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The Impact of Foliar Spray with Ascorbic Acid on Growth, Productivity, Anatomical Structure and Biochemical Constituents of Volatile and Fixed Oils of Basil Plant (*Ocimum basilicum* L.)

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ABSTRACT

The present investigation was carried out at the Agricultural Experiments and Researches Station, Faculty of Agriculture, Cairo University, Giza, Egypt during the two successive early summer growing seasons of 2015 and 2016 in order to study the effect of foliar spray with different concentrations of ascorbic acid (150, 300, 450 and 600 ppm) on morphological characters of vegetative growth (plant height, number of primary branches/plant, number of leaves /plant and total leaf area/plant), yield of fresh herb and seeds/plant, anatomical structures of the main stem and leaves and on percentages and composition of volatile and fixed oils of Basil plant (*Ocimum basilicum* L.). The obtained results revealed that ascorbic acid showed different effects on growth and productivity of Basil plant according to the tested concentration. Spraying Basil plants with 300 ppm ascorbic acid proved to be the most positive effective concentration with promoting vegetative growth, increased productivity from fresh herb and seeds/plant, induced favorable changes in anatomical structures of vegetative organs and increased percentages and improved quality of volatile and fixed oils of such important aromatic plant.

Key words: Ascorbic acid, *Ocimum basilicum* L., Growth, Productivity, Anatomy, Volatile oil, Fixed oil.

Introduction

Ocimum is one of the most important genera of the family Lamiaceae (often called Labiatae, the traditional name), native to India, Southern Asia and Middle East. Cultivated extensively in Southern, Central and Eastern Europe, North Africa and in the USA, particularly California (Kruger, 1992 and Singh and Panda, 2005). Economically, it is of great importance as a source of volatile aromatic oils, medicines and ornamentals. There are about 150 to 160 species in this genus broadly dispersed over the warm regions of the glob (Evans, 2001 and Kumar, 2009). They differ in growth habit, physiological appearance and chemical and aromatic composition. They grow in wide variety of soil and climatic conditions. All *Ocimum* species yielded essential oils which is responsible for the medicinal uses including antimicrobial, antioxidant, antifungal and anti-inflammatory activities (Nahak *et al*., 2011).

Ocimum basilicum L. (Sweet Basil or Basil) called Rehan in Arabic is one of the most important aromatic species in the genus *Ocimum* (Hugh, 2005) and chosen to be the subject of the present investigation because of its economic importance as an ornamental, spice, culinary and medicinal herb. It grow well in Egypt, the cultivated area is about 1091feddans (1 Feddan = 4200 m^2) produced about 1951 tons fresh herb per year and yielded about 29.263 tons essential oil (Arafa, 2007). Basil herb contains 0.5 - 1.5 % essential oil of varying composition out of which linalool as the main component (Hiltunen, 1999). The essential oil, obtained by steam distillation from the fresh herb, is used in perfumery, in making incense and in the food industry (Bunney, 1992). Basil seed containing fixed oil (reached to 22.5 %) that consists mainly of linoleic acid, linolenic acid and oleic acid (Hiltunen, 1999). Basil seed oil has some medicinal properties, it is useful in catarrh, chronic diarrhea, dysentery, gonorrhoea, nephritis, cystitis and internal piles (Panda, 2004 and Singh and Panda, 2005).

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 per unit area is highly recommended to meet the demand of human needs and exportation. In this concern, the use of antioxidants

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in promoting growth and increasing productivity of many plant species is highly recommended. These compounds have beneficial effect on catching the free radicals or the active oxygen (singlet oxygen, superoxide anion, hydrogen peroxide, hydroxyl radicals and ozone) that producing during photosynthesis and respiration processes (Zhang and Klessing, 1997). Leaving these free radicals without chelating or catching leads to lipids oxidation and the loss of plasma membrane permeability and the death of cell within plant tissues. Antioxidants have also an auxinic action. One of the most familiar antioxidants is ascorbic acid which being synthesized in higher plants and affects plant growth and development. It is a product of D-glucose metabolism which affects some nutritional cycles activity in higher plants and play an important role in the electron transport system (Givan, 1979).

Ascorbic acid, the subject of the present investigation, is currently considered to be a regulator on cell division and differentiation and is involved in a wide range of important functions as antioxidant defense, photoprotection and regulation of photosynthesis and growth (Bolkhina *et al*., 2003). 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 and senescence (Zhang, 2013).

As to the effect of foliar application with different concentrations of ascorbic acid on growth and productivity of Basil plant, El-Gamal (2005) found that ascorbic acid at concentration of 300 ppm have promotive effect on vegetative growth, photosynthetic pigments, herb productivity and essential oil percent of Basil plant grown either under normal conditions or grown under stress of salinity or drought. Likewise, Khalil *et al.* (2010) sprayed ascorbic acid at concentrations of 100,150 and 200 ppm on Basil plant, the obtained results indicated that all tested concentrations of ascorbic acid showed promotive effect on growth, herb fresh and dry weights, oil percentage and concentrations of photosynthetic pigments. Such effect was clear either Basil plants grown under normal conditions or grown under water deficit stress. The enhancement effect of ascorbic acid on vegetative growth, productivity and percentage of essential oil was also recorded on other medicinal and aromatic plants; for instance, Pundarikakshudu and Bhavsar (1991) on *Anethum sowa*, Reda *et al* (2007) on *Thymus vulgaris* L., Hendawy and Ezz El-Din (2010) on *Foeniculum vulgare* and Soltani *et al* (2013) on *Calendula officinalis*.

In this respect, many investigators reported that ascorbic acid application resulted in enhancement of growth, yield and chemical constituents of some different plant species especially field crops. Among of them, Rabie and Negm (1992) and Abdel-Messih and Eid (1999) on wheat, Zahran (1993) and Abdel-Aziz (1999) on lentil, Mahmoud (1994) and Nofal *et al*. (1996) on faba bean, Anton *et al*. (1999) and Abdo and El-Moselhy (2004) on barley, El-Kobisy *et al*. (2005) on pea, Nassar and Abdo (2009) on Egyptian lupine, Emam *et al*. (2011) and Nassar *et al*. (2016) on flax and Nassar (2013) on mungbean.

Therefore, the present investigation is an attempt to through to light more information about the effect of foliar application with different concentrations of ascorbic acid on vegetative growth, yield of fresh herb and seeds, stem and leaf anatomy as well as on percentages and composition of essential and fixed oils of Basil plant. This would be an effort to trace the beneficial effect for ascorbic acid on productivity of Basil, if any.

Materials and Methods

The present investigation was carried out at the Agricultural Experiments and Researches Station, Faculty of Agriculture, Cairo University, Giza, Egypt during the two successive early summer growing seasons of 2015 and 2016 in order to study the effect of foliar spray with different concentrations (150, 300, 450 and 600 ppm) of ascorbic acid on morphological characters of vegetative growth, yield of fresh herb and seeds/plant, anatomical structures of the main stem and leaves and on percentages and composition of essential and fixed oils of Basil plant (*Ocimum basilicum* L.).

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

Field work procedure:

Seeds of Basil were sown on 16th March, 2015 in the first season and replicated on 18th March, 2016 in the second one to provide the experimental plant materials. The experiment was made in a randomized complete block design with three replicates. The four levels of ascorbic acid beside the control required that the experimental land of each replicate be divided into five plots, each contain one treatment. The plot

was six ridges, four meters long, 60 cm apart. Seeds were sown in hills, spaced 20 cm. The plants were thinned to two plants per hill. All field practices were carried out as recommended for Basil crop in the vicinity.

