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Improved growth and productivity of basil plants grown under salinity stress by foliar application with ascorbic acid

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ABSTRACT

The present research aimed to enhancement the growth of basil plants grown under of salinity stress by foliar application with 300 ppm ascorbic acid.

The obtained results indicated that irrigation basil plants with salinized water retarded vegetative growth characters (plant height, number of primary branches/plant, number of leaves/plant and total leaf area/plant), yield of fresh herb, concentration of photosynthetic pigments and anatomical structure of basil leaf. Whereas, the concentration of free proline was increased due to salinity stress. Worthy to note that, sprayed ascorbic acid on basil plants grown under salinity stress showed better growth behavior than those of unsprayed, and induced significant recovery for the reduction occurred in most of the studied characters of salinized basil plants especially those grown under stress of 4000 ppm

***Keywords*:** Basil, Salinity stress, Ascorbic acid, Growth, Essential oil, Anatomy

Introduction

Ocimum basilicum L. (basil or sweet basil) called rehan in Arabic is one of the most important aromatic species in the genus *Ocimum* of the family Lamiaceae (Hugh, 2005) and chosen to be the subject of the present study because of its economic importance as an ornamental, culinary, spice and medicinal herb. Sweet basil is cultivated extensively in Southern, Central and Eastern Europe, North Africa and in the USA, particularly California (Singh and Panda, 2005). It grows well in Egypt, the cultivated area is about 1091 feddans (1 feddan = 4200 m^2) produced about 1951 tons fresh herb per year and yielded about 29.263 tons essential oil (Arafa, 2007). In this respect, UPEHC (2016) recorded that the cultivated area is about 7320 feddans. Basil herb contains 0.5-1.5 % volatile oil of varying composition out of which linalool as the main component (Hiltunen, 1999). The volatile oil, obtained by steam distillation from the fresh herb, is used in perfumery, in making incense and in the food industry (Bunney, 1992). The market for basil oil is dominated by European and Egyptian production (Nahak *et al*., 2011). Therefore, increasing productivity of basil plant from fresh herb and essential oil either per unit area, by using natural and safety growth promoting substances, or by expansion of the agricultural area is highly recommended to meet the demand of human needs and exportation.

The expansion of the agricultural area requires an enormous amount of irrigation water, which is not sufficient to meet all the expected demand.

Thus, the possibility of using saline water for irrigation especially from underground or drainage water is expected. The application of saline water for irrigation is dependent upon the concentration, composition of dissolved salts and the degree to which the plant species is salt tolerant.

Salinity stress adversely affects the morphological, physiological and biochemical responses of plant species (Nazar *et al*., 2011). It reduces photosynthetic attributes (Khan *et al*., 2009 and Saha *et al*., 2010) and disturbs plant growth and development (Sairam and Tyagi, 2004). Thus, it is well established that salinity inhibits plant growth, reduces yield in many crop plants and affected their commercial value (Zhang, 2013). In this concern, EL-Gamal (2005) stated that salinity stress inhibited growth of basil plant by decreasing significantly plant height, number of leaves/plant, number of branches/plant and dry weight of herb/plant. Concentrations of photosynthetic pigments (chl a, chl b and carotenoids) and total carbohydrates as well as NPK were reduced in leaves of salinized plants. Likewise, number of inflorescences, volatile oil percentage and oil yield/plant were significantly

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depressed under stress of salinity. By contrast, proline concentration and Na were significantly increased under salinity stress. The retardant effect of salinity stress on growth, physiological aspects and productivity was also recorded on other different plant species; for instance, Reda (2007) on *Senna occidentalis* , Dawood *et al*. (2014) on Faba bean, Bargaz *et al*. (2016) on *Phaseolus vulgaris*, Nassar *et al*. (2016) on Leucaena and Rady *et al*. (2016) on *Lupinus termis*.

The use of antioxidants in promoting growth and increasing productivity of many plant species grown under normal conditions or under abiotic stresses is highly recommended.

One of the most familiar antioxidants is ascorbic acid which being synthesized in higher plants and affects plant growth and development. Recently, it is recorded about its essential role in series of physiological processes such as plant defense against oxidization, cofactor of key enzyme, plant cell division, cell expansion, growth and development, senescence and counteracts the deleterious effects of abiotic stresses (Zhang, 2013). Therefore, it is chosen to be the subject of our present study.

As to the effect of foliar application with ascorbic acid on growth and productivity of basil plant, El-Gamal (2005) found that ascorbic acid at concentration of 300 ppm have a promotive effect on vegetative growth, photosynthetic pigments, herb productivity and percentage of essential oil of basil plant grown either under normal conditions or under stress of salinity or drought.

Therefore, the present investigation is an attempt to through to light more information about the influence of foliar spray with ascorbic acid on vegetative growth characters, fresh herb, physiological aspects, volatile oil composition and leaf anatomy of basil plant grown under salinity stress hoping to counteracting or at least minimizing the deleterious effects of salinity stress on growth and productivity of basil plant by ascorbic acid application.

Materials and Methods

The present research was carried out during the two growing seasons of 2015 and 2016 in order to enhancement the growth of basil plants grown under different levels of salinity stress by foliar application with ascorbic acid.

2.1. Source of basil seeds and ascorbic acid

Basil seeds were procured from the Experimental Station of Medicinal Plants, Faculty of Pharmacy, Cairo University, Giza, Egypt. The antioxidant ascorbic acid was obtained from Electro Sciente Company, Egypt. It is a powder containing 99.9% active ingredient.

