K^+
4.23	2709.60	-	2.17	22.02	15.77	9.47	7.75	21.75	0.99

Table (D): Meteorological data at Siwa Oasis during the two seasons (2016-2017)

Parameter / Months	Temperature at 2 Meters (C)			Daylight Hours (hours)	Wind Speed Range at 10 Meters (m/s)
	Max.	Min.	Mean		
January	17.39	6.07	11.00	10.52	3.19
February	19.04	6.50	12.22	11.18	3.71
March	23.07	9.02	15.71	11.98	3.95
April	28.37	13.09	20.55	12.87	4.12
May	32.77	17.25	25.10	13.60	4.10
June	36.30	20.21	28.51	13.97	3.91
July	37.12	21.43	29.56	13.82	3.82
August	36.81	21.90	29.50	13.18	3.73
September	34.42	20.45	27.32	12.37	3.60
October	29.39	16.62	22.76	11.48	3.23
November	23.46	11.84	17.10	10.73	3.04
December	18.69	7.71	12.49	10.32	3.11

III. Results And Discussion

1. Growth and yield characters

The presented data in Fig. (1) and Table (1) show the effect of foliar spraying by ascorbic acid (AA) and salicylic acid (SA) on growth and yield characters demonstrated that ascorbic acid at 150 & 300 ppm and salicylic acid at 100 & 200 ppm applications significantly increased all tested parameters (plant height (cm). Herb fresh yield per plant (g) and per feddan (kg) as well as herb dry yield per plant (g) and per feddan (kg) of marjoram plants) compared to control, in most cases. Regarding combination between AA and SA, the highest values of above mentioned parameters were obtained by AA acid 300 ppm + SA 200, as compared with other treatments . These results were in the same line in the two cuts of the two seasons.

These results are agreement with those found by **[30,31,32]** who recorded that foliar application of ascorbic acid and salicylic acid increased plant growth yield of Peas, roselle and fennel plants**. [33]** reported that the application of ascorbic acid in different concentrations showed significant increases in all growth parameters, fresh and dry weights of *Ocimum basilicum* plant. **[8,34]** pointed that, the application of SA increased the plant shoots, dry weight and yield of coriander and fennel plants.

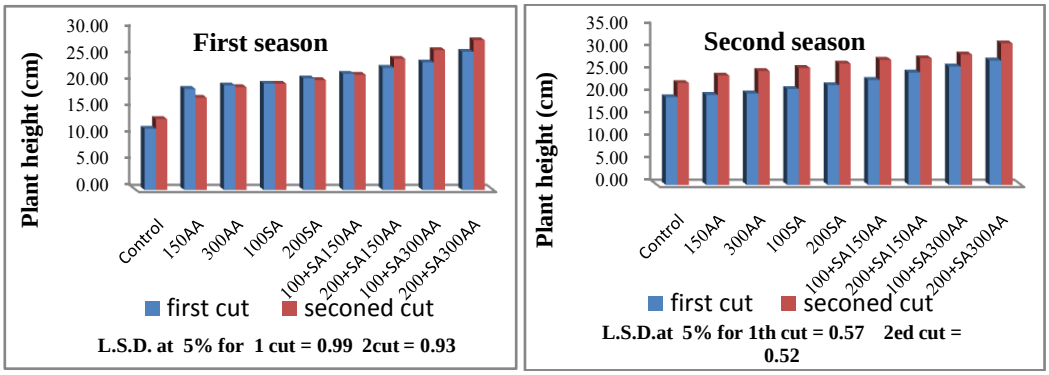

Fig.(1): Impact of foliar spraying of ascorbic acid, salicylic acid and their combination treatments on plant height of marjoram (*Majorana hortensis* L.) plant during two cuts in the two seasons (2016 and 2017)

Table (1): Impact of foliar spraying of ascorbic acid, salicylic acid and their combination treatments on yield of marjoram (*Majorana hortensis* L.) plant during two cuts in the two seasons (2016 and 2017)