The tested concentrations of ascorbic acid were applied twice. The first application at seven weeks from sowing date and the second application was done after three weeks from the first application. The control plants were sprayed with tap water, Tween-20 was added as a spreading agent for tested treatments. The volume of spraying solution per plot was almost 1.25 and 2.0 liters in the first and second application; respectively.

Recording of data:

I- Morphological characters of vegetative growth and yield of fresh herb per plant:

A random sample of 12 plants for each treatment (4 plants from each replicate) was assigned for investigation. Vegetative characters were recorded after 14 weeks from sowing date; *i.e.*, four weeks after second application of ascorbic acid. This age represents full blooming stage. The following characters were studied in both growing seasons.

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

II- Yield of seeds (g) per plant:

A random sample of 15 plants, for each treatment (5 plants from each replicate) was taken at harvest time (17 and 18 weeks from sowing date for first and second season; respectively) to determine yield of seeds (g) per plant.

III- Anatomical studies:

It was intended to carry out a comparative microscopical examination on plant material which showed the most positive response of plant growth to tested treatments with control. Tested materials included the main stem at its median portion and lamina of the corresponding leaf. Specimens were taken throughout the second growing season of 2016 at the age of 12 weeks from sowing date; *i.e.*, two weeks from the second application of ascorbic acid. Specimens were killed and fixed for at least 48 hours in F.A.A. (10 ml formalin, 5 ml glacial acetic acid and 85 ml ethyl alcohol 70%). The selected materials were washed in 50% ethyl alcohol, dehydrated in a normal butyl alcohol series, embedded in paraffin wax of melting point 56°C, sectioned to a thickness of 20 micro-meter (µm) double stained with crystal violet-erythrosin, cleared in xylene and mounted in Canada balsam (Nassar and El-Sahhar, 1998). Sections were read to detect histological manifestations of the noticeable responses resulted from spraying with 300 ppm ascorbic acid (the most positive effect concentration in this investigation) compared to control and photomicrographed.

IV- Chemical analysis:

a- Determination of percentage and constituents of volatile oil:

A chemical analysis was carried out to gain information about the effect of foliar spray with ascorbic acid on the percentage and composition of volatile oil of Basil herb at full blooming stage of the second growing season 2016 (the age of 14 weeks from sowing date). 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 one hour 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.

b- Determination of percentage and constituents of fixed oil:

A chemical analysis was carried out to gain information about the effect of foliar spray with ascorbic acid on the percentage and composition of fixed oil of Basil seed at harvest time of the second season 2016.

The method of AOAC (2000) was conducted for lipid extraction from Basil seeds using chloroform methanol (2 : 1 V /V) to extract the lipids. The associated non-lipids were removed by washing lipid extract three times with CH_3OH : H_2O (1 : 1 V/V). The lipids in chloroform were dried over anhydrous sodium sulfate, then the solvent was removed by heating at 60˚C under vacuum. The lipid samples were saponified over night with ethanoic KoH (20%) at room temperature. The fatty acids were freed from their potassium salts by acidification with hydrochloric acid (5N), followed by extraction with ether (or Pt. ether 40-60 ˚C), the ether extract was washed three times with distilled water then dried over anhydrous sodium sulfate, and filtered off (Vogel, 1975).

GC-MS technique was used to separate and detect the fixed 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- FAME capillary column (30 m × 0.25 mm; film thickness 0.25 µm). The oven temperature was programmed from 80°-230°C at 3°C/min. Helium was used as carrier gas at a flow rate of 1.5 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 200°C.

Qualitative identification of the different constituents was performed by comparison of their relative retention times and mass spectra with those of authentic reference compounds (fatty acid methyl esters, purity 98% by GC). Also, probability merge search software and the NIST MS spectra search program were used.

Statistical analysis:

Data on morphological characters and yield of fresh herb and seeds per plant 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

I- Morphological characters of vegetative growth:

Results of morphological characters of Basil plant as influenced by foliar application with various concentrations of ascorbic acid are given in Table (1). The investigated morphological characters included plant height, number of primary branches/plant, number of leaves/plant and total leaf area/plant at full blooming stage (age of 14 weeks from sowing date).

Data presented in Table (1) clearly show that sprayed ascorbic acid with any of the two first used concentrations of 150 and 300 ppm induced significant increase in all studied morphological characters of vegetative growth. The maximum significant promotion in vegetative growth characters was detected when Basil plants were sprayed with 300 ppm ascorbic acid (Fig. 1), being 20.1, 28.8, 30.0 and 31.5% more than the untreated plants for plant height, number of primary branches/plant, number of leaves/plant and total leaf area/plant; respectively. Worthy to mention that the difference between the two promotive

concentrations of ascorbic acid (150 and 300 ppm) proved significant. At the same time, the third sprayed concentration of 450 ppm ascorbic acid showed no significant effect in this respect.

Table 1: Morphological characters of vegetative growth and yield of fresh herb / plant at full blooming stage (age of 14 weeks from sowing date) and yield of seeds/ plant at harvest time (17 weeks from sowing date) of *Ocimum basilicum* L. as affected by foliar spray with various concentrations of ascorbic acid (average of the two seasons, 2015 and 2016 combined)

Treatments	Conc. (ppm)	Morphological characters of vegetative growth				Yield of fresh herb (g)/plant	Yield of seeds (g)/plant
		Plant height (cm)	No. of primary branches/plant	No. of leaves/plant	Total leaf area (cm^2)/plant		
Control	0	76.3 C	12.82 C	286.4 C	4324.6 C	465.11 C	27.19 C
Ascorbic acid	150	84.2 B	14.93 B	329.7 B	4996.2 B	549.72 B	34.26 B
	300	**91.6 A**	**16.51 A**	**372.3 A**	**5684.5 A**	**605.26 A**	**38.39 A**
	450	77.2 C	13.27 C	294.6 BC	4451.4 C	479.71 C	28.27 C
	600	64.9 D	11.14 D	247.2 D	3693.1 D	400.93 D	23.22 D
L.S.D. (0.05)		6.68	1.22	35.9	502.3	51.84	2.97