2.2 Procedure of the experiment

Seeds of Basil were sown on 15th March, 2015 in the first season and replicated on 19th March, 2016 in the second one to provide the experimental plant materials. Seeds were sown in plastic trays (40 x 60 cm) filled with peat moss and clean sand at the ratio of 1:1 by volume. Three weeks from sowing date, the emerged uniform seedlings were transplanted to plastic pots; one seedling per pot (25 cm diameter) filled with about 8 Kg of clay and clean sand at the ratio of 1:1 by weight. Each pot was received NPK at the recommended dose; the rate of 2g ammonium sulphate (20.6 % N), 1g calcium super phosphate (15.5 % P_2O_5) and 0.5g potassium sulphate (48 % K_2O). The experiment was made in a randomized complete block design with five replicates. The replicate contained 35 pots, each 5 pots were assigned for one treatment. The treatments were seven as follows:

1. Control, plants were irrigated with tap water.
2. Three levels of salinity in irrigation water; namely, 2000, 4000 and 6000 ppm of salt mixture (Na Cl : $Ca Cl_2$, 1:1 w/w).
3. One level of ascorbic acid (300 ppm) was applied twice on each of the tested three levels of salinity. The first application at seven weeks from sowing date (one month from transplanting) and the second application was done after three weeks from the first application.

Each level of salinity in irrigation water was added regularly (500 ml/pot/week) during whole period of the experiment (12 weeks from transplanting). Irrigation treatments were applied three times with salt-water followed by one irrigation with tap water, for leaching the accumulated salts, and then repeated in the same manner till the end of the experiment.

2.3. Recording of data

2.3.1. Growth characters

At the end of the experiment (full blooming stage) in each of the two growing seasons (three months from transplanting), ten plants from each treatment, two from each replicate, were lifted from pots for recorded the data of vegetative growth as follows:

1. Plant height (cm), measured from the cotyledonary node up to the upper most point of the plant.
2. Number of primary branches developed per plant.
3. Number of leaves per plant.
4. Total leaf area (cm^2) per plant, measured by means of leaf area meter.
5. Fresh weight of shoot (g) per plant, represents yield of fresh herb in grams per plant.

2.4. Physiological studies

Photosynthetic pigments and free proline were determined quantitatively in leaves of treated and untreated plants at the end of the experiment (full blooming stage) in each of the two growing seasons.

2.4.1. Photosynthetic pigments

Chlorophyll a, chlorophyll b and carotenoids were extracted from upper fresh leaves by using dimethyl formamide and determined according to Nornai (1982) as mg/g fresh weight (FW) of basil leaves.

2.4.2. Free proline

Free proline was determined in fresh leaves according to the method described by Bates *et al*. (1973). Bush and Lomb spectrophotometer (model spectranic 2000) was used. The absorbance was measured at 520 nm. Free proline was estimated as mg/g fresh weight of basil leaves.

2.5. Chemical analysis of volatile oil

A chemical analysis was carried out to gain information about the effect of salinity stress and foliar spray with ascorbic acid on salinized plants on the percentage and composition of volatile oil of basil herb at full blooming stage of the second growing season of 2016. Hydrodistillation of the volatile oil was conducted using the technique described by Densy and Simon (1990). For each studied treatment, plant material was placed in a 2-liter round bottomed flask with distilled deionized water (400 ml for 200 g fresh herb) and the volatile oil was extracted by water distillation using a modified Clevenger trap (ASTA, 1968). For smaller fresh plant sample, the distillation period was two hours and the volatile oil content was determined on an oil volume to tissue weight.

GC-MS technique was used to separate and detect the volatile oil constituents. Analysis was performed at Research Park, Faculty of Agriculture, Cairo University, Giza, Egypt. GC-MS analysis was carried out on a Hewlett-Packard 6890 gas chromatograph fitted with a fused silica HP-5MS capillary column (30 m × 0.25 mm; film thickness 0.25 μm). The oven temperature was programmed from 50°-180°C at 5°C/min. Helium was used as carrier gas at a flow rate of 1 ml/min. The gas chromatograph was coupled to a Hewlett-Packard 6890 mass selective detector. The MS operating parameters were ionization voltage, 70 eV; and ion source temperature 250°C.

2.6. Anatomical studied

Tested material included lamina of the leaf developed on the median portion of the main stem. Specimens were taken throughout the second growing season of 2016 at the age of 12 weeks from sowing date. The procedure of microtechique was carried out according to the method described by Nassar and El-Sahhar (1998).

2.7. Statistical analysis

Data on growth characters, yield of fresh herb, photosynthetic pigments and free proline were subjected to conventional methods of analysis of variance according to Snedecor and Cochran (1982). The data were statistically analyzed for each season and the homogeneity of experimental error, in both seasons, was tested. Then the combined analysis of the two seasons was done. The least significant difference (L.S.D.) at 0.05 level of probability was calculated for each determined character under different assigned treatments.

Results and Discussion

3.1. Vegetative growth characters

Data on morphological characters of vegetative growth of basil plants grown under different levels of salinity and sprayed with ascorbic acid are given in Table (1). The studied morphological characters included plant height, number of primary branches/plant, number of leaves / plant and total leaf area/plant at full blooming stage (15 weeks from sowing date).

It is realized from Table (1) that irrigated basil plants with salinized water retarded vegetative growth characters and the retardation was increased significantly with increasing salinity level up to 6000 ppm. The maximum decrease was achieved at 6000 ppm salinity (Figure.1, A and B), being 25.1, 42.9, 44.0 and 48.2 % less than the control for plant height, number of primary branches/plant, number of leaves/plant and total leaf area / plant; respectively.

Table 1: Effect of foliar application with ascorbic acid on morphological characters of vegetative growth and yield of fresh herb / plant, at full blooming stage, of basil grown under different levels of salinity stress (average of the two seasons, 2015 and 2016 combined)

Treatments	Morphological characters of vegetative growth				Yield of fresh herb (g)/plant
	Plant height (cm)	No. of primary branches/plant	No. of leaves/plant	Total leaf area (cm^2)/plant	
Control	61.7 B	9.62 AB	211.9 AB	3044.8 AB	315.35 B
2000 ppm salinity	60.9 BC	9.36 BC	203.8 AB	2962.6 BC	301.79 B
4000 ppm salinity	52.4 DE	7.17 D	161.3 C	2240.9 D	226.42 D
6000 ppm salinity	46.2 E	5.49 E	118.6 D	1577.2 E	155.78 E
2000 ppm salinity + 300 ppm ascorbic acid	70.9 A	10.82 A	238.4 A	3452.8 A	360.13 A
4000 ppm salinity+ 300 ppm ascorbic acid	59.7 BC	9.15 BC	199.9 BC	2895.6 BC	291.70 BC
6000 ppm salinity+ 300 ppm ascorbic acid	54.2 CD	8.26 CD	180.1 BC	2551.4 CD	255.12 CD
L.S.D. (0.05)	7.16	1.29	38.5	486.2	44.46