Charact. / Treatments	Herb fresh yield/plant (g)		Herb fresh yield/feddan (kg)		Herb dry yield/plant (g)		Herb dry yield/feddan (kg)	
	First season							
	1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut
Control	26.72	56.31	694.60	1464.10	13.11	21.49	340.86	558.83
AA*150	32.22	56.85	837.60	1478.20	15.54	23.15	403.95	601.99
AA300	33.66	58.85	875.10	1530.20	16.72	23.93	434.63	622.18
SA**100	39.25	66.81	1020.60	1737.00	17.15	25.60	445.81	665.69
SA200	43.17	74.01	1122.40	1924.30	18.36	26.84	477.36	697.84
AA150+SA100	43.63	78.42	1134.40	2038.80	20.59	27.50	535.43	715.00
AA150+SA200	46.77	80.96	1214.90	2105.00	23.27	30.14	605.11	783.73
AA300+SA100	48.62	82.71	1264.10	2150.50	26.65	33.28	692.99	865.37
AA300+SA200	52.01	86.26	1352.30	2242.80	28.46	34.05	739.96	885.39
L.S.D. at 5%	2.67	2.03	69.42	52.87	0.98	1.15	25.58	29.98
	Second season							
Control	35.67	42.47	963.10	1146.80	16.85	20.45	454.95	552.24
AA150	42.15	46.08	1138.00	1244.10	19.81	21.42	534.96	578.43
AA300	47.19	50.30	1274.00	1358.20	22.24	24.94	600.57	673.47
SA100	48.95	61.99	1321.60	1673.80	22.78	27.61	614.97	745.38
SA200	50.75	68.02	1370.30	1836.50	23.96	28.85	647.01	781.74
AA150+SA100	54.99	70.01	1484.90	1890.20	25.42	30.05	686.25	811.26
AA150+SA200	71.67	80.24	1935.00	2166.60	27.76	30.44	749.61	821.79
AA300+SA100	79.05	83.45	2134.30	2253.10	30.54	32.26	824.58	871.02
AA300+SA200	81.79	87.90	2208.40	2373.20	31.88	34.88	860.67	941.76
L.S.D. at 5%	1.82	1.42	49.04	38.45	0.79	0.99	21.40	26.94

*AA= Ascorbic acid ** SA= Salicylic acid

2. Chemical analysis

2.1. Essential oil productivity

The data for essential oil percentage and essential oil yield per plant and feddan are presented in **Table 2.** It clear that, foliar applications of AA and SA significantly improved the essential oil percentage and essential oil yield per plant and feddan compared to untreated plants in the two cuts of both seasons. The combination effect between ascorbic acid and salicylic acid foliar application with all concentrations gave the highest values of this respect as compared with either individual foliar application or control plants. These results were in the same line in the two cuts of the two seasons.

These results are in line with those obtained by **[31]** noticed that spraying fennel plants with ascorbic acid and/or salicylic acid improved oil production, **[32]** mentioned that, the application of ascorbic acid increased essential oil yield of *Ocimum basilicum* plant and **[8, 33]** on coriander and fennel plants using SA.

Table (2): Impact of foliar spraying of ascorbic acid, salicylic acid and their combination treatments on volatile oil yield of marjoram (*Majorana hortensis* L.) plant during two cuts in the two seasons (2016 and 2017)

Charact. / Treatments	Volatile oil %		Volatile oil yield/plant (ml)		Volatile oil yield/feddan(kg)	
	First season					
	1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut
Control	1.07	0.82	0.14	0.18	3.64	4.60
AA*150	1.14	1.43	0.18	0.33	4.61	8.59
AA300	1.52	1.72	0.25	0.41	6.59	10.68
SA**100	1.53	1.81	0.26	0.47	6.82	12.07
SA200	1.54	2.03	0.28	0.55	7.37	14.17
AA150+SA100	1.62	2.12	0.33	0.58	8.68	15.16
AA150+SA200	1.65	2.32	0.38	0.70	9.99	18.16
AA300+SA100	1.92	2.91	0.51	0.97	13.29	25.21
AA300+SA200	2.24	3.05	0.64	1.04	16.55	27.01
L.S.D. at 5%	0.02	0.03	0.02	0.03	0.46	0.75
	Second season					
Control	0.88	1.12	0.19	0.23	5.10	6.19
AA150	1.24	1.22	0.24	0.26	6.54	7.07
AA300	1.42	1.31	0.29	0.33	7.89	8.84
SA100	1.44	1.42	0.32	0.39	8.75	10.61
SA200	1.45	1.44	0.35	0.42	9.32	11.26
AA150+SA100	1.45	1.46	0.37	0.44	10.02	11.84
AA150+SA200	1.47	1.52	0.42	0.46	11.42	12.52
AA300+SA100	1.49	1.62	0.49	0.52	13.36	14.11
AA300+SA200	1.83	1.81	0.58	0.63	15.58	17.05
L.S.D. at 5%	0.01	0.02	0.01	0.01	0.30	0.38

