Effect of Water Stress, Ascorbic Acid and Spraying Time on Some Morphological and Biochemical Composition of *Ocimum basilicum plant.*

[1]Soha E. Khalil, [2] Nahed G. Abd El- Aziz and [1]Bedour H. Abou Leila

[1]Departments of Water Relation and Field Irrigation, National Research Centre, Dokki, Cairo, Egypt
[2]Department of Ornamental and Woody Trees, National Research Centre, Dokki, Cairo, Egypt
Soha_khalil2001@yahoo.com

Abstract: Pot experiments were conducted to study the effect of different soil moisture levels (30, 50 and 70% depletion of available soil moisture), different concentrations of ascorbic acid (0"sprayed with distilled water", 100, 150 and 200 ppm) and two spraying time (at vegetative or vegetative plus flowering stages) on some morphological and biochemical characteristics of basil plant. The experiments were conducted in a split-split plot design with 24 treatments in the greenhouse of National Research Centre. The results of statically analysis showed that Plant height, number of branches, number of leaves, leaf area, RWC % ,fresh and dry weights of the first cut showed significant increases under 50% soil moisture level. While in the second cut, the previously mentioned characters plus photosynthetic pigments showed progressive increases with increasing soil moisture levels as to reach their maximum values under 30% depletion of the available soil water. Reveres trend observed for oil% and proline content. The data also indicated that the application of ascorbic acid in different concentrations showed significant increases in all growth parameters, fresh and dry weights, relative water content, oil % and photosynthetic pigments compared with control treatment and revealed decrease in proline accumulation. [Journal of American Science. 2010;6(12):33-44]. (ISSN: 1545-1003).

Keywords: water stress; ascorbic acid; spraying time; biochemical composition; *Ocimum basilicum*

1. Introduction

Water stress is the most influential factors affecting crop yield particularly in irrigated agriculture in arid and semi arid regions, it is necessary to get maximum yield in agriculture by using available water in order to get maximum profit form per unit area because existing agricultural land and irrigation water are rapidly diminishing due to rapid industrialization and urban development. Optimizing irrigation management due to water scarcity together with appropriate crops for cultivation is highly in demand; the cost of irrigation pumping and inadequate irrigation scheme capacity as well as limited water sources is among the reasons that force many countries to reduce irrigation applications. Potential of water stress tolerance and the economical value of medicinal and aromatic plants, make them suitable alternative crops in dry lands (Ghanbari *et al.*, 2007).

Ocimum basilicum plant is one of the most important aromatic plants which used to flavor foods and in traditional medicines (Yusuf *et al.*, 1994). In aromatic plants, growth and essential oil production are influenced by various environmental factors, such as water stress (Burbott and Loomis, 1969). Efforts are being made to overcome this problem primarily by studding the tolerance of different plants to water stress or by using hormones, chemical and physical treatments as well as biological methods (El Saidi, 1997). Water stress is one of the important limiting factors of plant growth that has limited the production of 25% of world lands (Levitt, 1980). Solinas and Deiana (1996) reported that secondary products of plants can be altered by environmental factors and water stress is the major factor affecting the synthesis of natural products. Water stress resulted in significant reduction of fresh and dry matter, nutrient content and essential oil yield (Mirsa and Strivastov, 2000). Fresh and dry weights of *Ocimum bacilicum* L. were decreased as plant water deficit increased (Simon *et al.*, 1992). The linalool and methyl chavicol contents of sweet basil as percentage of total essential oil increased as water stress increased (Simon *et al.*, 1992) the essential oil yield of basil was increased by subjecting plants to water stress just before harvesting (Baeck *et al.*, 2001). Essential oil, total carbohydrates and proline contents were pronouncedly increased with increasing stress levels of *Salivia officinalis* L. (sage) plants (Hendawy and khalid, 2005) Moreover, Ashraf and Foolad (2007) reported that osomoprotectants such as proline and glycine betaine were increased under drought stress. Also, Tawfik (2008) indicated that osmoprotectants such as total soluble sugars, proline and glycine betaine increased in plants subjected to water stress.

Ascorbic acid is one of the water soluble reductants which is very important antioxidant which

protect plants by suppressing oxidative injury, by affecting many enzymes activities and also is required for regeneration of x-tocopheral (Smirnoff, 1995). Ascorbate occurs in the cell wall where it is a first line of defense against ozone; Ascorbate also has been implicated in regulation of cell division and photosynthesis. Ascorbate has benefits for human nutrition and possibly for tolerance of plants to photo oxidative stresses (Foyer *et al.*, 1993; Smirnoff, 1995 and Abou-Leila 1994).

Therefore, we aim in this investigation to study the effect of water stress, ascorbic acid concentrations and spraying time on vegetative growth, essential oil % and chemical content of *Ocimum basilicum* which is economically important plant in Egypt.

2. Material and Methods

The experiment was conducted during the two successive seasons of 2008 and 2009 in the greenhouse of National Research Centre (NRC), Giza, Egypt. Seeds of *Ocimum basilicum* c.v. Thai Magic were sown in the second week of April in plastic pots (30 cm diameter), each pot was filled with 10 kg of air dried soil, physical and chemical properties of the soil used are presented in Table (1) using the standard method described by Klute (1986).

Seeds of *Ocimum basilicum* c.v. Thai Magic were provided by the Department of Medicinal and Aromatic plants, Ministry of Agriculture, Giza, Egypt. ocimum seeds were irrigated regularly with tap water for three weeks until seedling emergency, then seedlings were thinned to two plants per pot, all pots received recommended does of NPK fertilizers.