Means having the same letter (s) are not significantly different at 0.05 level**.**

In this concern, El-Gamal (2005) stated that salinity stress inhibited growth of basil plant by decreasing significantly plant height, number of leaves/plant and number of branches/plant. The retardant effect of salinity stress on growth of other different plant species was also recorded; for instance, Reda (2007) on *Senna occidentalis*, Dawood *et al.* (2014) on faba bean, Baragaz *et al*. (2016) on *Phaseolus vulgaris*, Nassar *et al.* (2016) on leucaena and Rady *et al*. (2016) on *Lupinus termis.* All, being in agreement with the present findings.

At the same time, sprayed ascorbic acid on basil plants grown under salinity stress showed better growth behavior than those unsprayed with ascorbic acid (Figure.1, B and C). It is clear that ascorbic acid application at 300 ppm had the ability to induce beneficial effects on all studied morphological characters of vegetative growth of basil plants grown under salinity stress and

overcome the harmful effects induced by salinity stress on basil growth . It could be stated that ascorbic acid had the ability to induce significant recovery for the reduction occurred in vegetative growth of basil plants especially those grown under stress of 4000 ppm salinity. The previous report of El-Gamal (2005) found that ascorbic acid at concentration of 300 ppm has a promotive effect on vegetative growth of basil plants grown under salinity stress, being in agreement with the present investigation.

A **B** **C**

Fig. 1: Habit of mature plants, at full blooming stage, of basil as affected by ascorbic acid and salinity stress.
A- Untreated plant (control).
B- Plant grown under 6000 ppm salinity stress.
C- Plant grown under 6000 ppm salinity stress and sprayed with 300 ppm ascorbic acid.

3.2. Yield of fresh herb/plant

The mean values of yield of fresh herb per basil plant at full blooming stage as affected by different levels of salinity and sprayed with ascorbic acid are presented in Table (1).

Data presented in Table (1) clearly show that the assigned low concentration of 2000 ppm artificial salinized water had no statistical effect on yield fresh herb per basil plant. On the other hand, increasing salinity level more than 2000 ppm induced significant decrease in this respect. Worthy to note that the rate of reduction increased steadily as the salinity level increased reached its maximum at salinity level of 6000 ppm, being 50.6 % less than weight of fresh herb per basil plant of control treatment. The retardant effect of salinity stress on herb productivity per basil plant was also recorded by El-Gamal (2005), being in accordance with the present findings.

Results in Table (1) also indicate that foliar application with ascorbic acid at concentration of 300 ppm on basil plants grown under different levels of salinity had the ability to induce significant recovery for the reduction occurred in yield of fresh herb/plant especially in basil plants grown under stress of 4000 ppm salinity where the difference between such treatment and control proved

insignificant. In this respect, El-Gamal (2005) found that ascorbic acid at concentration of 300 ppm has a promotive effect on herb productivity of basil plants grown under salinity stress, being in harmony with the present findings.

3.3. Physiological aspects

Photosynthetic pigments (chlorophyll a, chlorophyll b and carotenoids) and free proline were determined quantitatively in leaves of treated and untreated plants of basil at full blooming stage. Data on these fractions are presented in Table (2).

3.3.1. Photosynthetic pigments

Concerning the effect of salinity stress on concentration of photosynthetic pigments in basil leaves (Table 2), it is obvious that the relatively low used concentration of 2000 ppm salinized water had no statistical effect on photosynthetic pigments (chlorophylla a, chlorophyll b and carotenoids) in leaves of basil plants. On the other hand, the other tested concentrations of salinized water (4000 and 6000 ppm) decreased significantly the concentration of chloroplast pigments and the rate of reduction increased proportionally as salinity level in irrigation water was increased reached its maximum at salinity level of 6000 ppm, being 30.7, 35.3 and 35.1% less than the control for chlorophyll a, chlorophyll b and carotenoids; respectively. Such results are in agreement with those reported by El-Gamal (2005) on basil plants as well as by Reda (2007) on *Senna occidentalis* and by Dawood *et al*. (2014) on faba bean and by Nassar *et al*. (2016) on leucaena plants.

Worthy to note that foliar application with 300 ppm ascorbic acid had the ability to minimize and counteract the harmful effect of salinity and improve the concentration of chloroplast pigments in leaves of salinized plants of basil especially of those grown under salinity stress of 2000 and 4000 ppm. The obtained results are in accordance with those reported by El-Gamal (2005). Such promotional effect of ascorbic acid upon the formation of chloroplast pigments might be due to the role of ascorbic acid as one of the protective systems to chloroplast pigments, for its antioxidant nature.

Table 2: Effect of foliar application with ascorbic acid on photosynthetic pigments and free proline concentrations in leaves of basil plant, at full blooming stage, grown under different levels of salinity stress (average of the two seasons, 2015 and 2016 combined)

Treatments	Photosynthetic pigments (mg/g FW)			Free proline (mg/g FW)
	Chl a	Chl b	Carotenoids	
Control	1.817 B	0.993 B	0.584 B	1.107 D
2000 ppm salinity	1.772 BC	0.984 B	0.596 AB	1.126 D
4000 ppm salinity	1.531 D	0.761 C	0.484 C	1.418 B
6000 ppm salinity	1.259 E	0.643 D	0.379 D	1.635 A
2000 ppm salinity + 300 ppm ascorbic acid	2.084 A	1.109 A	0.662 A	0.908 E
4000 ppm salinity+ 300 ppm ascorbic acid	1.792 B	0.986 B	0.594 B	1.113 D
6000 ppm salinity+ 300 ppm ascorbic acid	1.578 CD	0.775 C	0.488 C	1.292 C
L.S.D. (0.05)	0.206	0.099	0.067	0.125

Means having the same letter are not significantly different at 0.05 level.