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

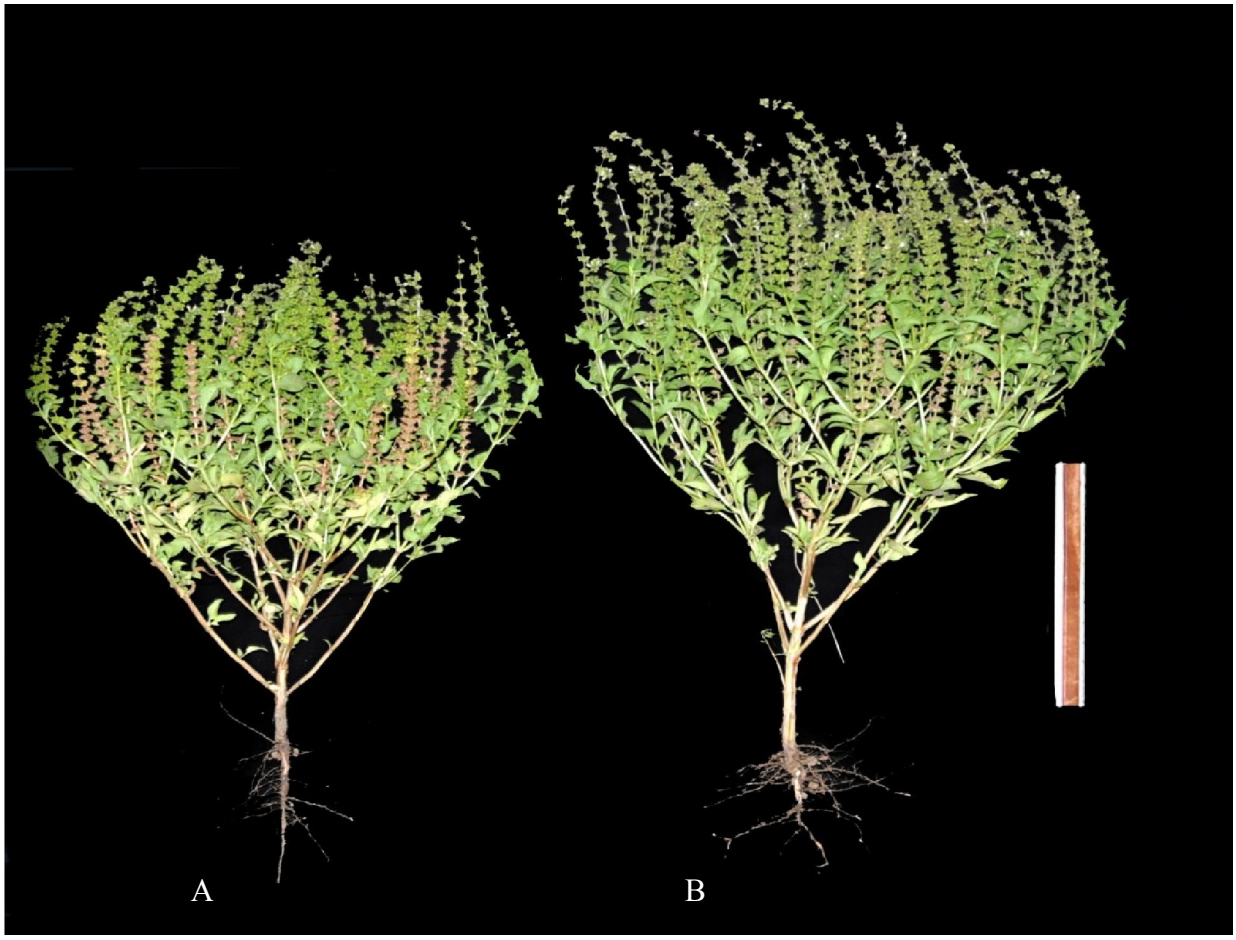

Fig. 1: Habit of mature plants, at the end of full blooming stage, of Basil as affected by foliar application with ascorbic acid.
A- Control plant. B- Plant sprayed with 300 ppm ascorbic acid.

By contrast, the relatively high used concentration of 600 ppm ascorbic acid induced significant decrease in all studied characters of vegetative growth of Basil plant. The decrements below the control were 14.9, 13.1, 13.7 and 14.6% for plant height, number of primary branches/plant, number of leaves/plant and total leaf area/plant; respectively.

Claims to the promotive effect of ascorbic acid on vegetative growth characters of Basil plant, El-Gamal (2005) using 300 ppm ascorbic acid as well as Khalil *et al.* (2010) using 150 and 200 ppm ascorbic acid stated that ascorbic acid showed promotive effect on vegetative growth characters of Basil plant; *i.e.*, increasing plant height, number of branches/plant, number of leaves/plant and leaf area, being in good

agreement with the present findings. The enhancement effect of ascorbic acid on vegetative growth was also recorded on other medicinal and aromatic plants; for instance, Pundarikakshudu and Bhavsar (1991) on *Anethum sowa*, Reda *et al.* (2007) on *Thymus vulgaris*, Hendawy and Ezz-El-Din (2010) on *Foeniculum vulgare* and Soltan *et al.* (2013) on *Calendula officinalis*. In this respect, many investigators reported that ascorbic acid application resulted in enhancement of growth of some other different plant species. Among of them, Rabie and Negm (1992) and Abdel Messih and Eid (1999) on wheat, Zahran (1993) and Abdel-Aziz (1999) on lentil, Mahmoud (1994) and Nofal *et al.* (1996) on faba bean, Anton *et al.* (1999) and Abdo and El-Moselhy (2004) on barley, El-Kobisy *et al.* (2005) on pea, Nassar and Abdo (2009) on Egyptian lupine, Emam *et al.* (2011) and Nassar *et al.* (2016) on flax and Nassar (2013) on mungbean. All, being in accordance with the present findings. Worthy to note that the promotive effect of ascorbic acid on growth of different plant species depending on tested concentration, plant species used as genotype, time of application and recommended practices applied for the used genotype.

II- Yield of fresh herb/plant:

Data concerning yield of fresh herb per Basil plant at full blooming stage as affected by foliar application with different concentrations of ascorbic acid are shown in Table (1).

It is obvious from Table (1) that foliar application with ascorbic acid at any of the two first assigned concentrations of 150 and 300 ppm induced significant promotion effect on fresh weight of Basil herb per plant with significant difference between these two sprayed concentrations. The maximum significant increase in Basil herb was achieved at treatment of 300 ppm ascorbic acid, being 30.1% more than fresh weight of herb per control plant. Whereas, sprayed Basil plants with the relatively high median used concentration of 450 ppm ascorbic acid showed no statistical effect on herb fresh weight per Basil plant. On the other hand, the relatively high used concentration of 600 ppm ascorbic acid decreased significantly yield of fresh herb per Basil plant below the control by 13.8%.

The above mentioned results indicated that the maximum significant increase in yield of fresh herb per Basil plant was achieved at treatment of 300 ppm ascorbic acid. Such result is an extension to that reported by El-Gamal (2005) who found that ascorbic acid application at concentration of 300 ppm have a promotive effect on herb productivity of Basil plant, being in good agreement with the present findings. In this respect, Khalil *et al.* (2010) sprayed ascorbic acid at concentrations of 100, 150 and 200 ppm on Basil plant, the obtained results revealed that all sprayed concentrations of ascorbic acid induced significant increases in fresh and dry weights of herb yield and the maximum significant increase was detected at concentration of 150 ppm ascorbic acid in the first cut and at 200 ppm ascorbic acid in the second cut.

III- Yield of seeds/plant:

The mean values of seed yield per Basil plant as affected by foliar spray with different concentrations of ascorbic acid are presented in Table (1).

It is noted from Table (1) that the effect of foliar application with the tested concentrations of ascorbic acid on yield of seeds per Basil plant showed the same trend that previously mentioned about the effect of ascorbic acid on morphological characters of vegetative growth as well as on yield of fresh herb per Basil plant. The first two assigned concentrations of 150 and 300 ppm ascorbic acid increased significantly yield of seeds per Basil plant with significant difference between these two tested concentrations and the maximum significant increase in seed yield was detected at 300 ppm ascorbic acid, being 41.2% more than seed yield per untreated plant. At the same time, sprayed Basil plants with the used relatively high median concentration of 450 ppm ascorbic acid had no significant effect on yield of seeds per Basil plant. By contrast, the relatively high used concentration of 600 ppm ascorbic acid decreased significantly the yield of seeds below the yield of untreated plant by 14.6%.

Information about the effect of foliar application with ascorbic acid on yield of seeds per Basil plant are not available. However, some investigators reported that ascorbic acid application resulted in increasing of seed yield per plant in some different plant species especially field crops. Among of them, Nofal *et al.* (1996) on faba bean, Abdel-Aziz (1999) on lentil, Abdel-Messih and Eid (1999) on wheat, Abdo and El-Moselhy (2004) on barley, Nassar and Abdo (2009) on Egyptian lupine, Nassar (2013) on mungbean and Nassar *et al.* (2016) on flax. All, being in harmony with the present findings.

IV- Anatomical studies:

It was aimed in this investigation to follow up the internal structure of vegetative growth which exhibited the most positive noticeable response to tested treatments. The aforementioned findings concerning the morphological characters of vegetative growth of Basil plant as affected by foliar application with ascorbic acid proved that 300 ppm ascorbic acid achieved the most positive remarkable effect among the various tested concentrations of ascorbic acid. This may justify a further study on the spraying effect with 300 ppm ascorbic acid on internal structure of Basil plant.

