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Response of Spearmint Plants Grown under Sandy Soils Condition to some Growth Stimulators

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Abstract: The present study was carried out at Ali Mubarak Experimental Farm of South Tahrir, Horticulture Research Station, El-Bostan Area, EL-Behira Governorate, during the two successive seasons of 2013 and 2014 to study the response of spearmint plants grown under sandy soil condition to some growth stimulators ,i.e (Salicylic acid, Ascorbic acid and Vitamin E) growth characters (plant height ,fresh and dry weights)were determined .Maximum plant height, fresh and dry weights ,essential oil content& composition were obtained with ascorbic acid at 400ppm .Anatomical study for stem and leaves were also carried out. As for the effect of foliar application with ascorbic acid at 400 ppm on anatomical structure of vegetative growth of sprearmint plants it could be stated that such treatment increased stem diameter. spraying ascorbic acid at 400 ppm increased thickness of both midvein and lamina of leaf blades**.** The main vascular bundle of the midvein increased in size , also increased the mean diameter of vessel.

1. Introduction

Spearmint (*Mentha arvensis* L.) plant belongs to the family *lamiaceae* is a small to moderate sized perennial herb .Mint is one of the most important herbs produced in Egypt. Spearmint oil has a long history of safe use both in medicinal preparation and as flavoring agent in foods and confectionery[1].Carminative , anti-spasmodic ,anti-peptic ulcer agent to treat indigestion ,skin diseases ,coughs and cold in folk medicine .Antioxidant activity of spearmint plants have been reported[2].

Ascorbic acid is one of the water soluble antioxidant which decreases the harmful effect of oxidative enzymes activities on plants[3]. Ascorbate occurs in the cell wall, it is a first line of defense against a zone, Ascorbate is very important in regulation of cell division and photosynthesis[4]. Plant cells are required to remove of free radicals to preserve the plants form acidities injury. Ascorbic acid also plays role in the removal of active oxygen free radicals[5].

Salicylic acid (SA) occurs naturally in plants, which identified as an important element involved in establishing the local and systematic disease resistance response of plants after pathogen attack[6]. Salicylic acid and ascorbic acid effectively enhanced mint productivity, and can reduce the use of chemical fertilizers and conserve natural resources[7].

Vitamin E is a strong antioxidant and assists in transport of electrons in photosystem- II protein complex[8].

Thus, the present investigation is an attempt to bring to more information about the effect of spraying salicylic acid, ascorbic acid and vitamin E on growth, anatomical structure, essential oil content and yield of mint plant.

Materials and Methods

The present work was carried out at the sandy soil of the Experimental Farm of South Tahrir Horticulture Research Station , at Ali Mubarak village , El-Bostan Area , EL-Behira Governorate, during the two successive seasons of 2013 and 2014. Rooted soil runners (rhizomes) of spearmint (*Mentha arvensis* L.*)* with at least 3 buds (10 –15 cm in length) were obtained from a private nursery, then transplanted in the experimental area on March 15th in both seasons under drip irrigation system The plot area was 3*4 m with 4 rows 60cm a part and the seedlings were distanced at 25 cm (48 plant / plot).The physical and chemical analysis the experimental soil is shown in table (1)

Table (1): Physical and chemical analysis of the experimental soil

Soluble cations (meg./100gm soil)	**Soluble cations**	**Sandy soil**		
			1st season	**2nd season**
		Ca+2	0.64	0.65
		Mg+2	0.30	0.32
		Na+	0.38	0.41
		K+	0.03	0.03
	Soluble Anions	Co3-	……..	…….
		Hco3-	0.54	0.50
		Cl-	0.38	0.39
		So4-	0.40	0.40
PH			8	7
E.C.(ds/m)			0.26	0.26
Sand%			93	92
Silt%			2.70	2.73
Clay%			2.92	2.90
Texture Class			Sandy	Sandy

This experiment included 10 treatments as follows:

1. Control (untreated).
2. Salicylic acid at 50 ppm.
3. Salicylic acid at100 ppm.
4. Salicylic acid at 200 ppm.
5. Ascorbic acid at 100 ppm.
6. Ascorbic acid at 200 ppm.
7. Ascorbic acid at 400ppm.
8. Vitamin E at 50ppm.
9. Vitamin E at 100 ppm.
10. Vitamin E at 200 ppm.