3.3.2. Free proline

It is realized from Table (2) that increasing salinity level in irrigation water more than 2000 ppm, induced significant increase in proline concentration in leaves of basil plant and the rate of accumulation increased steadily with increasing salt concentration in irrigation water and expressed

its maximum with salinity level of 6000 ppm which increased concentration of free proline in basil leaves by 47.7% more than the control. Similar results were recorded by El-Gamal (2005) on basil as well as by Reda (2007) on *Senna occidentalis* and by Nassar *et al.* (2016) on leucaena. In this respect, Pessarkli (1994) stated that the accumulation of soluble proline in leaves of many higher plant species could be induced by environmental stresses such as light, temperature, drought and salinity. Also, it was found that the amount of proline accumulation correlated with the degree of salinity, being in accordance with the present findings. Flowers *et al*. (1977) postulated that proline may function as a compatible solute which had an important role of balancing cytoplasmic and vascular water potential. In this concern, Ridge *et al*. (1993) pointed out that proline may serve as a substrate for respiration, an energy source and a storage compound for the recovering plant following stress.

Results in Table (2) also reveal that foliar application with 300 ppm ascorbic acid decreased the concentration of free proline in salinized plants near to the level of control plants especially in basil plants grown under salinity stress of 4000 ppm where the difference with control proved insignificant. The present results are in agreement with those reported by El-Gamal (2005) who applied ascorbic acid on basil plants grown under salinity stress.

3.4. Volatile oil

The percentage, yield and composition of volatile oil of basil herb at full blooming stage, age of 15 weeks, as affected by foliar spray with 300 ppm ascorbic acid and stressed by 6000 ppm salinity are given in Table (3). Likewise components of volatile oil analyzed by GC/MS are illustrated in Figures (2,3and 4).

Concerning the effect of salinity stress on volatile oil of basil herb, data presented in Table (3) clearly show that salinity stress increased volatile oil percentage and decreased the yield of volatile oil/plant. The percentage of volatile oil in stressed plants was 0.64 % against 0.56% in normal plants. Whereas, the yield of volatile oil /stressed plant was 0.997 g against 1.766 g/control plant. Although salinity stress increased the percentage of volatile oil by 14.3% over the control, it decreased yield of volatile oil/plant by 43.5% below the control. In this respect, El-Gamal (2005) stated that salinity stress reduced both percentage and yield of volatile oil/basil plant, being partially in agreement with the present findings.

Data presented in Table (3) revealed that salinity stress decreased the percentages of the three main components of volatile oil. Linalool recorded 33.67% of volatile oil of stressed plants against 38.14% of volatile oil of control plants. Likewise, geranial recorded 14.45% of the volatile oil of stressed plants against 16.55% of volatile oil of control plants. Also, the third main component neral recorded 12.54% of volatile oil of stressed plants against 14.85% of volatile oil of control plants. It realized that such three main components comprised 69.54 and 60.66 % of the volatile oil for control and stressed plants; respectively. Moreover, salinity stress at 6000 ppm affected other components of volatile oil where 12 different minor components are present in the composition of volatile oil of which three minor components belongs to volatile oil of control plants and comprised 2.16% of its volatile oil and nine other different minor components belongs to the volatile oil of stressed plants and comprised 3.98% of its volatile oil. Thus, it could be stated that stressed basil plants by 6000 ppm salinity reduced three minor compounds in the composition of volatile oil which were found in the composition of volatile oil of control plants. By contrast, such treatment induced nine minor compounds in the composition of volatile oil of stressed plants which were not found in the volatile oil of control plants. As far as the authors are aware, the pervious information about the effect of salinity stress on the composition of volatile oil of basil plants are not available in the literature.

Worthy to note that spraying ascorbic acid at concentration of 300 ppm increased percentage and yield of volatile oil per basil plant grown under salinity stress of 6000 ppm. Basil herb recorded 0.56% volatile oil of control against 0.76% volatile oil for herb of plants grown under 6000 ppm salinity and sprayed with 300 ppm ascorbic acid. Such percentages yielded 1.766 and 1.939 g for control and combined treatment; respectively.

It is clear that ascorbic acid had the ability to counteract the harmful effect of salinity stress on yield of volatile oil per salinized plant. Such treatment improved yield of volatile oil which surpassed that of the control by 9.8%. Similar results were also reported by El-Gamal (2005). As to the effect on volatile oil composition, ascorbic acid had the ability to induce prominent recovery for the reduction induced in the first main component linalool of the volatile oil obtained from fresh herb of salinized

basil plants. Linalool comprised 38.14% of the volatile oil of control plants against 39.84% of the volatile oil of salinized plants which sprayed with 300 ppm ascorbic acid. The three main components (Linalool, geranial and neral) comprised 69.54 and 65.77 % of the volatile oil for control and plants treated with 6000 ppm salinity + 300 ppm ascorbic acid; respectively. This means that spraying ascorbic acid on salinized basil plants counteracts the harmful effect of salinity stress on the percentages of the three major components of basil volatile oil.

Table 3: Volatile oil of basil herb at flowering stage, retention time, components, percentages and yield /plant

