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Pivotal Role of Ascorbic Acid in Promoting Growth, Increasing Productivity and Improving Quality of Flax Plant (*Linum usitatissimum* L.)

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ABSTRACT

The present investigation was carried out during the two winter growing seasons of 2013/2014 and 2014/2015 to study the effect of foliar spray with different concentrations (0, 150, 300, 450 and 600 ppm) of ascorbic acid on morphological characters, stem anatomy and yield of flax cv. Sakha-1 from straw, fiber and seeds as well as on their related characters. Also, the effect of ascorbic acid on seed oil percentage and composition of fatty acids were investigated. The obtained results revealed that all sprayed concentrations of ascorbic acid, except the relatively low used one of 150 ppm, promoted significantly all investigated morphological characters (plant height, technical length, length of fruiting zone and diameter of the main stem); enhanced anatomical structure of the main stem; increased significantly seed yield and its related characters except that of specific seed weight (number of capsules/ plant, number of seeds/capsule, seed yield/plant, seed yield/feddan, seed oil percentage and seed oil yield/feddan); promoted significantly straw and fiber yield per plant and per feddan as well as fiber length of flax cv. Sakha-1. The maximum promotion was detected at 450 ppm ascorbic acid.

Keywords: Ascorbic acid, Flax, *Linum usitatissimum* L., Growth, Stem anatomy, productivity, Quality

Introduction

The family Linaceae consists of 6 genera and about 220 species, widely distributed throughout the world, but most common in temperate and subtropical regions. By far the largest genus is *Linum*, with perhaps 200 species. Common flax, a traditional source of fiber and oil, is *Linum usitatissimum* L. (Cronquist, 1981).

Flax, the subject of our present investigation, is an erect annual herb plant grown to about 1.2 m tall, with slender stems. The leaves are glaucous green, slender lanceolate, sessile, 20-40 mm long and 3 mm broad. The blue or white flowers are arranged in erect terminal panicles. The fruit is a globose capsule with shiny, flattened, brown seeds with a short blunt beak (Bunney, 1992).

Flax is now unknown in the wild but originally it may have been a native of Asia. It has been cultivated since at least 5000 BC, probably first by ancient Mesopotamians and later by the Egyptians who wrapped their mummies in linen cloth. The Romans spread flax cultivation to Northern Europe and now the plant is grown allover the world for the oil extract from the seeds and for its fibers, which are made into linen and other cloths. Various parts of the plant have been used to make fabric, dye, paper, medicines, fishing nets, hair gels, and soap. It is also grown as an ornamental plant in gardens.

The seeds are widely used medicinally. Their constituents include 30-40% of a fatty oil (linseed oil) with esters of linoleic acid, linolenic acid, stearic acid and oleic acid; also mucilage, proteins, a cyanogenic glycoside (linamarin) and enzymes. Whole of crushed, the seeds are a reliable means of relieving constipation. Externally, crushed seeds mixed to a paste with water are used to make hot poultices to relieve pain and to heal septic wound, skin rashes and ulcers. The extracted oil is used in the pharmaceutical industry to make liniments for burns and rheumatic pain. The oil is also important in the manufacture of paints, soap and printer's ink (El-Kady, 2010 and El-Nagdy *et al.*, 2010).

In Egypt, flax is cultivated as a dual purpose (seeds for oil and stems for fibers). The cultivated area through the last 25 years was decreased from 60.000 to 30.000 feddans due to the great competition of other economic winter crops resulting in a gap between production and consumption. Therefore, it is necessary to increase flax productivity per unit area which could be achieved by using high yielding cultivars and improving the agricultural treatments (Hussein, 2007 and Ibrahim, 2009).

One of these treatments is the use of natural and safety substances in order to improve plant growth, flowering and fruit setting. In this concern, antioxidants has synergistic effect on growth, yield and yield quality of many plant species. These compounds have beneficial effect on catching the free radicals of the active

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oxygen (single oxygen, superoxide anion, hydrogen peroxide, hydroxyl radicals and ozone) that producing during photosynthesis and respiratory 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 cells 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). Many investigators reported that ascorbic acid application resulted in enhancement of growth, yield and chemical constituents of some 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) on flax and Nassar (2013) on mungbean.

Thus, the present investigation was carried out as an attempt to bring to light more information about the effect of foliar spray with different concentrations of ascorbic acid on morphological characters and yield from straw, fibers and seeds per plant and per feddan as well as on seed oil percentage, fatty acids and stem anatomy of flax cv. Sakha-1. This would be an effort to trace the beneficial effect for ascorbic acid on productivity and quality of flax, if any.

Materials and Methods

The present investigation was carried out at Sakha Agricultural Research Station, Kafr El-Sheikh Governorate, Egypt, during the two winter growing seasons of 2013/2014 and 2014/2015 in order to study the effect of foliar application with different concentrations of ascorbic acid on morphological characters and yield of flax cv. Sakha-1 from straw, fiber and seeds as well as on their related characters. The effect on stem anatomy, seed oil percentage and composition of fatty acids were also investigated.