Treatments were arranged in a complete randomized block design with three replicates.

Plants were sprayed at 45, 60 days from transplanting and 45,60 days after the first cut .Two cuts were harvested, the first one was on July 15th and the second one was on October 15th during both seasons and the vegetative parameters were taken as follows : plant height (cm) , fresh weight (g/plant) and dry weight (g/plant).

Essential oil extraction

Essential oil was isolated by hydro-distillation using a Clevenger type apparatus according to[9] and essential oil percentage was calculated on dry weight basis and the essential oil yield was thus calculated by multiplication of herb weight (g) x oil (%). The essential oil was dried with anhydrous sodium sulphate and subjected to gas chromatographic analysis.

Anatomical Studies:

A comparative microscopically examination on plant material was carried out to show the most prominent response of plant growth to the investigated treatments. Specimens of spearmint were taken from the median internode of the second branch as well as from the middle part of the third leaf developed on the second terminal branch. After 90 days of planting were taken through the second season for anatomical examination. Specimens were killed and fixed for at least 48 hr. in FAA (10 ml formalin, 5 ml glacial acetic acid and 85 ml ethyl alcohol 70%). The selected materials washed in 50% ethyl alcohol, dehydrated in normal butyl alcohol series, embedded in paraffin wax of 56oC melting point, sectioned to a thickness of 20 microns, double stained with safranin and fast green, cleared in xylene and mounted in Canada balsam[10]. Sections were examined to detected histological manifestations of the chosen treatments.

Statistical Analysis

Data of this experiment were statistically analyzed by using MSTAT statistical software and the treatments means were compared by using LSD at 0.5 level of probability according to[11].

Results and Discussion

Growth characteristics:

Data presented in Table (2) showed the effect of salicylic acid, ascorbic acid and vitamin E on plant height, fresh weight and dry weight of spearmint plants.

1. Plant height:

Data reveal that, all concentrations of salicylic acid, ascorbic acid and Vitamin E increased plant height in the first cut and second cut in both seasons compared to control . Vitamin E at 200 ppm gave the tallest plants in the first cut in both seasons i.e (46.00 cm and 44.60cm) respectively compared to the other treatments. Ascorbic acid at 400 ppm was more effective in the second cut on both seasons i.e (54.66 cm and 59.33 cm) respectively .The minimum value obtained with ascorbic acid at 100 ppm in the first cut on both seasons i.e (37.34 cm -36.00 cm) respectively and with vit E at100 ppm in the second cut on both seasons (46.00 cm-46.00 cm) respectively .Similar finding were obtained by [12,13] they found that foliar application of ascorbic acid 300 ppm enhanced most of pepper plant growth parameters.

2. Fresh weight:

Data presented in Table (2) indicated that, spraying spearmint plants with salicylic acid and ascorbic acid with different concentrations increased fresh weight throughout the experimental period of plant growth in the first cut and second cut of the two seasons compared to control. Maximum fresh weight was obtained with ascorbic acid at 400 ppm in the first and second cuts in both seasons i.e (59.13 gm and 247.00 gm) ,(65.52 gm and 235.83 gm), respectively .In addition the obtained results are harmony with those[12,13] on pepper plants. Plants treated with ascorbic acid at 100 ppm resulted in the least value compared to the other treatments in two cuts for both seasons i.e (40.66 gm - 151.83 gm)-(43.93 gm and 135.60 gm) respectively. Similar results reported by [14] on pepper plants.

3. Dry weight:

Data in Table (2) showed that, spraying spearmint plants with all concentration salicylic acid, ascorbic acid and vitamin E were insignificantly increase dry weight in the first cut of both growing seasons. Maximum dry weight was obtained with ascorbic acid at 400 ppm in the second cut for both seasons i.e (76.70 gm and

70.50 gm), respectively. On the other hand, the minimum values recorded when plants treated plants with ascorbic acid at 100 ppm in the second cut of the two seasons (39.70 gm and 35.40 gm), respectively . The results is accordance with those obtained by on tomato[15] who found that foliar application of salicylataldehyde at 10^{-5}M stimulated different morphological and growth characteristics of tomato plants , while inhibitory effects were observed at 10^{-3} M. Also, [16]showed that, soybean plants treated with salicylic acid increased significantly the number of leaves and plant height. Salicylic acid increased plant height, number of branches per plant, leaf area, fresh and dry weight of herbs, total carbohydrates, crude protein, total amino acids, free proline, photosynthetic pigments of basil and marjoram plants[17]. Salicylic acid and ascorbic acid effectively improved mint productivity and can reduce the use of chemical fertilizers and conserve natural resources[7].