No of peaks	Retention time (min)	Components (%)	Treatments		
			Control	Salinity (6000 ppm)	Salinity (6000 ppm) + Ascorbic acid (300 ppm)
1	8.42	Alpha-Pinene	0.74	0.78	0.63
2	10.37	Beta-Pinene	-	0.69	0.48
3	10.43	Beta- Myrcene	0.77	0.97	0.80
4	10.94	Alpha-Terpinen	0.69	0.39	0.31
5	11.12	Limonene	0.67	0.52	0.45
6	11.50	Eucalyptol (1,8-Cineol)	0.93	4.26	3.23
7	11.66	Beta- Ocimene	-	0.25	-
8	11.86	Gamma-Terpinene	1.21	1.09	0.87
9	12.54	Alpha-Terpinolene	0.20	0.20	0.15
10	12.76	Cis-Sabinene Hydrate	0.84	0.44	0.46
11	**13.17**	**Linalool**	**38.14**	**33.67**	**39.84**
12	14.62	Camphor	-	0.95	-
13	14.89	Trans-Chrysanthemal	1.00	-	0.70
14	14.92	Verbenol	-	0.53	-
15	15.04	2-(2-Methylenecyclohexyl)ethanal	0.59	-	-
16	15.05	Bicyclo(2,2,2)octane	-	-	0.47
17	15.27	4-Terpineol	6.95	5.05	4.60
18	15.99	Methyl chavicol	0.65	4.46	4.66
19	16.06	Alpha-Terpineol	0.20	0.57	0.43
20	16.26	Acetic Acid	1.00	0.37	0.31
21	16.64	Nerol	2.02	0.58	1.03
22	**16.81**	**Z-Citral(Neral)**	**14.85**	**12.54**	**11.52**
23	17.41	Geraniol	-	0.31	0.70
24	**17.55**	**E-Citral (geranial)**	**16.55**	**14.45**	**14.41**
25	**20.05**	**Phenol (Iso-eugenol)**	**1.06**	**5.38**	**4.53**
26	20.41	Neryl Alcohol	0.57	-	-
27	20.42	Neryl Acetate	0.58	0.48	0.37
28	20.74	Neryl Acetate	-	-	0.26
29	20.82	1,7-Octadien-3-one	-	0.21	-
30	20.85	1,2,6-Octadienol	-	0.26	0.20
31	21.04	Beta-Elemene	0.31	0.43	0.41
32	21.59	Trans-Caryophyllene	1.68	1.30	1.19
33	21.83	Bicyclo[3.1.1]Heptene	1.51	1.52	1.51
34	22.60	Alpha- Humulene	0.69	0.76	0.57
35	23.02	Germacrene-D	1.91	1.74	1.70
36	23.35	Germacrene B	-	-	0.39
37	23.68	Naphthalene	0.40	0.37	0.52
38	23.94	Gamma-Cadinene	-	0.55	-
39	24.12	Cis-Alpha-Bisabolene	1.80	1.29	1.30
40	26.00	Alpha-Gurjunene	-	0.23	-
41	26.01	Alpha-copaene	-	-	1.26
42	26.67	Alpha-Amorphene (Delta Cadinen)	0.51	1.42	-
% of volatile oil in Basil herb			**0.56**	**0.64**	**0.76**
Yield of volatile oil (g)/plant			**1.768**	**0.997**	**1.939**