The experiment including 24 treatments which were the combination between three levels of soil moisture (30, 50, and 70 % depletion of available soil water) and four concentrations of ascorbic acid spray (0"sprayed with distilled water", 100, 150 and 200 ppm.) which sprayed two times (at the vegetative stage or at vegetative plus flowering stages). The treatments arranged in a split- split plot design with three replicates, water stress was assigned at random in the main plots, while sub-plots were devoted to ascorbic acid concentrations and spraying time were allotted in the sub-sub plots. All pots were weighed daily and the needed amount of water was added. All plants received the soil moisture levels after three weeks from planting. The different concentrations of ascorbic acid were added for the first group at the vegetative stage only; the addition was twice, the first after 35 days from planting and the other two weeks later. While, for the second group the addition of ascorbic acid was at vegetative plus flowering stages (four times) where the third addition was applied at the begging of flowering stage and the fourth two weeks later. The spraying process was foliar and always performed early in the morning; the plants were sprayed until run off.

The plants were harvested two times (first and second cuttings) by cutting plants 5 cm above the soil with three replications in each season, the first cut on the first week of July and the second cut on the first week of October. The growth parameters which recorded for each cut were plant height (cm), number of leaves/plant, number of branches/plant, leaf area (cm^2) and fresh and dry weights (g) of herb yield. The amount of chlorophyll (a, b, a+b and carotenoids) was determined according to Metzener *et al*., (1965).

The fresh plants were collected from each treatment during the first and second cuttings and weighed to extract the essential oil, the fresh plant material from each replicate of all treatments was subjected to steam distillation for 3h using petroleum ether, which was removed carefully and the essential oil was obtained according to (Guenther, 1961). Proline was determined in dry leaves in the first and second cuts using the method of Troll (1995). The relative water content was also measured according to Weatherly (1962). The averages of data from two seasons were tested by analysis of variance according to (Snedecor and Cochran 1980) and the means separations were compared by using Least Significant Difference (LSD) at 5% level.

Table (1): Mechanical and chemical analyses of the soil used during the experiment.

Mechanical characteristics	first season	second season
Clay %	17.00	16.00
Sand %	23.75	25.25
Chemical Properties		
PH (1:2.5)	7.25	7.9
E.C. (1:5)	1.1 dsm-1	1.0
Available macro nutrients (ppm)		
Na	3.22	5.01
N	169.10	172
P	3.04	4.00
K	242.25	244.15
Ca	62.15	65.21
Mg	63.18	65.22
Available micro nutrient (ppm)		
Fe	12.14	15.21
Mn	18.81	19.32
Zn	1.18	1.34
Cu	1.00	1.31
Cl	0.58	0.66
Soil Texture	Sandy	Sandy

3. Results

Effect on growth criteria:

Data presented in Tables 2&3 revealed in the first cut that all growth characters increased significantly so as to reach their maximum values mostly under 50% soil moisture level followed by decrease under 30% depletion of the available soil

moisture level. While for the second cut, the data showed mostly progressive increase in growth criteria with increasing soil moisture level so as to reach their maximum values under 30% depletion of the available soil moisture level. Moreover, all growth criteria were significantly reduced under the highest stressed level (70% depletion of the available soil moisture level) in both cuts.

Data in the same tables revealed also that plants treated with 150 or 100 ppm ascorbic acid showed the highest significant increases in growth criteria of the first cut compared with control treatment, where the difference between the two concentrations was insignificant except for plant height. While in the second cut, increasing ascorbic acid concentration showed gradual increases in growth criteria compared with control treatment.

Spraying plants with ascorbic acid at the vegetative stage only showed the highest significant means of growth criteria in the first cut. While in the second cut, spraying plants with ascorbic acid at vegetative plus flowering stages revealed the highest significant means of growth criteria compared with the other treatment.

In addition, the data of the interaction between water stress and ascorbic acid concentrations indicated that the highest significant increases in growth criteria of the first cut (except for number of branches/plant which revealed insignificant increases) observed mostly under 50% soil moisture level interacted with 100 or 150 ppm ascorbic acid where the difference between the two treatments was insignificant. While in the second cut, the highest significant means observed under 30% depletion of the available soil moisture level interacted with 200 ppm ascorbic acid except for plant height.

The data of interaction between water stress and spraying time showed mostly that plants grown under 50% soil moisture level and sprayed with ascorbic acid at the vegetative stage only revealed the highest significant means in the first cut. While, plants grown under 30% depletion of the available soil moisture level and sprayed with ascorbic acid at vegetative plus flowering stages showed the highest significant means in the second cut.

Also, the data of interaction between ascorbic acid concentrations and spraying time revealed in the first cut that the highest significant means observed in plants sprayed with 150 or 100 ppm ascorbic acid (where the difference between the two concentrations was insignificant) at the vegetative stage only. As for the second cut, spraying plants with 150 or 200 ppm ascorbic acid at vegetative plus flowering stages showed the highest significant means of growth criteria compared with the other treatments, where the difference between the two concentrations was mostly insignificant.

The interaction between the three studied factors showed that the highest significant means in growth criteria of the first cut observed mostly when plants grown under 50% soil moisture level and sprayed with 100 ppm (followed by 150 ppm under the same level) ascorbic acid at the vegetative stage only. While for the second cut, the highest significant means observed in plants grown under 30% depletion of the available soil moisture level and sprayed with 200 ppm ascorbic acid at vegetative plus flowering stages except for plant height.

Table (2): Effect of water stress, ascorbic acid concentrations, spraying time and their interactions on growth criteria of *Ocimum basilicum* plant in the first cut (combined analysis of two seasons).

Charact. / Treatments		Plant height (cm)	No of leaves /plant	No of branches /plant	Fresh weight /plant (g)	Dry weight /plant (g)	Leaf area (cm^2)
Water stress							
70%		37.58	71.75	2.46	28.63	7.26	1.06
50%		44.38	83.50	3.38	39.58	10.78	1.44
30%		42.42	79.33	3.08	40.08	10.72	1.40
LSD0.05		1.46	1.37	0.73	2.47	0.66	0.18
Ascorbic acid concentrations							
0		36.35	60.25	2.16	26.39	6.67	1.05
100ppm		42.44	88.56	3.56	41.12	11.13	1.32
150ppm		45.61	92.28	3.44	40.93	11.07	1.49
200ppm		41.44	71.67	2.72	35.96	9.49	1.34
LSD0.05		1.36	4.20	0.71	2.73	0.74	0.18
Spraying time							
Vegetative		43.94	84.08	3.19	39.22	10.37	1.62
Vegetative+Flowering		38.98	72.31	2.75	32.98	8.80	0.99
LSD0.05		0.61	2.10	0.23	1.48	0.41	0.08
W.S	ASC	Water stress X Ascorbic acid con.					
70%	0	31.01	56.11	1.97	20.94	5.03	0.91
	100	33.50	79.50	2.33	33.71	8.75	0.91
	150	44.17	95.17	3.00	31.47	8.06	1.24
	200	41.67	56.17	2.50	28.41	7.14	1.18