The same results obtained on the effect of ascorbic acid were obtained by[18] who found that, ascorbic acid at 100, 150 and 200 ppm showed significant increase all growth parameters i.e, fresh and dry weights and reduced the harmful effects of reactive oxygen species and improved plant resistance to water stress. In brief, ascorbic acid reduced the injury action of drought and decreased enzyme activity due to reactive oxygen species[19]. Ascorbic acid enhanced plant growth in colza plant, it can be due to removing active oxygen[20]. [21]found that, salicylic acid and vitamin E separately or in their combination increased number of branches and leaves per plant, also combination of salicylic acid at 50 ppm and vitamin E at 100 ppm gave the highest leaf area and dry weight per plant in tomato[22]. reported that foliar application with α- tocopherol (vitamin E) on *faba bean* plants induced increased in morphological characters and yield components.

Table (2): Effect of some growth stimulators on morphological characters of spearmint plants during 2013 and 2014 seasons

Treatments	Plant height (cm)				Fresh weight (g)				Dry weight(g)			
	First season		Second		First season		Second		First season		Second	
	1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut
Control	34.00	42.66	35.33	45.33	39.30	107.76	42.33	95.16	15.06	34.80	15.13	31.10
Salicylic acid 50 ppm	40.60	48.80	39.20	54.40	51.82	191.18	48.76	188.28	19.60	47.50	16.96	42.60
Salicylic acid 100 ppm	40.65	50.60	41.66	53.67	57.76	224.93	59.16	232.86	19.00	55.00	19.51	53.00
Salicylic acid 200 ppm	43.10	52.66	42.70	56.43	56.20	230.10	52.46	208.50	18.03	53.70	17.86	46.20
Ascorbic acid 100 ppm	37.34	48.33	36.00	47.63	40.66	151.83	43.93	135.60	15.33	39.70	15.86	35.40
Ascorbic acid 200 ppm	37.68	50.66	39.65	48.00	54.90	246.86	50.33	208.06	18.80	45.10	19.46	44.10
Ascorbic acid 400 ppm	42.32	54.66	41.30	59.33	59.13	247.00	65.52	235.83	18.93	76.70	25.90	70.50
Vitamin E 50 ppm	37.35	49.66	38.66	53.66	41.50	158.23	46.70	136.50	17.86	42.20	23.53	36.70
Vitamin E 100 ppm	40.80	46.00	38.66	46.00	39.40	165.70	52.60	163.80	16.70	42.80	25.70	38.80
Vitamin E 200 ppm	46.00	48.33	44.60	51.50	48.82	190.34	50.12	171.61	17.63	68.30	18.42	69.10
L.S.D	6.36	7.97	7.23	10.85	11.01	71.73	10.16	67.68	N.S	16.17	N.S	13.38

Essential oil percentage and yield

Data presented in Table (3) showed clearly the effect of salicylic acid, ascorbic acid and vit. E on oil percentage and essential oil yield. Higher essential oil percentage was obtained in first cut than that of the second cut in all treatments. The maximum mean values of essential oil percentage in 1st cut were obtained by

spraying spearmint plants with ascorbic acid at 400 ppm with values (1.21% and 1.11%) in the 1st and 2nd seasons respectively. These results ensure that foliar application with ascorbic acid at 400 ppm was the favorable treatment which recorded the highest values of oil percentage and oil yield per plant in 1st and 2nd cuts of both seasons. Foliar application of ascorbic acid caused increases in the percent and yield of essential oil of lemongrass. These results are in accordance with[23,24].

Essential oil composition

Effect of foliar spray with saliyclic acid, ascorbic acid and vit. E on essential oil composition are shown in Table (4), the GC., profile of the essential oil for all treatments showed seven compounds. It could be noticed that carvon represented the main constituent of spearmint oil, followed by P-cyemene, 1,8 cineale, Y-terpincne, β- caryophyllene, myrcene and β-pinene.