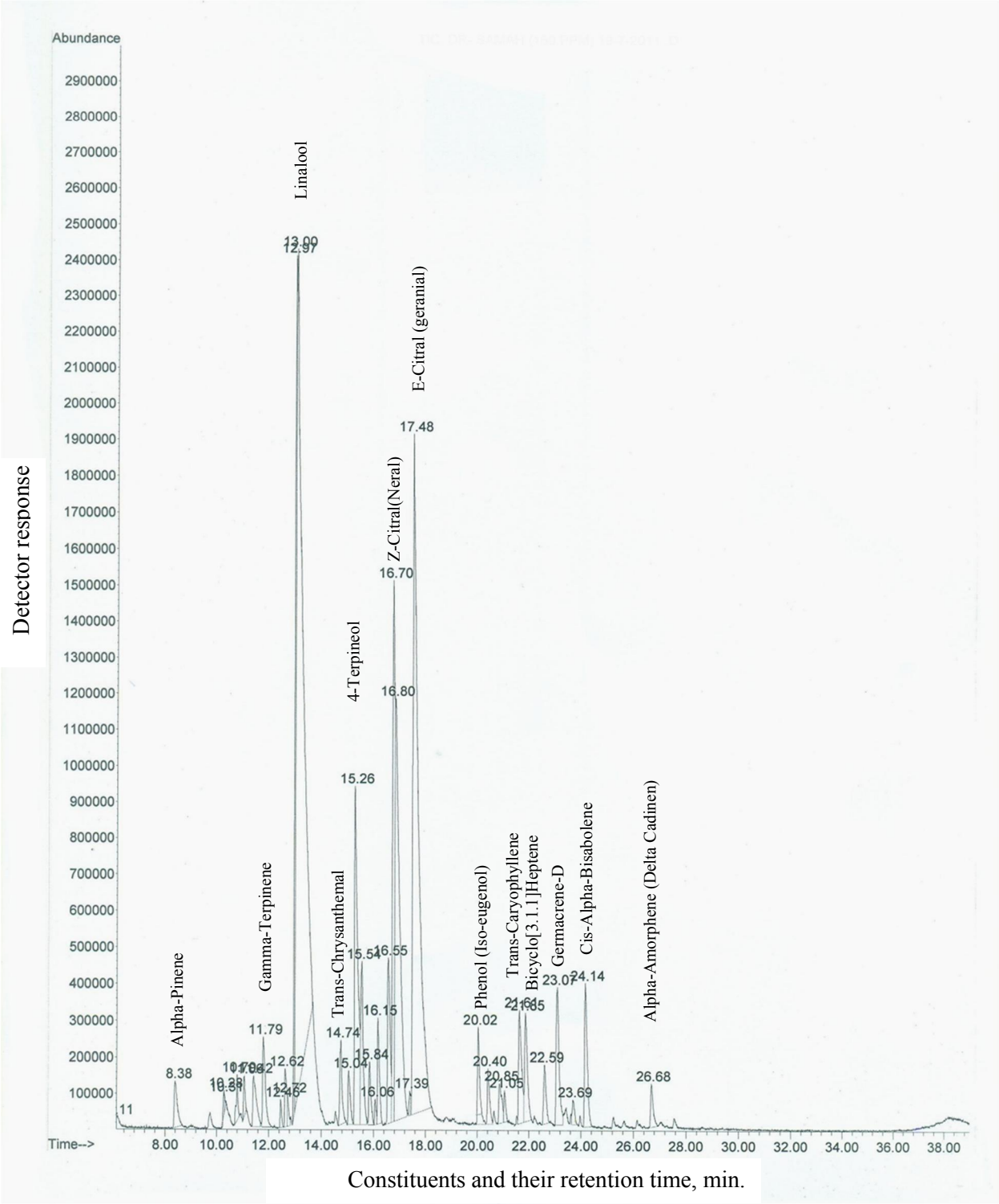
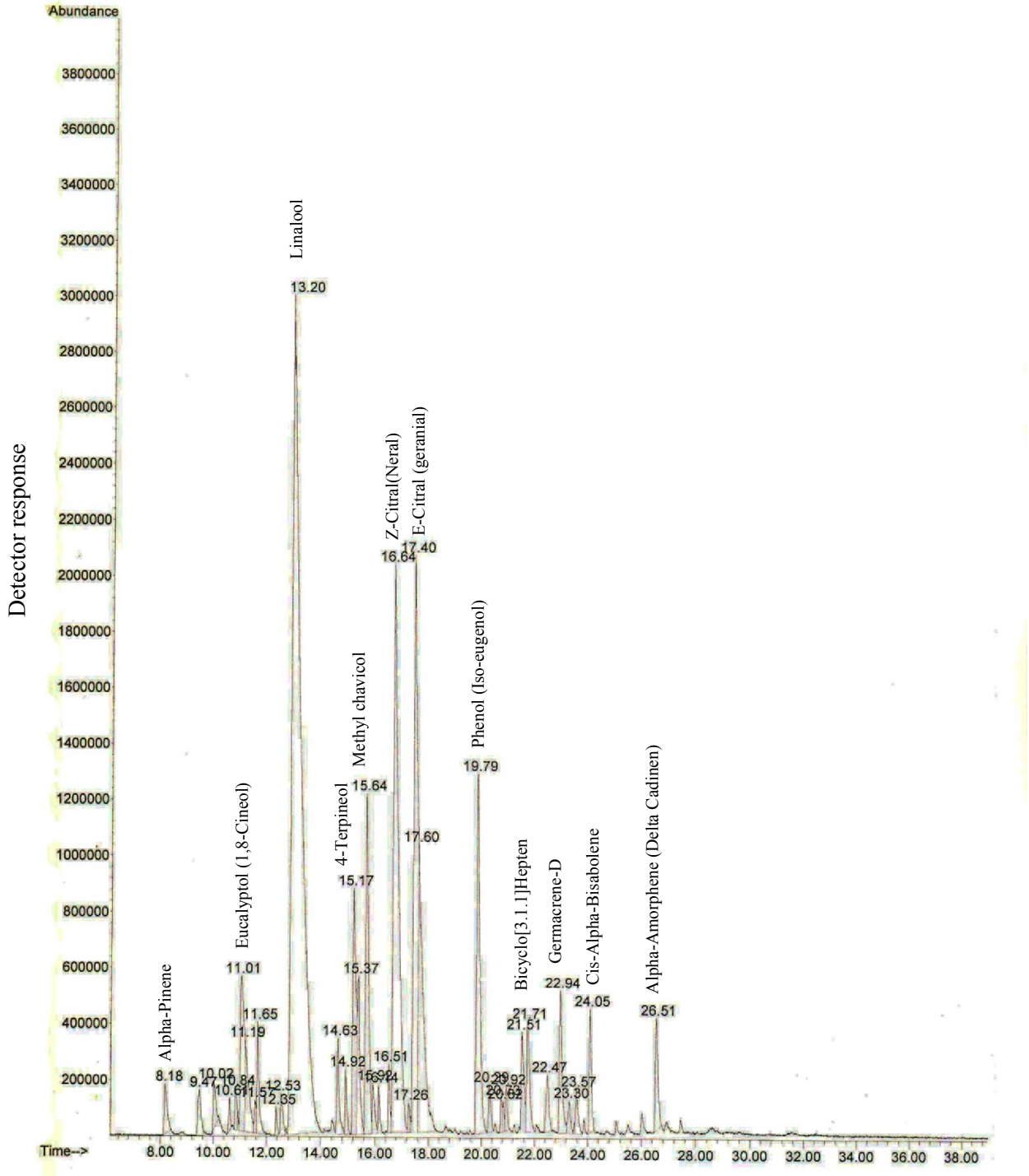


Fig 2: GC/MS of volatile oil of Basil herb at full blooming stage of untreated plants (control).



Constituents and their retention time, min.

Fig 3. GC/MS of volatile oil of Basil herb at full blooming stage of plants grown under salinity stress of 6000 ppm.