50%	0	37.67	59.83	2.00	23.38	5.78	1.09
	100	47.33	99.67	4.83	41.12	13.63	1.72
	150	47.67	98.50	4.17	42.03	13.88	1.61
	200	42.17	76.00	2.50	42.34	9.88	1.36
30%	0	40.33	64.83	2.50	34.84	9.10	1.15
	100	46.50	86.50	3.50	48.54	11.00	1.33
	150	45.00	83.17	3.17	49.28	11.32	1.61
	200	40.50	82.83	3.17	37.14	11.45	1.49
LSD0.05		2.36	7.27	N.S	4.73	1.28	0.32
W.S	Spraying time	Water stress X Spraying time					
70%	Vegetative	39.42	71.33	2.58	30.56	7.62	1.41
	Veg.+Flow.	35.71	72.17	2.33	26.71	6.90	0.72
50%	Vegetative	46.42	95.17	3.83	46.00	12.62	1.79
	Veg.+Flow.	42.33	71.83	2.92	33.16	8.95	1.10
30%	Vegetative	46.00	85.75	3.17	41.10	10.87	1.65
	Veg.+Flow.	38.83	72.92	3.00	39.07	10.57	1.14
LSD0.05		1.06	3.63	0.40	2.57	0.70	0.14

Cont. Table 2.

Charact. / Treatments			Plant height (cm)	No of leaves /plant	No of branches /plant	Fresh weight /plant (g)	Dry weight /plant (g)	Leaf area (cm^2)
ASC	Spraying time		Ascorbic acid conc. X Spraying time					
0	Vegetative		36.34	60.27	2.16	26.40	6.67	1.05
	Veg.+Flow.		36.34	60.27	2.16	26.40	6.67	1.05
100	Vegetative		44.00	97.22	4.22	45.25	12.27	1.64
	Veg.+Flow.		40.89	79.88	2.89	36.99	9.99	1.00
150	Vegetative		47.33	101.22	3.89	44.14	11.63	1.82
	Veg.+Flow.		43.89	83.33	3.00	37.71	10.52	1.16
200	Vegetative		45.67	81.33	2.56	40.28	10.65	1.66
	Veg.+Flow.		37.22	62.00	2.89	31.64	8.33	1.02
LSD0.05			1.23	4.19	0.46	2.97	0.81	0.17
W.S	ASC	Spraying time	Water stress X Ascorbic acid con. X Spraying time					
70%	0	Vegetative	31.01	53.46	2.30	18.57	4.29	0.49
		Veg.+Flow	31.01	53.46	2.30	18.57	4.29	0.49
	100	Vegetative	34.33	83.67	2.33	37.56	9.65	1.24
		Veg.+Flow	32.67	75.33	2.33	29.86	7.85	0.59
	150	Vegetative	45.67	102.33	2.33	34.40	8.52	1.60
		Veg.+Flow	42.67	88.00	2.67	28.55	7.59	0.88
	200	Vegetative	46.00	57.33	2.67	29.27	7.26	1.44
		Veg.+Flow	37.33	55.00	2.33	27.55	7.03	0.91
50%	0	Vegetative	40.34	63.00	2.00	25.15	6.16	1.35
		Veg.+Flow	40.34	63.00	2.00	25.15	6.16	1.35
	100	Vegetative	48.33	115.00	4.67	56.66	16.15	2.01
		Veg.+Flow	47.67	84.67	3.33	45.02	11.11	1.35
	150	Vegetative	50.00	112.33	6.33	53.53	14.86	2.08
		Veg.+Flow	47.00	84.33	3.67	40.42	12.83	1.20
	200	Vegetative	45.67	97.33	2.33	48.68	13.30	1.71
		Veg.+Flow	38.67	54.67	2.67	25.59	6.45	1.00
30%	0	Vegetative	37.67	64.33	2.67	35.45	9.57	1.33
		Veg.+Flow	37.67	64.33	2.67	35.45	9.57	1.33
	100	Vegetative	47.00	101.33	4.00	41.53	11.00	1.62
		Veg.+Flow	43.00	71.67	3.00	40.71	11.00	1.04
	150	Vegetative	48.00	89.00	3.67	44.49	11.50	1.84
		Veg.+Flow	42.00	77.33	2.67	39.56	11.13	1.38
	200	Vegetative	45.33	89.33	3.67	42.91	11.51	1.83
		Veg.+Flow	35.67	76.33	2.67	41.78	11.39	1.15
LSD0.05			2.13	7.26	0.79	5.14	1.41	0.29

Table 3: Effect of water stress, ascorbic acid concentrations, spraying time and their interactions on growth criteria of *Ocimum basilicum* plant in the second cut (combined analysis of two seasons)