Microscopical characters were examined through specimens of the median portion of the main stem as well as of the blade of the corresponding leaf. Sampling was carried out during the second season of 2016 at the age of 12 weeks from sowing date.

1. Anatomy of the main stem:

Microscopical measurements of certain histological characters in transverse sections through the median portion of the main stem of Basil plant sprayed with 300 ppm ascorbic acid and those of control are given in Table (2). Likewise, microphotographs illustrating these treatments are shown in Figure (2).

Table 2: Measurements in micro-meters (μm) of certain histological characters in transverse sections through the median portion of the main stem of Basil plant, aged 12 weeks, as affected by foliar spray with ascorbic acid (Means of three sections from three specimens)

Histological characters	Treatments		
	Control	Ascorbic acid (300 ppm)	± % to control
Stem diameter	4692.0	5516.0	+ 17.6
Cortex thickness	157.9	198.4	+ 25.7
Fiber strands thickness	78.5	116.9	+ 48.9
Phloem tissue thickness	102.2	137.5	+ 34.5
Xylem tissue thickness	658.4	1125.8	+ 71.0
Vessel diameter	52.6	65.3	+ 24.1
Pith diameter	2598.0	2249.0	- 13.4

It is realized from Table (2) and Figure (2) that foliar application with 300 ppm ascorbic acid increased stem diameter by 17.6% over the control. This increment in stem diameter was mainly due to the prominent increase in thickness of all included tissues except that of pith diameter which showed a noticeable decrease by 13.4% less than the pith diameter of cross section of the main stem of untreated plant. The increase in stem diameter, which was observed due to foliar application with 300 ppm ascorbic acid, was accompanied with 25.7, 48.9, 34.5 and 71.0% increments in thickness of cortex, fiber strands, phloem tissue and xylem tissue compared to the control; respectively. Likewise, vessel diameter was increased over the control by 24.1% due to foliar application with 300 ppm ascorbic acid.

As far as the authers are aware, previous information about the effect of spraying ascorbic acid on anatomical structure of the main stem of Basil plant or other related species are not available in the literature. However, some investigators confirmed the present findings using ascorbic acid on other different plant species; for instance, El-Kobisy *et al.* (2005) using 400 ppm ascorbic acid on pea plants, Nassar and Abdo (2009) using 400 ppm ascorbic acid on Egyptian lupine plants, Nassar (2013) using 450 ppm ascorbic acid on mungbean plants and Nassar *et al.* (2016) using 450 ppm ascorbic acid on flax plants. They recorded favorable changes in anatomical structure of the main stem of each of the investigated plant species due to the effect of ascorbic acid which induced prominent increases in thickness of most of included tissues of the main stem of each investigated species, being in harmony with the present findings.

2- Anatomy of the leaf blade:

Microscopical counts and measurements of certain histological features in transverse sections through the blade of the mature foliage leaf developed at the median portion of the main stem of Basil plant sprayed with 300 ppm ascorbic acid and those of untreated plant are presented in Table (3). Likewise microphotographs illustrating these treatments are shown in Figure (3).

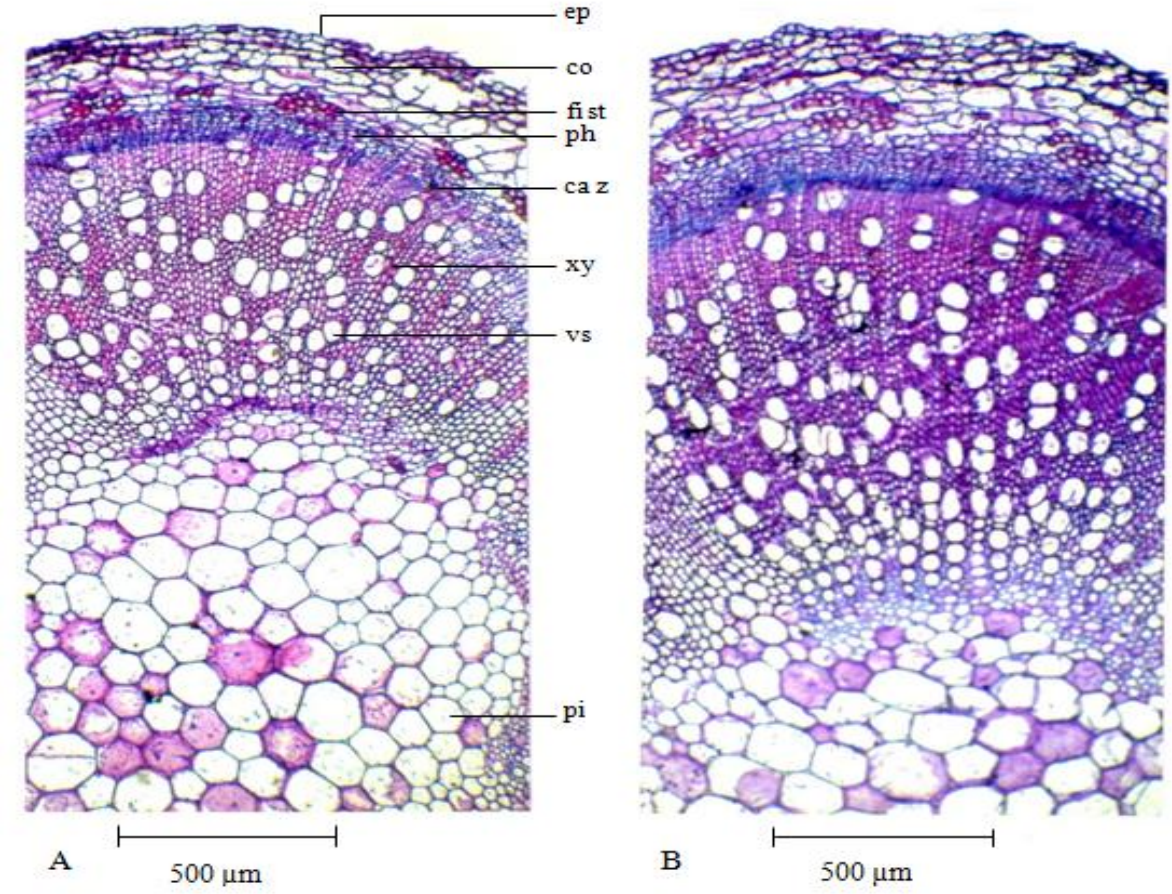

Fig. 2: Transverse sections through median portion of the main stem of Basil plant, aged 12 weeks, as affected by foliar spray with ascorbic acid.
A- From untreated plant (control).
B- From plant sprayed with 300 ppm ascorbic acid.
Details : ca z, cambium zone; co, cortex; ep, epidermis; fi st, fiber strands; ph, phloem; pi, pith; vs, vessel and xy, xylem.