The main components were Carvone (52-73% to 68.38%), P-cyeme (9.12%-17.94%) ,1.8 cineole (5.79%-9.79%),y-terpine (0.79%-4.61%),

Table (3): Response of Spearmint plants growth under sandy soils conditions to some growth stimulators on essential oil and oil yield during the two seasons 2013 and 2014.

Treatments	Essential oil				Oil yield			
	First season		Second season		First season		Second season	
	1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut
Control	1.04	0.68	0.86	0.70	15.68	23.66	13.01	21.79
Salicylic acid 50 ppm	1.08	0.85	0.91	0.80	21.16	40.37	15.43	34.10
Salicylic acid 100 ppm	1.08	1.01	0.92	0.76	20.50	55.55	17.94	40.26
Salicylic acid 200 ppm	1.08	0.88	0.99	0.82	19.47	47.25	17.68	37.88
Ascorbic acid 100 ppm	1.02	0.97	0.94	0.76	15.63	38.51	14.90	26.90
Ascorbic acid 200 ppm	1.11	0.78	1.00	0.82	20.86	35.17	19.46	36.18
Ascorbic acid 400 ppm	1.21	1.10	1.11	0.95	22.97	84.32	28.74	66.97
Vitamin E 50 ppm	0.98	1.03	0.92	0.87	17.50	43.41	21.64	31.92
Vitamin E 100 ppm	1.02	0.95	0.96	0.90	17.04	40.67	24.67	34.50
Vitamin E 200 ppm	1.00	1.02	1.00	0.90	17.59	69.66	18.42	62.20
L.S.D	0.06	0.10	0.08	0.10	N.S	16.29	2.07	13.11

Table (4): Response of spearmint plants growth under sandy soils conditions to some growth stimulators on essential oil composition.

Characters	Carvone	P-cymene	1,8 cineole	Y-terpince	β-caryophylenc	Myrcene	β-pinene
Control	59.65	13.32	5.89	4.61	2.22	1.98	1.92
Salicylic acid 50 ppm	63.08	11.79	5.99	2.79	2.37	1.70	0.23
Salicylic acid 100 ppm	64.02	11.92	6.12	1.88	2.03	2.21	1.43
Salicylic acid 200 ppm	63.10	11.78	6.92	1.45	1.70	2.01	1.31
Ascorbic acid 100 ppm	63.64	12.31	6.92	1.65	1.96	2.02	1.54
Ascorbic acid 200 ppm	63.38	12.98	5.79	0.79	1.97	1.83	1.40
Ascorbic acid 400 ppm	68.38	9.12	6.15	0.86	2.01	1.48	0.95
Vit. E 50 ppm	61.20	13.23	8.86	0.80	2.05	2.46	1.16
Vit. E 100 ppm	52.73	17.94	9.79	0.93	2.69	2.95	2.01
Vit. E 200 ppm	57.83	12.33	9.37	0.76	2.47	2.00	0.86

B-caryophylene (1.70%-2.69%), Myrcene (1.48%-2.95%) and B- pinene (0.86%-2.01%), while other components were present in amounts less than 2 % .The main component in spearmint is carvon[2]. The same data in Table (4) show that spraying spearmint plants with ascorbic acid at the highest concentration (400ppm) recorded the highest values of carvone, whereas it was decrease p- cymene as compared to control . In addition, foliar application of Vit. E at100 ppm increase cineole 1.8 than other treatments, while, the maximum percentage of y- terpince (4.61%) was obtained by control. Moreover, the highest content of B-caryphylene was obtained when spraying plants with Vit. E at100 ppm. It could be suggested from the obtained results that spraying spearmint plants with salicylic acid at 400 ppm increased the essential oil components percentage as a result of the improvement on growth characters of treated plants.

Anatomical studies:

1-Anatomy of stem:

Microscopically measurements of certain histological characters of the median internode of the second branch of spearmint plant of control plants and sprayed with ascorbic acid at 400 ppm are given in Table (5). Likewise, microphotographs depict these treatments are shown in Fig. (1).