Charact. / Treatments		Plant height (cm)	No of leaves /plant	No of branches /plant	Fresh weight /plant (g)	Dry weight /plant (g)	Leaf area (cm^2)
Water stress							
70%		37.19	218.41	7.04	68.82	13.97	3.57
50%		53.65	382.08	9.50	92.75	21.81	4.77
30%		53.37	465.25	10.79	101.57	25.32	5.16
LSD0.05		0.59	22.56	1.50	12.04	2.27	0.19
Ascorbic acid concentrations							
0		41.87	274.83	6.78	73.72	16.04	3.96
100ppm		48.44	358.33	9.00	88.09	20.21	4.42
150ppm		50.98	384.00	9.94	92.46	22.29	4.60
200ppm		50.99	403.83	10.72	96.57	22.91	5.02
LSD0.05		2.18	19.63	0.81	3.61	1.63	0.13
Spraying time							
vegetative		42.21	306.56	7.61	73.35	15.51	4.30
veg.+f lowering		53.93	403.94	10.61	102.07	25.22	4.70
LSD0.05		1.23	14.59	0.75	2.80	1.21	0.09
W.S	ASC	Water stress X Ascorbic acid conc.					
70%	0	31.25	189.83	5.17	61.41	11.98	3.34
	100	39.15	206.50	7.00	66.70	13.62	3.57
	150	40.48	222.17	7.67	68.10	12.47	3.69
	200	37.87	255.17	8.33	79.07	17.80	3.69
50%	0	50.33	303.00	7.33	76.33	17.15	4.14
	100	51.25	324.17	9.67	90.43	20.99	4.64
	150	54.27	351.33	9.00	88.91	20.75	4.44
	200	58.23	549.83	12.00	115.31	28.34	5.66
30%	0	44.02	331.67	7.83	83.42	18.99	4.40
	100	54.92	544.33	10.33	107.14	26.03	5.06
	150	58.77	406.50	11.83	95.33	22.61	5.54
	200	56.30	578.50	13.17	120.38	33.65	5.84
LSD0.05		3.79	34.00	1.40	6.25	2.83	0.22
W.S	Spraying time	Water stress X Spraying time					
70%	vegetative	33.80	202.00	5.75	49.11	9.55	3.49
	veg.+Flowering	40.58	234.83	8.33	88.53	18.38	3.66
50%	vegetative	49.22	351.00	8.25	83.94	18.07	4.51
	veg.+Flowering	58.09	413.17	10.75	101.55	25.55	5.02
30%	vegetative	48.76	366.67	8.83	87.01	18.90	4.91
	veg.+Flowering	57.98	563.83	12.75	116.13	31.73	5.42
LSD0.05		2.13	25.27	1.29	4.86	2.09	0.16

Cont. Table 3.

Charact. / Treatments			Plant height (cm)	No of leaves /plant	No of branches /plant	Fresh weight /plant (g)	Dry weight /plant (g)	Leaf area (cm2)
ASC	Spraying time		Ascorbic acid conc.X Spraying time					
0	vegetative		41.87	274.84	6.78	73.73	16.04	3.96
	veg.+Flowering		41.87	274.84	6.78	73.73	16.04	3.96
100	vegetative		44.88	299.33	7.56	75.43	15.96	4.26
	veg.+Flowering		52.00	417.33	10.44	100.74	24.46	4.59
150	vegetative		43.67	313.89	7.89	74.00	15.76	4.48
	veg.+Flowering		58.32	454.11	12.00	110.93	28.81	4.78
200	vegetative		47.87	373.44	9.22	83.74	18.18	4.72
	veg.+Flowering		54.09	434.22	12.22	109.40	27.65	5.27
LSD0.05			2.46	29.17	1.50	5.61	2.42	0.19
W.S	ASC	Spraying time	Water stress X Ascorbic acid conc. X Spraying time					
70%	0	vegetative	30.61	94.13	5.33	61.28	6.14	3.38
		veg.+Flowering	30.61	94.13	5.33	61.28	6.14	3.38
	100	vegetative	36.30	195.00	6.33	47.46	9.06	3.53
		veg.+Flowering	42.00	218.00	7.67	85.93	18.17	3.61
	150	vegetative	28.67	201.00	5.67	50.23	9.84	3.51
		veg.+Flowering	52.30	243.33	9.67	85.97	15.09	3.88
	200	vegetative	40.73	232.33	7.00	58.21	11.61	3.68
		veg.+Flowering	35.00	278.00	9.67	99.93	23.98	3.71

50%	0	vegetative	47.67	350.67	7.00	65.75	20.00	4.00
		veg.+Flowering	47.67	350.67	7.00	86.90	20.00	4.00
	100	vegetative	48.00	292.67	8.00	87.59	18.74	4.35
		veg.+Flowering	54.50	355.67	11.33	93.57	23.23	4.93
	150	vegetative	49.67	298.00	7.00	78.36	16.53	4.15
		veg.+Flowering	58.87	404.67	11.00	99.47	24.94	4.73
	200	vegetative	51.53	558.00	11.33	104.06	23.64	5.54
		veg.+Flowering	66.00	541.67	12.67	126.57	33.04	5.54
30%	0	vegetative	47.33	379.67	8.00	74.44	22.00	4.50
		veg.+Flowering	47.33	379.67	8.00	92.40	22.00	4.50
	100	vegetative	50.33	410.33	8.33	91.24	20.07	4.90
		veg.+Flowering	59.50	678.33	12.33	123.03	31.98	5.22
	150	vegetative	52.67	442.67	11.00	93.40	20.92	5.78
		veg.+Flowering	63.80	483.00	14.33	101.70	25.93	5.97
	200	vegetative	51.33	330.00	9.33	88.95	19.28	5.11
		veg.+Flowering	61.27	714.33	15.33	147.37	46.37	6.14
LSD0.05			4.26	50.53	2.59	9.71	4.19	0.33

2- Effect on some physiological process:
Photosynthetic pigments content:

Data presented in Tables 4 & 5 showed that the concentration of photosynthetic pigments i.e. chla, chlb and total chl (a+b) as well as carotenoids was increased significantly by increasing soil moisture water in both cuts.

The data in the same tables indicated also that plants sprayed with ascorbic acid showed significant increases in photosynthetic pigments content compared with control ones in both cuts. For the first cut, increasing ascorbic acid conc. above 100 ppm caused significant decrease in photosynthetic pigments compared with 100 ppm (except for Chl.b which increased with increasing ascorbic acid conc.). While in the second cut, increasing ascorbic acid conc. caused significant increases in chla, chlb and total chl (a+b), as for carotenoids the data revealed decrease in its content with increasing ascorbic acid conc. above 100 ppm.