Table 3: Counts and measurements in micro-meters (µm) of certain histological characters in transverse sections through the blade of the foliage leaf developed at the median portion of the main stem of Basil plant, aged 12 weeks, as affected by foliar spray with ascorbic acid (Means of three sections from three specimens)

Histological characters	Treatments		
	Control	Ascorbic acid (300 ppm)	± % to control
Midvein thickness	815.9	947.5	+ 26.1
Lamina thickness	368.5	513.2	+ 39.3
Palisade tissue thickness	144.8	197.4	+ 36.3
Spongy tissue thickness	157.9	236.8	+ 50.0
Dimensions of midvein bundle:			
Length	289.5	368.5	+ 27.3
Width	486.9	710.6	+ 45.9
No. of xylem rows/midvein bundle	18.3	25.2	+ 37.7
Vessel diameter	23.7	26.4	+ 11.4

It is obvious from Table (3) and Figure (3) that spraying ascorbic acid at concentration of 300 ppm on Basil plants resulted in thicker leaves than those of the untreated plants. Such effect could be attributed mainly to the increase induced in thickness of both midvein and lamina of Basil leaf by 16.1 and 39.3% more than those of the control; respectively. It is clear that the thicker lamina induced by ascorbic acid treatment was mainly due to the prominent increase observed in thickness of both palisade and spongy tissues by 36.3 and 50.0% over those of the control; respectively. Data also revealed that the main vascular

bundle of the midvein was increased in size as a result of spraying ascorbic acid. Such increment was mainly due to the increase in length by 27.3% and in width by 45.9% as well as in its number of xylem rows by 37.7% and in vessel diameter by 11.4% over the control.

In this respect, El-kobisy *et al*. (2005) using 400 ppm ascorbic acid on pea as well as Nassar and Abdo (2009) using the same previous concentration (400 ppm ascorbic acid) on Egyptian lupine and Nassar (2013) using 450 ppm ascorbic acid on mungbean stated that ascorbic acid treatment resulted in thicker leaflets than those of the control. Such effect was attributed to the increase induced in thickness of both leaflet lamina and midvein, being in conformity with the present findings.

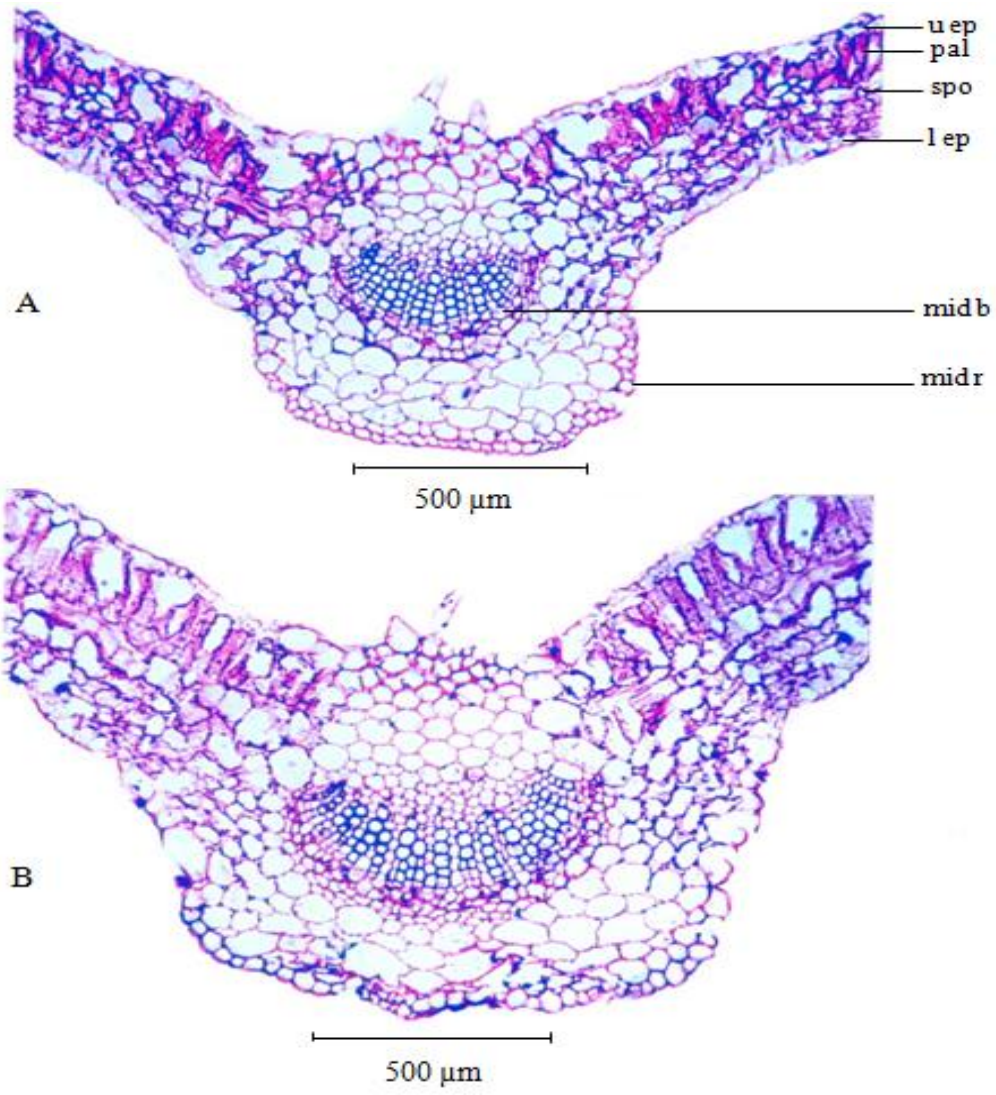

Fig. 3. Transverse sections through lamina of the leaf developed at the median portion of the main stem of Basil plant, aged 12 weeks, as affected by foliar spray with ascorbic acid.
A- From untreated plant (control).
B- From plant treated with 300 ppm ascorbic acid.
Details: l ep, lower epidermis; mid b, midvein bundle; mid r, midvein region; spo, spongy tissue; pal, palisade tissue and u ep, upper epidermis.

V- Volatile oil:

The composition and percentage of volatile oil of Basil herb at full blooming stage, age of 14 weeks, as influenced by foliar spray with 300 ppm ascorbic acid are presented in Table (4). Likewise, components of volatile oil analyzed by GC-MS are shown in Figures (4 and 5).

The volatile oil of Basil herb at full blooming stage was obtained by means of water-steam distillation. Basil herb at this stage yielded 0.65% volatile oil for herb of untreated plants (control) against 0.85% volatile oil for herb of plants treated with 300 ppm ascorbic acid. The previous reports of El-Gamal (2005) using 300 ppm ascorbic acid as well as of Khalil *et al.* (2010) using 100, 150 and 200 ppm ascorbic acid stated that such treatments caused significant increase in percentage of volatile oil of Basil herb, being in agreement with the present findings.

Table 4: Volatile oil of *Ocimum basilicum* L. herb at flowering stage as affected by foliar application with 300 ppm ascorbic acid, retention time, components and their percentages

No of peaks	Retention time (min)	Components (%)	Treatments	
			Control	Ascorbic acid (300 ppm)
1	8.42	Alpha-Pinene	0.75	0.63
2	9.79	Sabinene	-	0.62
3	10.43	Beta- Myrcene	0.47	0.87
4	10.94	Alpha-Terpinen	0.31	0.27
5	11.12	Limonene	0.54	0.60
6	11.50	Eucalyptol (1,8-Cineol)	2.15	3.25
7	11.86	Gamma-Terpinene	1.46	1.20
8	12.76	Cis-Sabinene Hydrate	0.52	-
9	12.85	Alpha-Thujene	-	0.46
10	**13.17**	**Linalool**	**35.58**	**41.68**
11	14.81	2,2 Dimethylocta-3,4-Dienal	0.82	-
12	14.89	Trans-Chrysanthemal	-	0.86
13	15.23	Bicyclo(3.1.1)hept-3-en-2-ol (Verbenol)	0.55	-
14	15.39	4-Terpineol	4.56	3.16
15	15.69	4-Carvomenthenol (3-cyclohexen-1-ol)	1.87	-
16	15.72	Cyclohexanone	-	1.04
17	15.93	Methyl Chavicol	0.41	2.55
18	16.21	P- Mentha-1.3-diene	-	0.61
19	16.26	Acetic Acid	0.44	0.25
20	16.64	Nerol	0.81	0.39
21	**16.81**	**Z-Citral(Neral)**	**14.13**	**10.95**
22	**17.55**	**E-Citral (geranial)**	**17.41**	**12.73**
23	20.05	Phenol (Iso-eugenol)	3.38	4.95
24	20.42	Neryl Acetate	0.27	0.30
25	20.85	1,2,6-Octadienol	0.53	0.11
26	21.04	Beta-Elemene	0.45	0.68
27	21.59	Trans-Caryophyllene	2.50	1.25
28	21.83	Bicyclo[3.1.1]Heptene	2.45	2.07
29	22.56	Alpha-Caryophyllene	1.23	0.99
30	23.02	Germacrene-D	2.67	2.13
31	23.42	Germacrene B	-	0.72
32	23.68	Naphthalene	-	0.94
33	24.12	Cis-Alpha-Bisabolene	2.57	1.38
34	26.67	Alpha-Amorphene (Delta Cadinen)	1.16	2.38
% of volatile oil in Basil herb			**0.65**	**0.85**