It is noted that foliar application with ascorbic acid at 400 ppm increased stem diameter by 16.4% more than the control. The increase in stem diameter due to foliar application with ascorbic acid at 400 ppm could be attributed to increase in phloem thickness, xylem thickness and pith thickness were 42.8%, 51.6% and 17.4% over the control, respectively. Vessel diameter was also increased by 50.2% over the control. On the other hand, foliar application with ascorbic acid did not effect on epidermis and cortex thickness. In this respect, [25]reported that, ascorbic acid at 400 ppm sprayed on Egyptian lupine cv. Giza 2, resulted in increasing stem diameter due to mainly to the increasing in thickness of stem wall and in diameter of hollow pith, increase in stem wall due to ncrease in thickness of epidermis, cortex, fiber strands, vascular tissue and parenchymatous area of pith.

Table (5): Effect of ascorbic acid at 400 ppm on stem anatomy of Spearmint plant at the age of 90 days. (Measurements in microns)

Characters	Control	Ascorbic acid at 400 ppm	± % to cont.
Stem diameter	1426.00	1660.60	+16.4
Epidermis thickness	18.40	18.40	-
Cortex thickness	55.20	55.20	-
Phloem thickness	42.90	61.30	+42.80
Xylem thickness	165.90	251.40	+51.60
Vessel diameter	24.50	36.80	+50.20
Pith thickness	809.70	950.60	+17.40

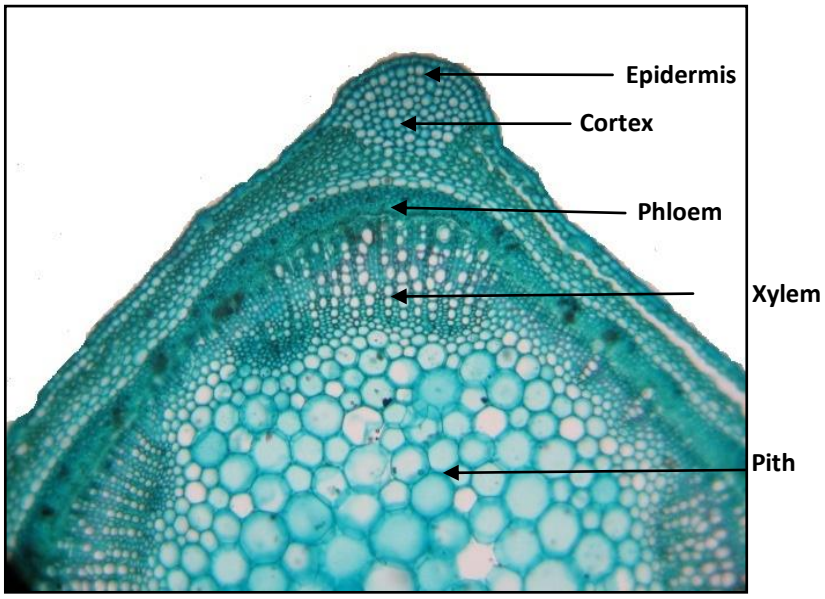
2-Anatomy of the leaf:

Microscopically measurements of certain characters in transverse sections through the middle part of the third leaf developed on the second terminal branch of spearmint plant of control and sprayed with ascorbic acid at 400 ppm are presented in Table (6). Likewise, microphotographs illustrating these treatments are shown in Fig. (2).