It could be also noticed that spraying plants with ascorbic acid at the vegetative stage only during plant's life of the first cut revealed the highest significant means in chla, chlb, chl (a+b) as well as carotenoids. Furthermore, spraying plants with ascorbic acid at vegetative plus flowering stages showed the highest significant means in photosynthetic pigments of the second cut.

Concerning the effect of interaction between water stress and ascorbic acid concentrations, the data demonstrated that plants grown under 30% soil moisture level and sprayed with 150 ppm ascorbic acid showed mostly the highest significant increases in photosynthetic pigments of the first cut and 30% soil moisture level interacted with 200 ppm ascorbic acid for the second cut, except for carotenoides.

Also, the data of interaction between water stress and spraying time showed that the highest significant increases in photosynthetic pigments recorded in plants grown under 30% soil moisture level in both cuts, spayed with ascorbic acid at the vegetative stage only for the first cut and sprayed with ascorbic acid at vegetative plus flowering stages for the second cut.

Irrespective to water stress, the data revealed that the best records in photosynthetic pigments of the first cut obtained mostly in plants sprayed with 100 or 150 ppm ascorbic acid at the vegetative stage only. While for the second cut, the highest significant means in photosynthetic pigments obtained mostly in plants prayed with 200 ppm at vegetative plus flowering stages (except for carotenoids).

The data of tri-interaction indicated that the best treatment for the first cut observed mostly when plants grown under 30% soil moisture level and sprayed with 150 ppm ascorbic acid at the vegetative stage only. While, the best records for the second cut obtained mostly when plants grown under 30% soil moisture level and sprayed with 200 ppm ascorbic acid at vegetative plus flowering stages.

Relative Water Content (RWC %):

Data in Tables 4 & 5 also revealed that the highest RWC % records for the basil leaves obtained under 50% depletion of the available soil moisture in the first cut and 30% depletion of the available soil moisture for the second cut.

The obtained data also indicated that all ascorbic acid concentrations showed significant increase in RWC% compared with untreated plants in both cuts. Where the highest significant increase in RWC% obtained in plants sprayed with 100 ppm ascorbic acid in the first cut and 200 ppm in the second cut.

For the effect of spraying time, the data revealed that spraying plants with ascorbic acid at vegetative plus flowering stages revealed the highest means in RWC% in both cuts.

Regarding the effect of interaction between water stress and ascorbic acid concentrations, the data illustrated that the highest significant means of RWC% in the first cut obtained under 50% soil moisture level

combined with 100 or 150 ppm ascorbic acid where the difference between the two concentrations was insignificant, and 30% depletion of the available soil moisture combined with 200 ppm ascorbic acid for the second cut. It was also clear from data that spraying plants with different concentrations of ascorbic acid caused increase in RWC % under different soil moisture levels and with significant difference.

The data of interaction between water stress and spraying time revealed that plants grown under 50% soil moisture level and sprayed with ascorbic acid at the vegetative plus flowering stages showed the highest significant means of RWC % in the first cut and under 30% depletion of the available soil moisture and sprayed with ascorbic acid at the vegetative plus flowering stages in the second cut.

Irrespective to water stress, the data illustrated that spraying plant twice with 100 ppm ascorbic acid at the vegetative plus flowering stages proved to be effective in increasing RWC% significantly in the first cut. While for the second cut, spraying plants with 200 ppm at vegetative and flowering stage revealed the highest significant means of RWC % in the second cut.

The combined effect between the three studied factors indicated in the first cut that the highest significant values of RWC% attained when plants grown under 50% soil moisture level and sprayed with 100 ppm ascorbic acid at the vegetative plus flowering stages. While for the second cut, the highest significant means observed in plants grown under 30% depletion of the available soil moisture level and sprayed with 200 ppm ascorbic acid at the vegetative and flowering stages.

Proline content:

Examination of data in Tables 4 & 5 showed that increasing water stress level caused progressive and significant increase in proline content of basil leaves in both cuts.

Treated basil plants with different concentrations of ascorbic acid revealed significant decrease in proline accumulation compared with untreated plants. For both cuts, the highest means observed under the control treatment, where the lowest mean in the first cut obtained under 150 ppm ascorbic acid, while for the second cut the difference between the different concentrations of ascorbic acid was insignificant.

Spraying plants with ascorbic acid at the vegetative stage only revealed the lower means in proline accumulation in both cuts, compared with the other treatment.

Concerning the bi-interaction between water stress and ascorbic acid concentrations, the obtained data revealed that the different concentrations of ascorbic acid caused significant decrease in proline accumulation under different water stress levels compared with control plants. Furthermore, the highest significant increase in proline accumulation obtained in control treatment of 70% depletion of the available soil moisture level, while the lowest accumulation obtained under 30% depletion of the available soil moisture level, these results were true for both cuts.

The data of interaction between water stress and spraying time also proved that plants sprayed with ascorbic acid at the vegetative stage only revealed the lowest means in proline accumulation under different soil moisture levels compared with the other treatments, this result was true for both cuts.

It could be also observed from the data of interaction between ascorbic acid concentration and spraying time that the highest accumulation of proline obtained mostly in untreated plants (control plants) in both cuts, while the lowest accumulation obtained when plants treated with different concentrations of ascorbic acid at the vegetative stage only.

The effect of tri-interaction illustrated in both cuts that the lowest significant means in proline accumulation obtained when plants grown under 30% depletion of the available soil moisture level and sprayed with 150 or 100 ppm ascorbic acid (where the difference between the two concentrations was insignificant) at the vegetative stage only, while the highest means obtained in control plants of 70% depletion of the available soil moisture level.

Oil percent:

The results in Tables 4 & 5 showed in both cuts that water stress induced significant and progressive increase in oil % of basil leaves, where the highest significant increase in oil % of both cuts obtained in plants grown under 70 % depletion of the available soil moisture level.

Ascorbic acid treatments caused significant increase in oil % compared with untreated plants in both cuts, where 100 ppm ascorbic acid proved to be the most effective concentration that affected oil % significantly in both cuts.

A significant increase was also recorded when plants treated with ascorbic acid twice at vegetative and flowering stages in both cuts.