Usig GC-MS technique in analyzing volatile oil of Basil herb (Figs. 4 and 5) proved the presence of 27 compounds in control plants against 30 compounds in plants sprayed with 300 ppm ascorbic acid (Table 4). The main constituents are linlool, geranial and neral. Linalool (the first major component) comprised 35.58% of the volatile oil of control plants against 41.68% of the volatile oil of treated plants. Geranial (the second major component) comprised 17.41% of the volatile oil of control plants against 12.73% of the volatile oil treated plants. Whereas, neral (the third main component) constitute 14.13% of the volatile oil of control plants against 10.95% of the volatile oil of treated plants. Such three main components comprised 67.12% and 65.63% of the volatile oil for control and treated plants; respectively. This means that spraying Basil plants with 300 ppm ascorbic acid showed slight effect on the major constituents of volatile oil of Basil herb. However, such treatment affected other constituents where 11 different components are present in the volatile oil of which 4 components belongs to control and comprised 3.76% of its volatile oil and 7 other different components belongs to the treated plants and comprised 5.25% of its volatile oil. Thus, it could be stated that spraying Basil plants with 300 ppm ascorbic acid reduced 4 minor compounds in the composition of volatile oil which were found in control. Moreover, such treatment induced 7 minor compounds in the composition of volatile oil which were not found in control.

As far as the authors are aware, information about the effect of foliar application with ascorbic acid on the composition of volatile oil of Basil herb are not available.

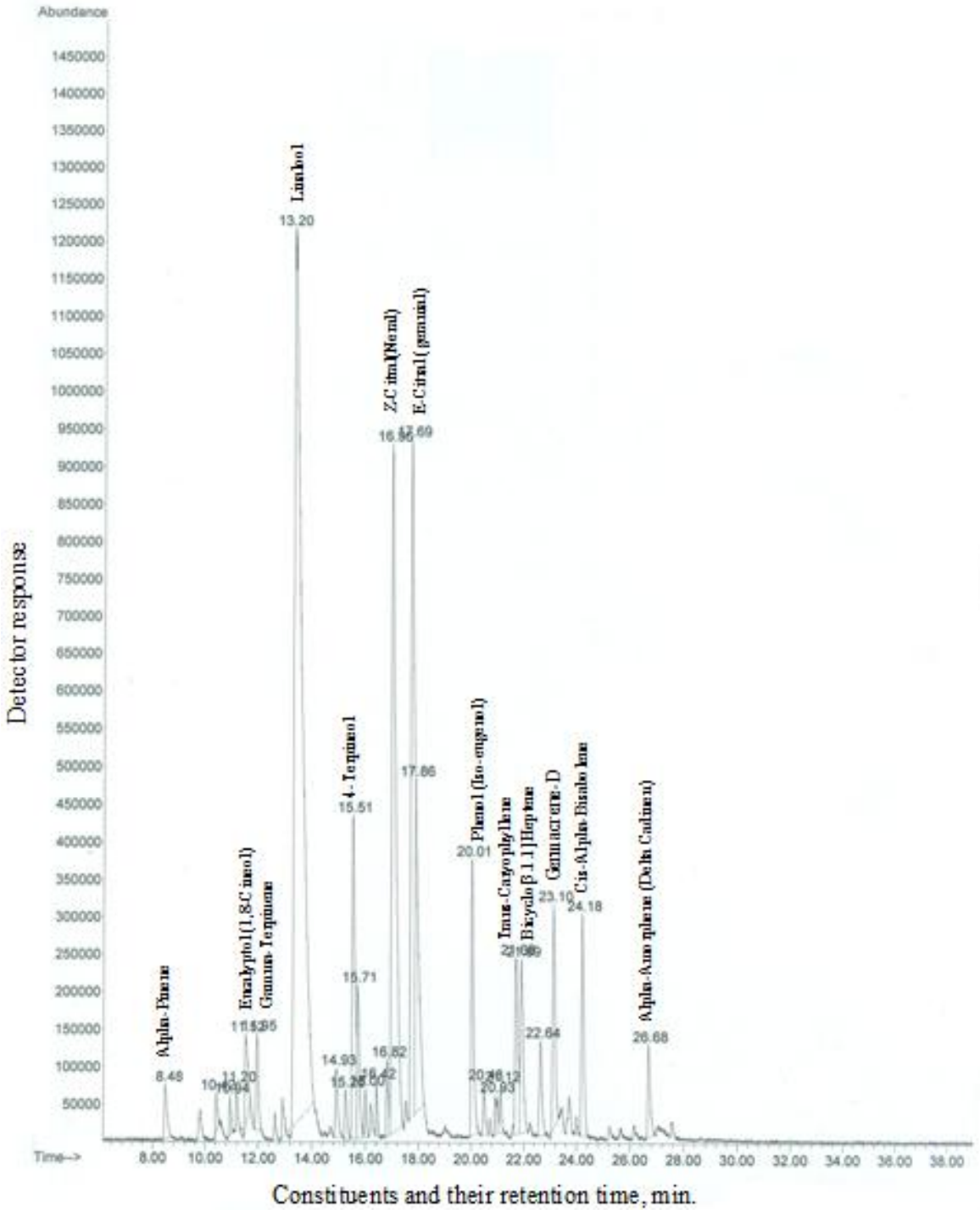

Fig. 4: GC / MS of volatile oil of Basil herb (*Ocimum basilicum* L.) at full blooming stage of untreated plants (control).