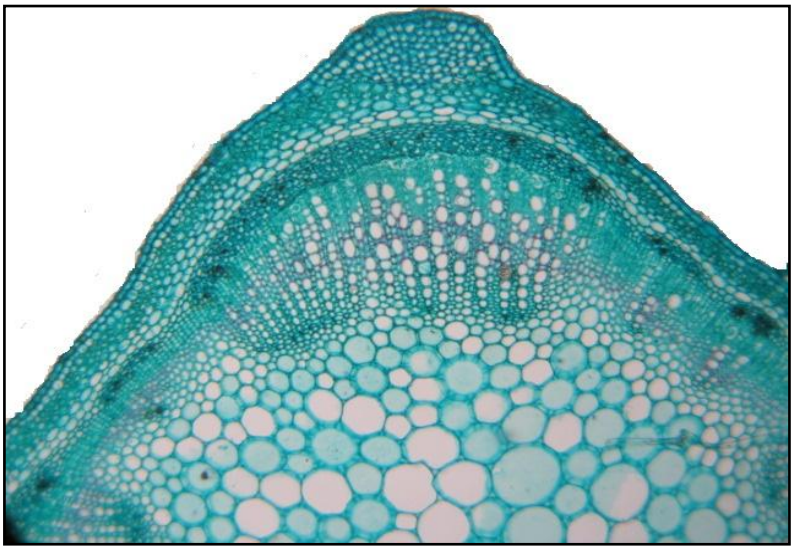
It is realized from Table (6) and Fig. (2) that sprayed with ascorbic acid at concentration of 400 ppm increase thickness of both midvein and lamina of leaf blades of spearmint cv. by 22% and 15.9% more than those of the control, respectively. It is noted that increase in lamina thickness which was induced due to spraying with ascorbic acid was accompanied with 50.7% and 29.1% increments in thickness of upper epidermis and spongy tissues compared with the control. The main vascular bundle of the midvein increased in size, the increment was mainly due to the increase in length by 66.7% and in width by 27.1% more than the control. The mean diameter of vessel was increased by 9.2% more than the control. Palisade tissue decreased by 5.2% below the control. These results are in accordance with, [25]found that, foliar application with ascorbic acid at 400 ppm on leaf of Egyptian lupine cv. Giza 2 , increased thickness of both midvein and lamina, also the main vascular bundle of the midvein increased in size. In this concern[26] who found that wider vessels have enhanced conductivity and this can increase xylem transport from the roots to the leaves. Also, [27]showed that change in vessels size have an important role in the adaptation to unfavorable environmental conditions.

Table (6): Effect of ascorbic acid at 400 ppm on leaf anatomy of spearmint plant at the age of 90 days (measurements in microns)

Characters	Control	Ascorbic acid at 400 ppm	± % to cont.
Thickness of midvein	452.80	552.70	+22.00
Thickness of lamina	166.50	193.10	+15.90
Thickness of palisade tissue	63.20	59.90	-5.20
Thickness of spongy tissue	79.90	103.20	+29.10
Dimensions of the main vascular buudle of midvein			
Length	104.80	183.10	+66.70
Width	253.00	939.50	+27.10
Vessels diameter	18.30	20.00	+9.20
Thickness of upper epidermis	13.20	19.90	+50.70
Thickness of lower epidermis	9.90	9.90	-

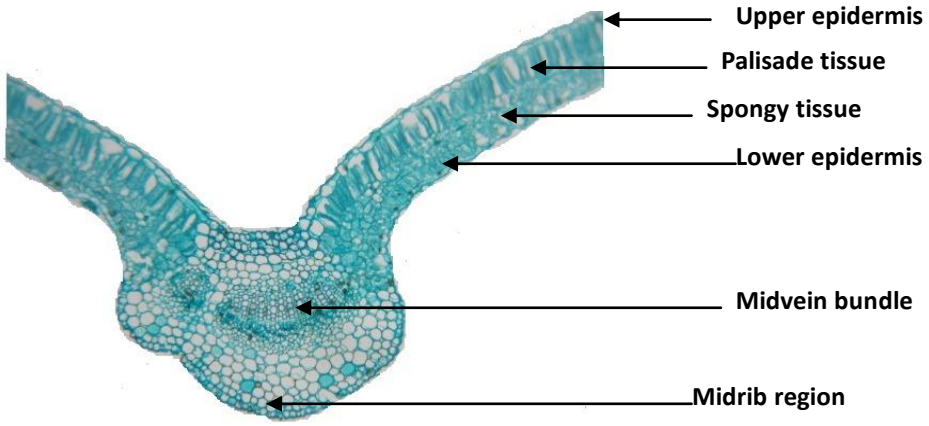


A



B

Fig. 1: Transverse sections through the median portion of the second branch of spearmint at the age of 90 days, as affected by foliar application with ascorbic acid. (x 40).
A- From untreated Plant (control). B- From Plant sprayed with 400 ascorbic acid.



A

B

Fig. 2 : Transverse sections through the midvein portion of the leaf blade developed on the second branch of spearmint at the age of 90 days, as affected by foliar application with ascorbic acid. (x 100).
A- From untreated Plant (control).
B- From Plant sprayed with 400 ascorbic acid.

Conclusion

From the previous results of this investigation, it could be concluded that spraying spearmint plants grown in summer season under sandy soil conditions with ascorbic acid at 400 ppm significantly enhanced plant growth, fresh weight, dry weight, essential oil percentage and yield, essential oil composition. Also, using the abovementioned treatment affected positively on stem and leaf structure.

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