In addition, the interaction between water stress and ascorbic acid concentrations indicated that oil % increased significantly with increasing stress levels, also spraying plants with ascorbic acid induced significant increase in oil % under different soil moisture levels, where the highest significant increase in oil % appeared in plants grown under 70% depletion of the available soil moisture and sprayed with 100 ppm ascorbic acid in both cuts.

For the interaction between water stress and spraying time the data in both cuts illustrated that the highest significant means of oil % obtained in plants grown under 70% depletion of the available soil moisture and sprayed with ascorbic acid at vegetative and flowering stages.

Moreover, the data of interaction between ascorbic acid concentrations and spraying time showed that the highest significant means of oil % in both cuts appeared when plants sprayed twice (at vegetative and flowering stages) with 100 ppm.

The effect of tri-interaction indicated that 70% depletion of the available soil moisture level interacted with 100 ppm ascorbic acid when sprayed twice at both vegetative and flowering stages proved to be the most effective treatment in oil % compared with the other treatments and with significant difference, this result was true for both cut.

Table (4): Effect of water stress, ascorbic acid concentrations, spraying time and their interactions on some physiological process of *Ocimum basilicum* plant in the first cut (combined analysis of two seasons).

Charact. / Treatments		Photosynthetic pigments				RWC %	Proline content	Oil %
		Chl.a	Chl.b	Chl.a+b	Carot.			
Water stress								
70%		1.61	1.57	3.15	0.49	55.29	0.18	0.22
50%		2.15	1.95	4.15	0.99	67.95	0.16	0.13
30%		2.58	2.31	4.89	1.53	63.07	0.13	0.07
$LSD_{0.05}$		0.05	0.11	0.16	0.04	1.76	0.04	0.003
Ascorbic acid concentrations								
0		1.41	1.59	2.95	0.73	58.40	0.18	0.08
100ppm		2.63	2.02	4.70	1.25	66.79	0.15	0.20
150ppm		2.52	2.04	4.55	1.13	65.46	0.14	0.16
200ppm		1.90	2.14	4.04	0.91	61.17	0.15	0.12
$LSD_{0.05}$		0.03	0.07	0.11	0.03	1.01	0.03	0.002
Spraying time								
Vegetative stage		2.22	2.17	4.15	1.03	61.81	0.15	0.13
Veg.+flowering stages		2.02	1.72	3.97	0.98	64.29	0.16	0.15
$LSD_{0.05}$		0.02	0.05	0.08	0.01	0.49	0.01	0.001
WS.	ASC.	Water stress X Ascorbic acid conc						
70 %	0	1.33	1.25	2.42	0.36	54.67	0.21	0.11
	100	2.01	1.62	3.64	0.69	56.46	0.18	0.32
	150	1.77	1.71	3.48	0.50	57.12	0.16	0.25
	200	1.34	1.71	3.05	0.42	52.91	0.16	0.21
50 %	0	1.33	1.50	2.83	0.82	61.48	0.17	0.07
	100	2.83	2.00	5.00	1.18	73.57	0.15	0.21
	150	2.46	2.01	4.47	1.00	71.94	0.15	0.15
	200	2.00	2.30	4.29	0.95	64.80	0.15	0.08
30 %	0	1.58	2.02	3.60	1.01	59.06	0.15	0.06
	100	3.05	2.43	5.48	1.88	70.34	0.12	0.08
	150	3.32	2.39	5.71	1.91	67.31	0.11	0.09
	200	2.37	2.40	4.77	1.35	65.81	0.13	0.06
$LSD_{0.05}$		0.05	0.13	0.20	0.05	1.75	0.05	0.003
WS.	ASC.	Water stress X Spraying time						
70 %	Vegetative	1.50	1.63	3.27	0.63	57.37	0.17	0.21
	Veg.+flow.	1.72	1.52	3.02	0.36	53.22	0.18	0.24
50 %	Vegetative	2.31	2.18	4.19	0.69	64.25	0.14	0.10
	Veg.+flow.	2.00	1.72	4.11	1.29	71.65	0.17	0.15
30 %	Vegetative	2.86	2.70	5.00	1.77	63.25	0.13	0.08
	Veg.+flow.	2.29	1.91	4.78	1.29	68.02	0.13	0.07
$LSD_{0.05}$		0.04	0.09	0.14	0.07	0.85	0.07	0.004

Cont. Table (4).

Charact. / Treatments		Photosynthetic pigments				RWC %	Proline content	Oil %
		Chl.a	Chl.b	Chl.a+b	Carot.			
ASC.	Spraying time	Ascorbic acid conc. X Spraying time						
0	Vegetative	1.41	1.59	3.00	0.73	58.42	0.17	0.09
	Veg.+flow.	1.41	1.59	3.00	0.73	58.42	0.17	0.09
100	Vegetative	3.31	2.05	5.36	1.16	65.60	0.13	0.17
	Veg.+flow.	1.95	1.98	3.93	1.34	67.98	0.17	0.24
150	Vegetative	2.42	2.42	4.84	1.36	66.17	0.13	0.16
	Veg.+flow.	2.61	1.65	4.26	0.91	64.75	0.15	0.16
200	Vegetative	1.95	2.46	4.41	0.79	60.04	0.15	0.09
	Veg.+flow.	1.85	1.81	3.66	1.02	62.31	0.15	0.14
$LSD_{0.05}$		0.04	0.11	0.16	0.06	0.99	0.06	0.006