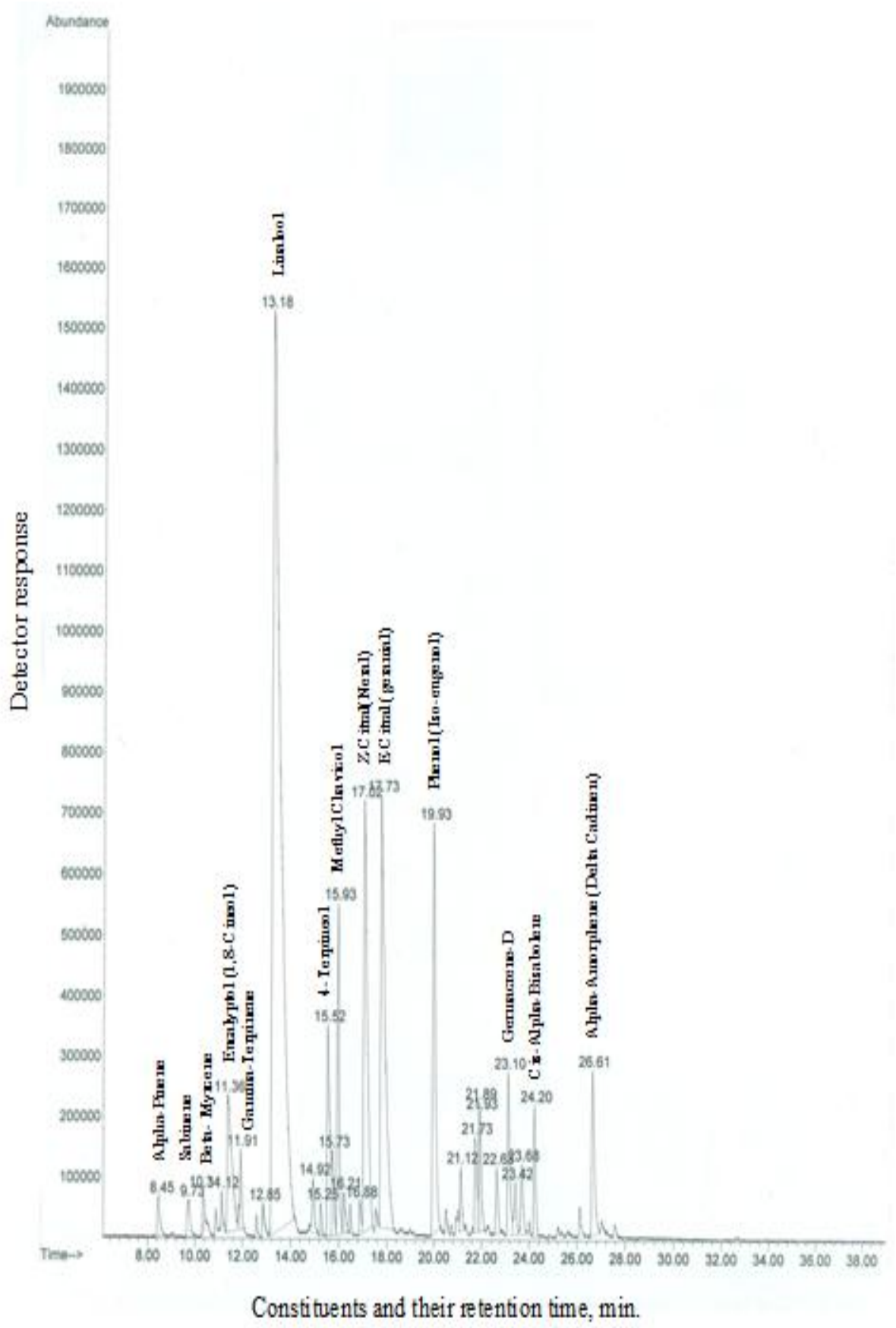

Fig. 5: GC / MS of volatile oil of Basil herb (*Ocimum basilicum* L.) at full blooming stage of plants treated with 300 ppm ascorbic acid.

VI- Fixed oil:

The composition and percentage of fixed oil of Basil seeds at harvest time, 17 weeks from sowing date, as affected by foliar application with 300 ppm ascorbic acid, the most positive effect concentration on growth and productivity of Basil plant, are presented in Table (5). Likewise, components of Basil seed oil from fatty acids analyzed by GC-MS are shown in Figures (6 and 7).

The chemical analysis proved that mature seeds of Basil contain 17.68% fixed oil for seeds obtained from untreated plants (control) against 20.65% fixed oil for seeds obtained from Basil plants sprayed with 300 ppm ascorbic acid (Table 5). This means that ascorbic acid treatment at concentration of 300 ppm increased yield of fixed oil by 16.8% over the control.

Using GC-MS technique in analyzing fixed oil of Basil seeds (Figures 6 and 7) proved the presence of 11 compounds (7 were identified and 4 were unknown) in control against 14 compound (8 were identified and 6 were unknown) in ascorbic acid treatment (Table 5).

Data presented in Table (5) indicate that the unsaturated linolenic acid (C18:3) was the first major fatty acid and comprised 49.58% of seed total fatty acids for control against 46.17% of seed total fatty acids for ascorbic acid treatment. The second major fatty acid was the unsaturated lenoleic acid (C18:2) which comprised 23.46% (for control) against 22.14% (for ascorbic acid treatment) of seed total fatty acids. The third major components was the unsaturated oleic acid (C18:1) which comprised 10.13% for control against 12.64% for ascorbic acid treatment of seed total fatty acids. The fourth major component was the saturated palmitic fatty acid (C16:0) which comprised 8.28% for control against 9.69% for ascorbic acid treatment of seed total fatty acids of Basil.

Worthy to note that the fixed oil of Basil seeds contain 12.27% saturated fatty acids for control against 15.26% saturated fatty acids for ascorbic acid treatment. Whereas, the unsaturated fatty acids comprised 85.01% for control against 81.10% for ascorbic acid treatment of the total fatty acids of Basil seeds. These results reveal that ascorbic acid increased the percentage of total saturated fatty acids and decreased the percentage of total unsaturated fatty acids of fixed oil of Basil seeds by about 3.0% more or less than the control.

As far as the authors are aware, information about the effect of foliar spray with ascorbic acid on percentage and composition of fixed oil of Basil seeds are not available.

Table 5: Fatty acids of *Ocimum basilicum* L. seeds as affected by foliar application with 300 ppm ascorbic acid, retention time, components and their percentages

No of peaks	Retention time (min)	Components (%)		Treatments	
				Control	Ascorbic acid (300 ppm)
1	20.86	Propanic acid	(C 3:0)	-	0.78
2	23.73	Palmitic acid	(C 16:0)	8.28	9.69
3	24.69	Palmitoleic acid	(C 16:1)	1.84	0.15
4	24.81	Unknown	-	-	2.14
5	25.40	Margaric acid	(C 17:0)	0.14	-
6	25.42	Unknown	-	-	0.21
7	28.49	Stearic acid	(C 18:0)	3.85	4.61
8	29.09	Oleic acid	(C 18:1)	9.23	11.37
9	29.27	Oleic acid (x_c , x_c)	(C 18:1)	0.90	1.27
10	29.39	Unknown	-	0.49	0.32
11	29.95	Unknown	-	1.67	-
12	30.15	Unknown	-	0.18	-
13	30.44	Linoleic acid	(C 18:2)	18.54	21.37
14	31.23	Linoleic acid (x_c , x_c)	(C 18:2)	4.92	0.77
15	31.50	Unknown	-	-	0.41
16	32.11	Linolenic acid	(C 18:3)	35.49	43.73
17	32.77	Linolenic acid (x_c , x_c)	(C 18:3)	14.09	2.44
18	33.44	Arachodic acid	(C 20:0)	-	0.18
19	33.79	Unknown	-	0.08	0.30
20	34.58	Unknown	-	-	0.25
% of fixed oil in seeds of Basil plant				**17.68**	**20.65**