*AA= Ascorbic acid ** SA= Salicylic acid

2.2. Essential oil composition.

Forty four compounds, accounting for more than 99% of the total volatiles in most marjoram samples were detected and identified (Table 3). There were differences in oil composition as affected by ascorbic acid and/or salicylic acid applications. The predominant compounds present under all treatments were the sabinene (6.55-10.28%), 4- Thajanol (10.11-25.04%), Terpine-4-ol (28.39-44.86%), α-Terpineol (3.68-5.32%), α-Terpinolene (1.61-2.05%), C-Terpinene (9.05-15.89%), D-Limonene (1.16-1.45%), α-Terpinene (4.79-8.29%), α-Myrcene (0.80-1.98) and α-Phenllandrene (0.90-3.18). These results are in line with those obtained by **[35]**. Contents of other oil constituents varied without a clear trend. Ascorbic acid and/or salicylic acid treatments gave an increase in the level of Terpinen-4-ol (the major compound in marjoram oil) , 4-Thajanol, C-Terpinene, α-Terpinene and Sabinene by a decrease in the proportions of α-Terpinolene and D-Limonene, relative to untreated plants. The highest value in Terpinen-4-ol was obtained from SA100ppm treatment followed by AA150ppm+SA200ppm and the lowest value recorded from AA150 ppm treatment.

Table (3): Impact of foliar spraying of ascorbic acid, salicylic acid and their combination treatments on essential oil constituents of marjoram (*Majorana hortensis* L.) plant during first seasons (2016 and 2017)

Components / Treatments		Control	AA150	AA300	SA100	SA200	AA150 + SA100	AA150 + SA200	AA300 + SA100	AA300 + SA200
1	β-Pinene	0.45	0.48	0.42	0.38	0.30	0.41	0.39	0.46	0.39
2	**α-Phellandrene**	**3.18**	**1.60**	**1.14**	**2.59**	**0.65**	**0.87**	**2.74**	**0.90**	**2.79**
3	α-Pinene	0.71	0.84	0.72	0.61	0.49	0.71	0.68	0.71	0.66
4	Camphene	0.03	-	-	0.03	-	-	0.03	-	0.03
5	**Sabinene**	**8.86**	**10.28**	**9.10**	**6.55**	**7.82**	**9.65**	**7.72**	**7.25**	**7.82**
6	**α-Myrcene**	**1.17**	**1.39**	**0.84**	**0.96**	**0.80**	**1.11**	**1.98**	**1.20**	**1.08**
7	**α-Terpinene**	**6.04**	**6.96**	**6.48**	**7.37**	**4.79**	**6.95**	**7.53**	**7.82**	**8.29**
8	**o-Cymene**	**1.31**	**2.49**	**2.13**	**0.97**	**1.36**	**1.26**	**0.70**	**0.79**	**0.92**
9	**D-Limonene**	**1.35**	**1.34**	**1.16**	**1.17**	**1.23**	**1.45**	**1.22**	**1.44**	**1.23**
10	TransOcimene	-	--	-	-	-	-	0.02	-	0.01
11	**Ç-Terpinene**	**11.19**	**11.70**	**11.86**	**14.08**	**9.05**	**12.73**	**13.96**	**13.59**	**15.89**
12	**4-Thujanol**	**23.56**	**17.66**	**17.74**	**10.14**	**25.04**	**17.54**	**12.24**	**15.45**	**10.11**
13	**α-Terpinolene**	**1.70**	**2.27**	**2.01**	**1.89**	**1.61**	**2.05**	**1.92**	**2.21**	**2.04**
14	Pinocarvone	-	-	-	0.01	-	-	-	-	-
15	1Cyclohexanone	-	-	0.48	-	-	0.08	0.02	-	-
16	Isoborneol	-	-	-	-	0.07	0.05	-	0.06	0.04