W.S	ASC.	Spraying time	Water stress X Ascorbic acid conc. X Spraying time						
70%	0	Vegetative	0.92	1.25	2.17	0.40	50.67	0.20	0.15
		Veg.+flow.	0.92	1.25	2.17	0.40	50.67	0.20	0.15
	100	vegetative	2.31	1.53	3.84	0.62	58.25	0.16	0.24
		veg.+flow.	1.72	1.71	3.43	0.77	54.68	0.20	0.40
	150	vegetative	1.31	1.95	3.26	0.77	58.98	0.15	0.26
		veg.+flow.	2.22	1.48	3.70	0.22	55.27	0.16	0.24
	200	vegetative	1.21	1.72	2.93	0.60	53.79	0.17	0.18
		veg.+flow.	1.48	1.70	3.18	0.24	52.03	0.15	0.24
50%	0	Vegetative	1.30	1.50	2.80	0.80	60.00	0.20	0.07
		Veg.+flow.	1.30	1.50	2.80	0.80	60.00	0.20	0.07
	100	vegetative	3.78	1.80	5.58	0.83	71.40	0.13	0.16
		veg.+flow.	1.88	2.20	4.08	1.53	75.73	0.18	0.25
	150	vegetative	2.03	2.49	4.52	0.46	72.23	0.15	0.11
		veg.+flow.	2.89	1.53	4.42	1.53	71.04	0.14	0.18
	200	vegetative	2.24	2.76	5.00	0.66	61.04	0.13	0.05
		veg.+flow.	2.75	1.83	4.58	1.24	68.57	0.17	0.11
30%	0	Vegetative	1.50	2.00	3.50	1.00	64.88	0.13	0.05
		Veg.+flow.	1.50	2.00	3.50	1.00	64.88	0.13	0.05
	100	vegetative	3.85	2.62	6.47	2.03	67.15	0.11	0.10
		veg.+flow.	2.25	2.24	4.49	1.72	73.54	0.12	0.06
	150	vegetative	3.92	2.83	6.75	2.83	67.30	0.10	0.10
		veg.+flow.	2.71	1.95	4.66	0.98	67.32	0.13	0.07
	200	vegetative	2.40	2.89	5.29	1.11	65.29	0.14	0.05
		veg.+flow.	2.33	1.92	4.25	1.58	66.33	0.13	0.07
$LSD_{0.05}$			0.08	0.19	0.27	0.09	1.71	0.09	0.008

Table (5): Effect of water stress, ascorbic acid concentrations, spraying time and their interactions on some physiological process of *Ocimum basilicum* plant in the second cut (combined analysis of two seasons)

Charact. / Treatments		Photosynthetic pigments				RWC %	Proline content	Oil %
		Chl.a	Chl.b	Chl.a+b	Carot.			
Water stress								
70%		0.50	0.29	0.79	0.29	58.73	0.17	0.26
50%		0.67	0.38	1.01	0.38	67.64	0.12	0.16
30%		0.96	0.53	1.49	0.49	70.23	0.12	0.11
$LSD_{0.05}$		0.03	0.10	0.13	0.04	1.51	0.03	0.04
Ascorbic acid concentrations								
0		0.63	0.34	0.97	0.35	53.04	0.16	0.13
100ppm		0.70	0.42	1.12	0.41	67.82	0.13	0.21
150ppm		0.73	0.40	1.13	0.40	70.18	0.13	0.19
200ppm		0.78	0.44	1.16	0.39	71.08	0.13	0.18
$LSD_{0.05}$		0.02	0.07	0.11	0.03	0.97	0.02	0.03
Spraying time								
Vegetative stage		0.43	0.25	0.68	0.27	64.67	0.11	0.15
Veg.+flowering stages		0.99	0.55	1.52	0.50	66.40	0.17	0.20
$LSD_{0.05}$		0.02	0.07	0.07	0.01	0.65	0.01	0.01
WS	ASC.	Water stress X Ascorbic acid conc.						
70 %	0	0.45	0.32	0.78	0.27	47.11	0.19	0.22
	100	0.47	0.31	0.78	0.29	67.03	0.16	0.30
	150	0.49	0.20	0.69	0.28	60.43	0.15	0.27
	200	0.60	0.33	0.93	0.32	60.35	0.16	0.24
50 %	0	0.60	0.21	0.81	0.29	56.37	0.15	0.10
	100	0.69	0.42	1.11	0.43	66.49	0.12	0.19
	150	0.71	0.45	1.15	0.42	73.70	0.12	0.21
	200	0.68	0.44	0.96	0.40	73.99	0.12	0.16
30 %	0	0.85	0.48	1.33	0.50	55.64	0.14	0.07
	100	0.94	0.55	1.49	0.51	69.95	0.11	0.14
	150	1.00	0.54	1.54	0.50	76.40	0.11	0.10
	200	1.06	0.55	1.61	0.44	78.91	0.13	0.14
$LSD_{0.05}$		0.04	0.13	0.20	0.05	1.68	0.03	0.05
WS.	Spraying time	Water stress X Spraying time						
70 %	Vegetative	0.27	0.20	0.47	0.16	55.44	0.11	0.22
	Veg.+flow.	0.73	0.38	1.11	0.42	62.02	0.22	0.29
50 %	Vegetative	0.37	0.19	0.56	0.30	69.44	0.10	0.14
	Veg.+flow.	0.97	0.57	1.45	0.47	65.84	0.14	0.19
30 %	Vegetative	0.65	0.35	0.99	0.35	69.13	0.10	0.09
	Veg.+flow.	1.28	0.71	1.99	0.62	71.33	0.14	0.12
$LSD_{0.05}$		0.03	0.13	0.13	0.07	1.12	0.04	0.07

Cont. Table (5).