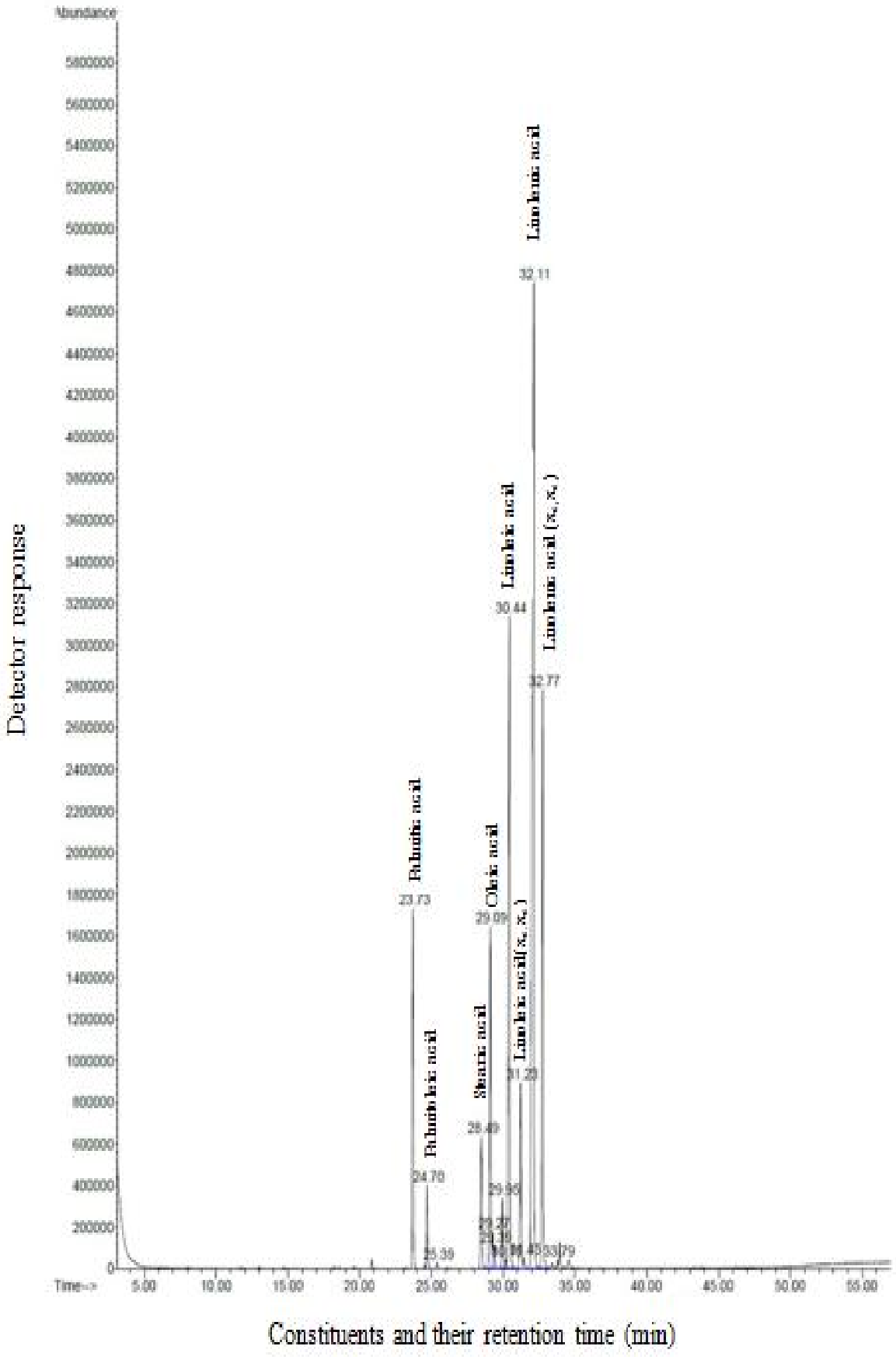

Fig. 6: GC-MS of fixed oil of *Ocimum basilicum* L. seeds from control plant.

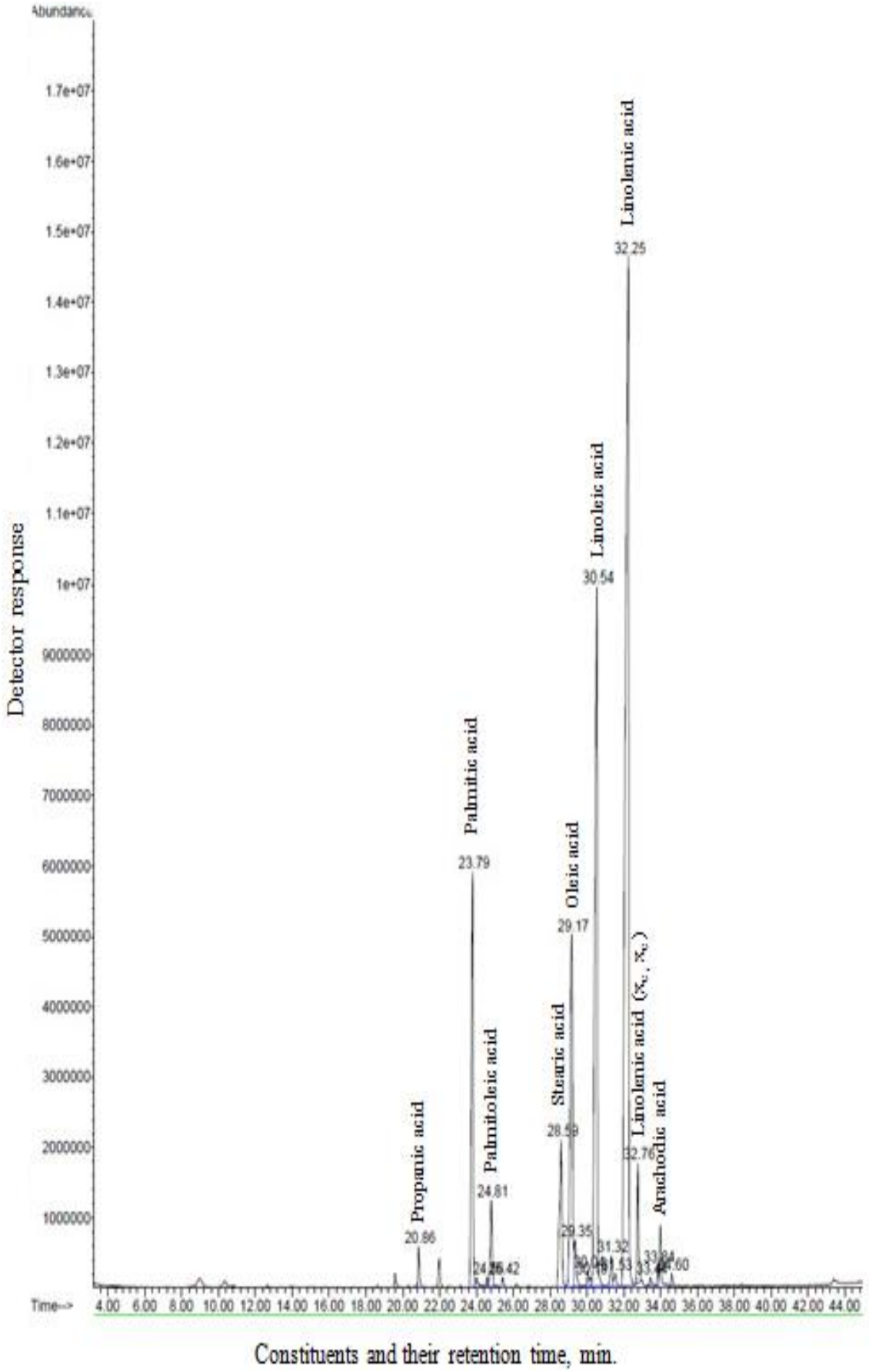

Fig. 7: GC-MS of fixed oil of *Ocimum basilicum* L. seeds from plants treated with 300 ppm ascorbic acid.

Recommendation

Spraying ascorbic acid twice, the first application at 7 weeks from sowing date and the second application after three weeks from the first application, at concentration of 300 ppm on Basil plant is highly recommended for promoting vegetative growth, increasing productivity from fresh herb and seeds, induced favorable changes in anatomical structures of vegetative organs and increasing percentages and improved quality of volatile and fixed oils of such important aromatic and medicinal plant.

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