17	Endo-Borneol	-	-	-	0.06	-	-	0.06	-	-
18	**Terpinen-4-ol**	**30.77**	**28.39**	**34.61**	**44.86**	**35.08**	**34.84**	**40.66**	**37.95**	**39.83**
19	**α-Terpineol**	**5.22**	**4.67**	**5.19**	**4.48**	**4.87**	**3.68**	**4.76**	**5.32**	**4.41**
20	Isopulegol	0.04	-	0.08	-	-	0.07	0.06	-	0.05
21	Dihydrocarvone	0.07	-	-	0.06	0.07	-	-	0.07	-
22	Piperitol isomer I	0.14	-	0.17	0.18	0.12	0.19	0.16	0.18	0.19
23	Ascaridole	-	-	0.04	0.03	0.04	-	-	-	0.01
24	Linalyl acetate	-	-	2.06	1.00	2.97	2.95	-	1.81	-
25	Cis-sabinene hydrate acetate	1.96	3.85	-	-	-	-	1.54	-	1.62
26	Citronellal	0.02	-	-	-	-	-	-	-	-
27	Bornyl acetate	0.06	-	0.12	0.07	0.13	0.09	0.06	0.08	0.06
28	4-Terpinenyl Acetate	-	-	0.53	0.30	0.28	0.39	0.28	0.32	0.30
29	Caryophyllene	1.03	1.17	1.84	0.91	1.23	1.26	0.87	1.06	0.97
30	α-Farnesene	0.03	-	-	-	-	-	-	-	-
31	Humulene	0.05	-	0.06	0.04	0.05	0.05	0.04	0.04	0.04
32	Ç-Elemene	0.83	-	-	0.01	-	-	0.01	-	0.01
33	Ledene	0.01	-	-	-	-	-	-	-	-
34	Bicyclogermacrene	-	1.12	1.31	1.04	1.73	1.45	1.05	1.15	1.02
35	4Isopropyl1phenylhexa 1,5dien3ol	-	-	-	0.01	-	-	-	-	-
36	Doconexent	-	-	-	-	-	-	0.01	-	-
37	Cyclooctasiloxane, hexadecamethyl	-	2.65	-	-	-	-	-	-	-
38	Spathulenol	0.10	-	0.12	0.08	0.10	0.07	0.09	0.06	0.07
39	Caryophyllene oxide	0.10	-	0.15	0.08	0.11	0.10	0.07	0.07	0.09
40	Lanceol, cis	-	-	-	-	-	-	0.03	-	-
41	Alloaromadendrene oxide	-	-	-	0.03	-	-	-	-	-
42	Cinobufagin,	-	-	-	-	-	-	0.01	-	-
43	Octasiloxane	-	0.65	-	-	-	-	-	-	-
44	Heptasiloxane, hexadecamethyl	-	0.48	-	-	-	-	-	-	-
Hydrocarbons Compounds		37.94	41.64	39.11	38.60	31.11	39.95	39.91	38.62	43.19
Oxigenated Compounds		62.04	58.35	61.29	61.39	68.88	60.06	60.05	61.37	56.78
Total		99.98	99.99	100.00	99.99	99.99	100.00	99.96	99.99	99.97

*AA= Ascorbic acid ** SA= Salicylic acid

2.3. Total antioxidant activity

It is clear from Fig. (2) that total phenolic compounds and antioxidant activity of marjoram plant was significantly affected by different application of ascorbic acid and / or salicylic acid at all concentrations in both seasons in comparison to untreated plants. In this respect, the combination between AA at 300ppm and SA at 200ppm was superior treatment and gave significant increase in total phenolic content and antioxidant activity compared to other treatments under study and control. These results are agreement with those demonstrated by **[36,37].** The antioxidant activity of plant extracts containing polyphenol components is due to their capacity to be donors of hydrogen atoms or electrons and to capture free radicals. Some of the flavonoid glycosides prevent oxidant injury and cell death by several mechanisms **[38,39].**