Treatments \ Charact.			Photosynthetic pigments				RWC %	Proline content	Oil %
			Chl.a	Chl.b	Chl.a+b	Carot.			
ASC	Spraying time		Ascorbic acid X Spraying time						
0	Vegetative		0.64	0.34	0.98	0.35	53.04	0.16	0.13
	Veg.+flow		0.64	0.34	0.98	0.35	53.04	0.16	0.13
100	Vegetative		0.43	0.20	0.63	0.30	67.78	0.10	0.17
	Veg.+flow		0.97	0.65	1.62	0.54	67.87	0.15	0.24
150	Vegetative		0.51	0.26	0.77	0.26	71.59	0.10	0.16
	Veg.+flow		0.94	0.54	1.48	0.52	68.65	0.15	0.21
200	Vegetative		0.41	0.27	0.69	0.31	70.58	0.10	0.15
	Veg.+flow		1.15	0.60	1.64	0.46	71.71	0.16	0.21
$LSD_{0.05}$			0.03	0.15	0.15	0.06	1.30	0.03	0.06
W.S	ASC.	Spraying time	Water stress X Ascorbic acid X Spraying time						
70%	0	Vegetative	0.42	0.24	0.66	0.17	50.95	0.18	0.21
		Veg.+flow	0.42	0.24	0.66	0.17	50.95	0.18	0.21
	100	vegetative	0.24	0.10	0.34	0.16	67.72	0.10	0.26
		veg.+flow	0.70	0.51	1.21	0.42	66.34	0.22	0.33
	150	vegetative	0.31	0.16	0.47	0.10	59.71	0.12	0.23
		veg.+flow	0.66	0.24	0.90	0.45	61.15	0.19	0.30
	200	vegetative	0.28	0.15	0.44	0.25	54.69	0.11	0.20
		veg.+flow	0.91	0.51	1.43	0.39	66.00	0.21	0.29
50%	0	Vegetative	0.50	0.20	0.70	0.40	53.16	0.14	0.10
		Veg.+flow	0.50	0.20	0.70	0.40	53.16	0.14	0.10
	100	vegetative	0.42	0.19	0.61	0.38	67.72	0.11	0.16
		veg.+flow	0.96	0.64	1.60	0.49	65.26	0.13	0.21
	150	vegetative	0.45	0.27	0.72	0.34	78.53	0.10	0.17
		veg.+flow	0.96	0.62	1.59	0.49	68.88	0.14	0.24
	200	vegetative	0.32	0.21	0.53	0.32	78.34	0.10	0.14
		veg.+flow	1.04	0.67	1.38	0.47	69.65	0.13	0.19
30%	0	Vegetative	1.00	0.60	1.60	0.50	55.03	0.13	0.07
		Veg.+flow	1.00	0.60	1.60	0.50	55.03	0.13	0.07
	100	vegetative	0.62	0.31	0.93	0.37	67.89	0.10	0.10
		veg.+flow	1.26	0.78	2.04	0.64	72.01	0.12	0.17
	150	vegetative	0.78	0.34	1.12	0.33	76.88	0.09	0.09
		veg.+flow	1.21	0.74	1.95	0.52	75.93	0.12	0.10
	200	vegetative	0.63	0.46	1.10	0.35	78.71	0.10	0.12
		veg.+flow	1.49	0.63	2.11	0.67	79.12	0.15	0.15
$LSD_{0.05}$			0.05	0.26	0.25	0.09	1.30	0.08	0.09

4. Discussion:

From the results, it was clear that the highest water stress level (70% depletion of the available soil moisture level) caused an observed adverse action on growth characters, fresh and dry weights, relative water content % as well as photosynthetic pigments of basil plants in both cuts. Previous results were supported by Fatima *et al.*, (1999); Mirsa and Strivastava (2000); Khalid (2006) and Tawfik (2008). This result could be due to that one of the first signs of water shortage was the decrease of turgor which resulted in decrease in growth and development of cell especially in stem and leaves (Alishah *et al.*, 2006). When the leave level decreased the plant lose less water through transpiration so the restriction of leaves level could be the first mechanism against drought (Levitt, 1980). Farooqi *et al.*, (1998) and Fatima *et al.*, (1999) supported previous results. Moreover, when the leaf level decrease the light attraction decrease and the total capacity of photosynthesis decrease so plant growth became less and plant performance decrease (Hsiao, 1973), which leads also to the decrease in dry matter production (Cox and Joliff, 1987), this result agrees with Fatima *et al.* (1999); khalid (2006) and Alishah *et al.*,(2006). Drought stress made chloroplast break down and the amount of chlorophyll decrease, therefore formation of chlorophyll a, b and carotenoids decrease. Our finding was in harmony with Cox and Jolliff (1987); Begum and Apaul (1993); Sepehri and Modarres (2003) and Alishah *et al.*, (2006). Furthermore, proline content showed significant increase with increasing water stress level and these results agree with Blum and Ebercon (1976) whom indicated that proline is regarded as a source of energy, carbon, and nitrogen for recovering tissue, so it increased under water shortage, Aspinal and Paleg (1981) stated that under water deficit condition the concentration of amino acid proline increase. Since chlorophyll and proline are both synthesis from the same substance therefore the increase in synthesis of proline leads to the decrease in synthesis of chlorophyll under drought conditions. Bajji *et al*, (2001); Begum and Paul (1993); Irigoyen *et al.*, (1992); khalid (2006) and Tawfik (2008) reached the same conclusions.

Moreover, the essential oil percentage was increased significantly with increasing water stress level; these results were in line with those of Sabih *et al*., (1999); Baher *et al*., (2002) and Khalid (2006). Our results also indicated difference between results of two cuts, since the results of the first cut revealed that 50% soil moisture level showed the highest records in growth parameters, fresh and dry weights and RWC %. While in the second cut, the data revealed progressive decrease in previously mentioned characters (plus photosynthetic pigments) with increasing stress levels which may due to that plants in the second cut exposed to higher temperature degrees than plants in the first cut because of summer season which revealed increase in the amounts of water that loss through transpiration and evapotranspiration and increasing needs for more water.

Water stress causes various physiological and biological changes in plants, one of which is the accumulation of reactive oxygen species in the cell, the reactive oxygen radicals are toxic and may result in a series of injuries to plant metabolism, it damages photosynthetic components, inactivates protein and enzymes, destroys cell membrane structure and permeability by causing lipid peroxidation, also excess accumulation of reactive oxygen species results in a series of oxidative injuries to plant prolines, polysacchorides and nucleic acids (Price and Henry, 1987, 1989, 1991, Winston, 1990), as a result normal cell metabolism can be seriously disturbed. The results of the present study indicated that ascorbic acid reduced the harmful effects of reactive oxygen species and improved plant resistance to water stress. In brief, ascorbic acid treatment reduced the damaging action of drought and decreased enzyme activity due to scavenging of reactive oxygen species; thereupon it may be effective for improvement of stressed plants in arid and semi-arid regions (Dolatabadian *et al*, 2009).

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