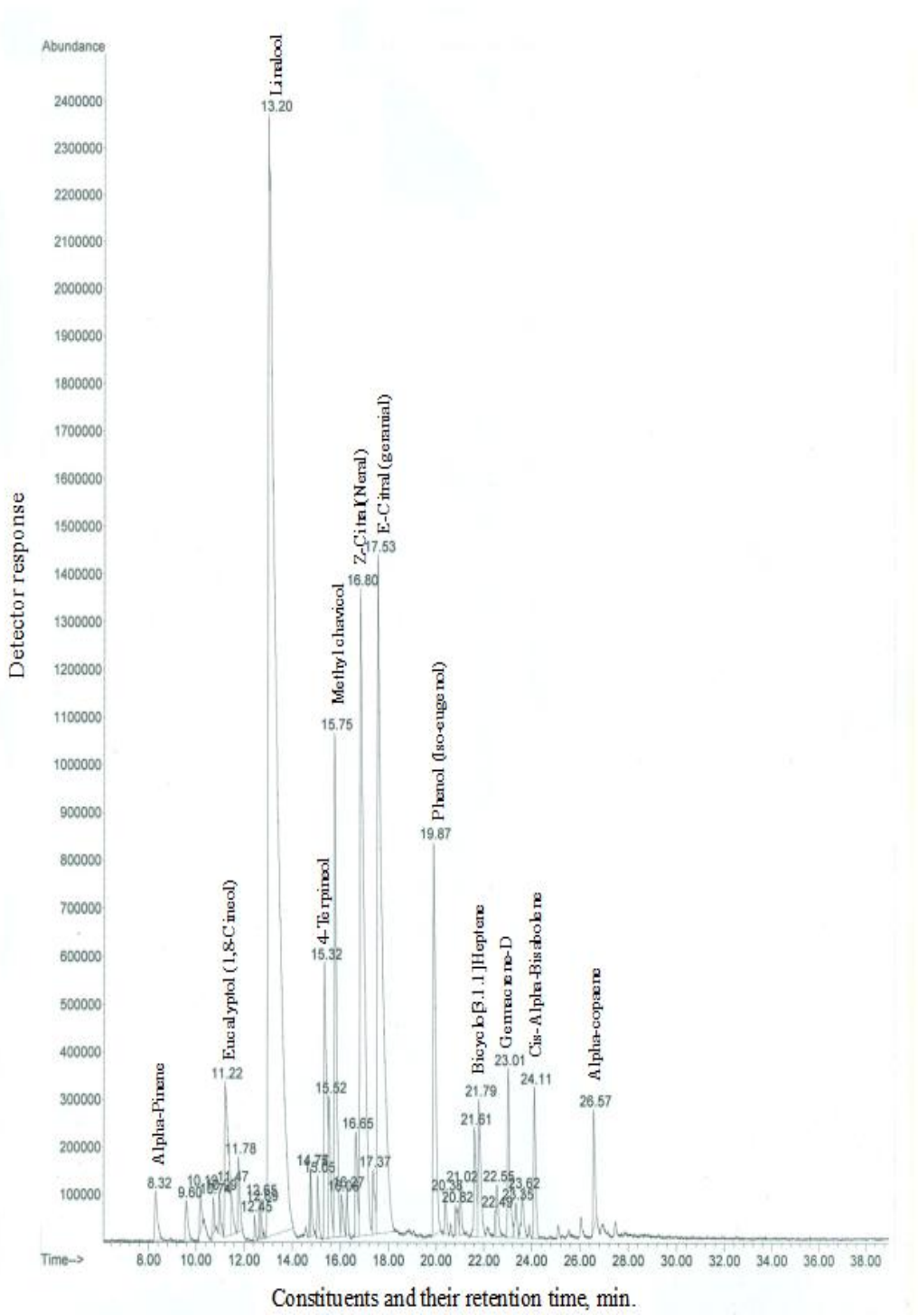


Fig 4: GC/MS of volatile oil of Basil herb at full blooming stage of plants grown under 6000 ppm salinity stress and sprayed with 300 ppm ascorbic acid

Also, such treatment affected other constituents where 10 different minor compounds are present in the composition of volatile oil of which three minor compounds belongs to volatile oil of control plant and comprised 1.67% of its volatile oil and other seven different minor compounds belongs to volatile oil of treated plants and comprised 3.76% of its volatile oil. Thus, it could be stated that spraying salinized basil plants with 300 ppm ascorbic acid reduced three minor components in the composition of volatile oil which were found in control, and induced seven minor components in the composition of volatile oil which were not found in control. As far as the authors are aware, previous

information about the effect of ascorbic acid on the composition of volatile oil of salinized basil plants are not available.

3.5. Leaf anatomy

Microscopical counts and measurements of some histological characters in transverse sections through the blade of the mature foliage leaf developed on the median portion of the main stem of basil plant, at the age of 12 weeks, grown under salinity stress of 6000 ppm and/or sprayed with 300 ppm ascorbic acid and those of untreated plant are given in Table (4). Likewise, microphotographs illustrating these treatments are shown in Figure (5).

Table 4: Counts and measurements in micro-meters (μm) of certain histological characters in transverse sections through the blade of the foliage leaf developed on the median portion of the main stem of basil plant, aged 12 weeks, grown under salinity stress and affected by foliar application with ascorbic acid

(Means of three sections from three specimens)

Histological characters	Treatments					
	Control	6000 ppm Salinity	±% to control	6000 ppm salinity +300 ppm ascorbic acid	±% to control	±% to 6000 ppm salinity
Midvein thickness	855.3	631.6	- 26.2	828.9	- 3.1	+ 31.2
Lamina thickness	421.1	302.4	- 28.2	375.2	-10.9	+ 24.1
Palisade tissue thickness	139.6	129.8	- 7.0	131.6	-5.7	+ 1.4
Spongy tissue thickness	203.7	118.4	- 41.9	184.3	- 9.5	+ 55.7
Dimensions of midvein bundle:						
Length	263.2	249.5	-5.2	276.3	+ 5.0	+ 10.7
Width	459.8	328.9	- 28.5	434.2	- 5.6	+ 32.0
No. of xylem rows/midvein bundle	22.0	14.2	- 35.5	20.8	- 5.5	+ 46.5
Vessel diameter	21.9	17.5	- 20.1	24.3	+ 11.0	+ 38.9

Results presented in Table (4) and photomicrographs shown in Figure (5) reveal that salinity stress at the level of 6000 ppm reduced the thickness of both lamina and midvein of the mature foliage leaf developed on the median portion of the main stem of basil plant by 26.2 and 28.2 % less than the control; respectively. The thinner leaves induced by salinity stress could be attributed to the decrease induced in thickness of mesophyll tissues (in both of palisade and spongy tissues) as well as in size and components of midvien bundle. The decrements below the control were 7.0, 41.9, 5.2, 28.5, 35.5 and 20.1 % for the thickness of palisade tissue, spongy tissue, length of midvein bundle, width of midvein bundle, number of xylem rows/midvein bundle and vessel diameter; respectively. As far as the authors are aware, information concerning anatomical structure of leaves of basil plants grown under stress of salinity are not available. However, some investigators confirmed the present findings using other different plant species grown under salinity stress; for instance, Reda *et al*. (2000) working on leucaena plants as well as Dawood *et al*. (2014) working on faba bean.

Worthy to note that the foliar application of 300 ppm ascorbic acid on basil plants grown under salinity stress of 6000 ppm caused enhancement in anatomical structure of leaves, due to increasing in their thickness, of salinized plants. Such treatment caused recovery of the reduction occurred in all included tissues of leaf blade where their mean values were almost reached the level of the control.