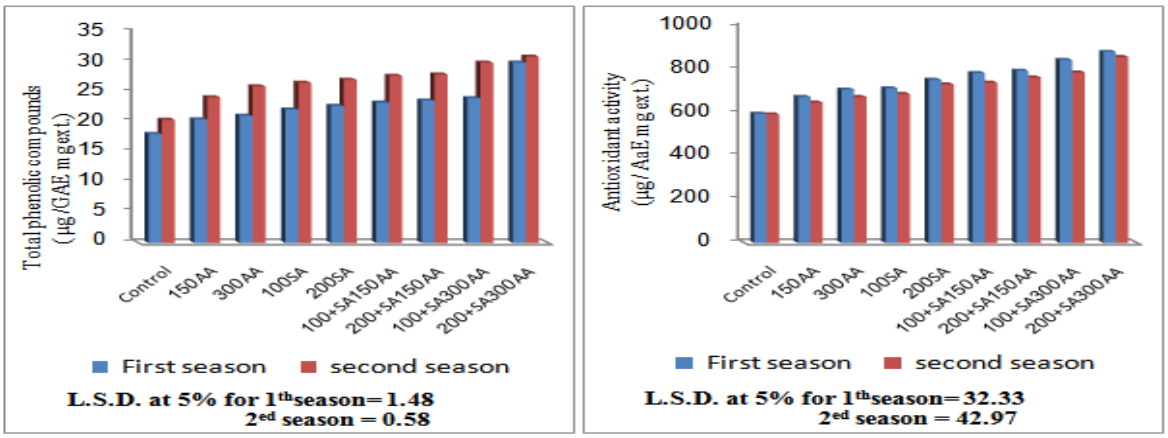

Fig.(2): Impact of foliar spraying of ascorbic acid, salicylic acid and their combination treatments on total antioxidant activity of marjoram (*Majorana hortensis* L.) plant during the two seasons (2016 and 2017)

3. Antimicrobial activity of marjoram essential oil:

Antimicrobial activity of essential oils was detected against some pathogenic microorganisms namely: Gram negative bacteria *Escherchia coli*, *Enterobacter*, Gram positive bacteria: *Bacillus subtilis* and *Staphylococcus aureus* and Fungi : *Verticillium dahlia* and *Fusarium solani*. *Escherchia coli* was most resistant to marjoram oil, the other tested pathogen were sensitive to oil as shown in (Table 4). Antioxidants application gave an increase the antagonistic activity of marjoram oil against some pathogenic microbes **[40]**. The treatment of (AA300+SA200) was superior in this regard compared to other treatments under study.

Table (4): Impact of foliar spraying of ascorbic acid, salicylic acid and their combination treatments on antimicrobial activity of marjoram (*Majorana hortensis* L.) volatile oil during the first season (2016)

	G+ve		G-ve		Fungi	
	E.coli	*Enterobacter*	*B.subtilis*	*S.aureus*	*V.dahliae*	*F.solani*
	Inhibition zone diameter					
Control	0.5	1.3	1.9	0.7	1.3	1.7
AA*150	0.8	1.4	2.3	1	1.6	1.8
AA300	0.8	1.8	2.4	1.5	1.8	2.1
SA**100	1.1	2.1	2.4	1.8	1.8	2.3
SA200	1.2	2.1	2.6	2.1	2.2	2.5
AA150+SA100	1.4	2.2	3	2.3	2.4	3
AA150+SA200	2.1	2.2	3.1	2.3	2.8	3.8
AA300+SA100	2.4	2.3	3.4	2.7	3	4.2
AA300+SA200	2.6	2.5	3.5	3.2	4	4.6

*AA= Ascorbic acid ** SA= Salicylic acid

From the obtained results previously, it is clear that the treatment of marjoram plants with ascorbic acid and/or salicylic acid increase antioxidant content which reduced the damage caused by thermal stress in Siwa Oasis and led to improved growth and productivity of the plant under these conditions.

IV. Conclusion

Under Siwa Oasis conditions, *Marjorana hortensis* L. plants should be sprayed by ascorbic acid at 300ppm + salicylic acid at 200 ppm after planting and after each cut to obtain the highest yields of herb and oil per feddan.

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