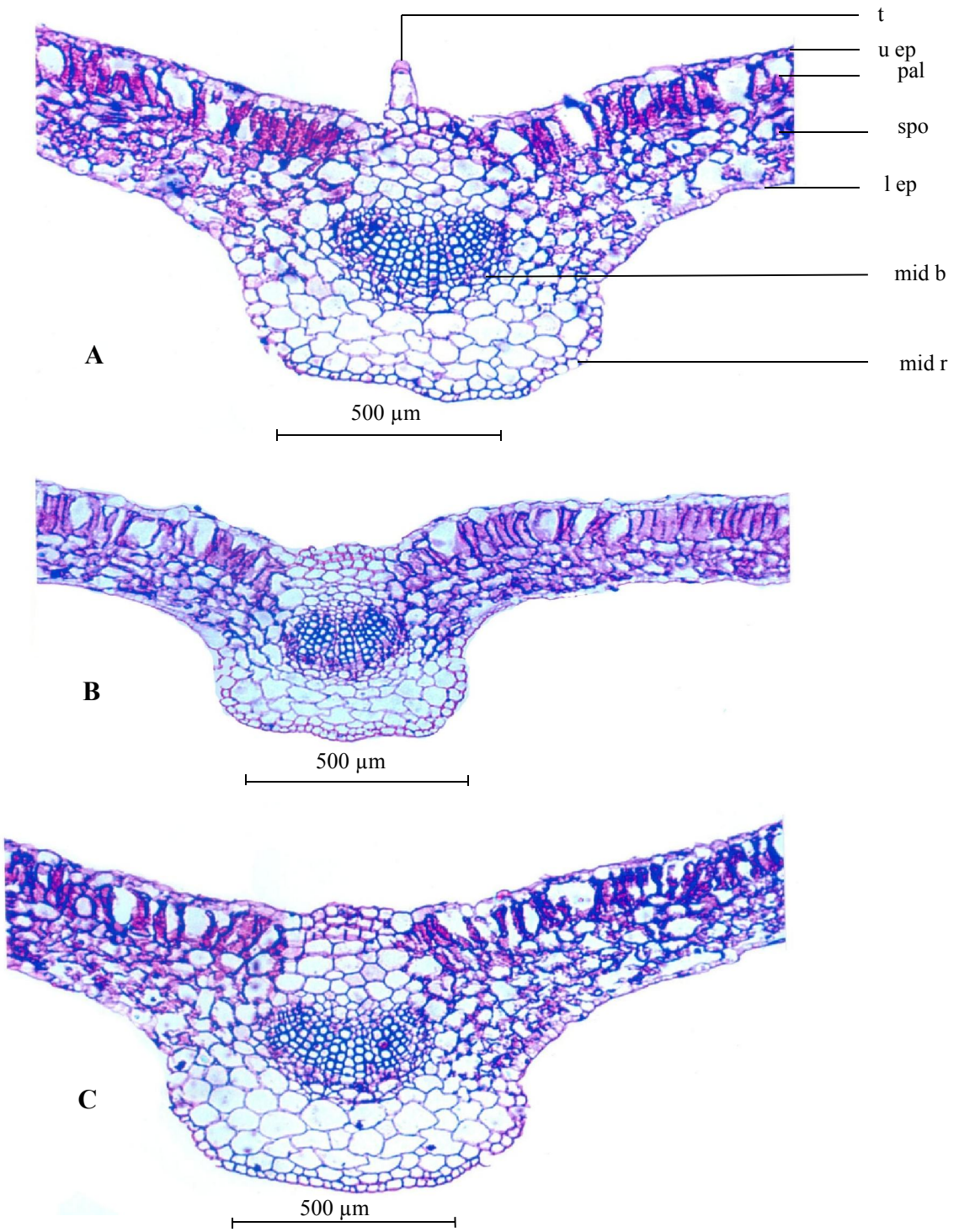


Fig. 5: Microphotographs of cross sections through lamina of the leaf developed at median portion of the main stem of basil plant, aged 12 weeks, as affected by ascorbic acid and salinity stress.
A- From untreated plant (control). B- From plant grown under salinity stress of 6000 ppm.
C- From plant grown under 6000 ppm salinity stress and sprayed with 300 ppm ascorbic acid.

Details: l ep, lower epidermis; mid b, midvein bundle; mid r, midvein region; spo, spongy tissue; pal, palisade tissue; t, trichome and u ep, upper epidermis.

Therefore, it could be stated that ascorbic acid had the ability to counteract the harmful effect of salinity stress on anatomical structure of basil leaf. Such treatment caused small decrease in thickness

of both midvein and lamina of leaves of salinized plant by 3.1 and 10.9 % less than the control due to decrements in most of the included tissues of leaf blade. Thickness of palisade and spongy tissues, width of midvein bundle as well as number of xylem rows/midvein bundle were slightly decreased by 5.7, 9.5, 5.6 and 5.5 % less than the control ; respectively. The beneficial effect of such treatment was also appeared by increasing length of midvein bundle by 5.0 % more than the control and vessel diameter by 11.0 % over the control.

Worthy to mention that the values of all tissues in leaves of salinized plants which were sprayed with ascorbic acid were decidedly higher over those of salinized plants. The thickness of midvein and lamina was increased by 13.2 and 24.1 % ; respectively over those of salinized plant due to increments in all included tissues by 1.4, 55.7, 10.7, 32.0, 46.5 and 38.9 % for thickness of palisade and spongy tissues, length and width of midvein bundle, number of xylem rows/midvein bundle and vessel diameter; respectively.

As far as the authors are aware, information concerning the effect of foliar spray with ascorbic acid on the anatomical structure of leaves of basil plants grown under salinity stress are not available in the literature.

Conclusion

From the above mentioned results, it could be concluded that foliar spray with ascorbic acid at concentration of 300 ppm had the ability to counteract the harmful effect of salinity stress on growth, fresh herb productivity, Photosynthetic pigments, volatile oil composition and leaf anatomy of basil plant and induced significant recovery for the reduction induced in vegetative growth and herb productivity as well as improved the concentrations of photosynthetic pigments, enhanced volatile oil composition and induced favorable changes in anatomical structure of basil leaves.

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