Seeds of flax cv. Sakha-1, a dual purpose field crop for oil and fiber production, were procured from Fiber Crops Research Section, Field Crops Research Institute, Agricultural Research Center, Giza, Egypt.

Ascorbic acid was sprayed at concentrations of 150, 300, 450 and 600 ppm. The control plants were sprayed with tap water.

Field experiment was carried out in each of the two growing seasons. Flax seeds of the selected cultivar were sown on 11^{th} November 2013 in the first season and replicated in 13^{th} November 2014 in the second one to provide the experimental plant materials. Seeds were drilled at the rate of 50 kg seeds / feddan (1 feddan = 4200 m^2). 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 contained one treatment. The plot area was 6 m^2 (2×3 m) consisted of 10 rows, 20 cm apart. Land preparation, fertilizer application and agricultural operations followed the normal practices of flax cultivation in the vicinity.

Ascorbic acid concentrations were applied by means of an atomizer sprayer at the age of 60 days from sowing date and repeated one month later. Volume of spraying solution was 1.5 liters per plot in the first application and it was 2.5 liters in the second application. This volume was adequate to wet the plants of the plot thoroughly, and excess solution was dripping. Tween-20 was added as a spreading agent for tested treatments.

Recording of data

At maturity, 5 months from sowing date, 10 guarded plants were taken randomly from each plot, totaling 30 plants for each treatment, for recording the following morphological and yield characters per plant in addition to straw, fiber and seed yields/feddan (each estimated according to yield / 1 m^2 of each plot). Seed oil percentage and composition of fatty acids as well as stem anatomy were also under consideration.

The recorded characters included

1. Plant height (cm); from cotyledonary node up to the terminal top of the plant.
2. Ultimate diameter of the main stem (mm); the diameter was measured with a caliper at its median portion.
3. Technical length of the main stem (cm); from the cotyledonary node up to the beginning of apical branching zone of the main stem.
4. Length (cm) of apical branching zone of the main stem (fruiting zone).
5. Number of capsules per plant.
6. Number of seeds per capsule; recorded on 50 random capsules from each plot.

7. Specific seed weight (average weight of 1000 seeds in grams); recorded on 10 random samples from each plot.
8. Seed yield (g) per plant.
9. Seed yield (kg) per feddan; estimated according to seed yield per 1 m^2 of each plot.
10. Seed oil percentage; determined in seed samples taken randomly from each plot according to the method described by AOAC (1990), using petroleum ether as a solvent in soxhelt apparatus.
11. Oil yield (kg/feddan); calculated by multiplying seed oil percentage × seed yield (kg) per feddan.
12. Analysis of fatty acids was carried out in Central Laboratory of Cairo University Research Park (CURP), Faculty of Agriculture, Giza, Egypt, from seed oil specimens of the second season according to Vogel (1975). GLC was carried out under the same conditions mentioned by Nassar and Hassan (2002).
13. Straw yield (g) per plant; weight of air dried straw per plant in grams.
14. Straw yield (ton) per feddan; estimated according to weight of air dried straw yield per 1 m^2 of each plot.

After harvesting and removing the capsules from plants of each plot, retting process took place at Fiber Crops Research Section, Sakha Agricultural Research Station. Straw of each plot was arranged in bundles, put in retting basins and soaked in water for about 12 hours. After soaking, the water was changed to leach out all the soluble materials. Retting period took one week in the summer season. The degree of water temperature during retting process ranged from 28 to 30ºC at pH 6-7. The retted straw was washed with water and finally dried in air. Thus, the fibers were easily extracted from above the woody part of the stem. The following fiber characters were recorded:

15. Fiber yield (g) per plant; weight of air dried fibers per plant in grams.
16. Fiber yield (kg) per feddan; estimated according to fiber yield per 1 m^2 of each plot.
17. Fiber length (cm); fiber ribbon of 10 plants from each plot were extracted individually and mean length of each ribbon was measured.

Statistical Analysis

Appropriate statistical analysis according to Snedecor and Cochran (1982) was done. 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 tested treatments.

Anatomical Studies

It was intended to carry out a comparative microscopical examination on plant material which showed the most prominent response of plant growth to investigated treatments. Specimens of flax cv. Sakha-1 were taken from the middle part of the main stem at the age of 105 days from sowing date of the second growing season 2014/2015.

Specimens were killed and fixed for at least 48 hours in FAA (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 normal butyl alcohol series, embedded in paraffin wax of 56ºC melting point, sectioned to a thickness of 20 micro-meter (μ m), double stained with safranin / light green. Cleared in xylene and mounted in Canada balsam (Nassar and El-Sahhar, 1998). Sections were examined to detect histological manifestations of the chosen treatments and photomicrographed.

Results and Discussion

I- Morphological characters

Results of morphological characters at harvest time of flax cv. Sakha-1 as affected by foliar application with various concentrations of ascorbic acid are given in Table (1). The investigated characters included plant height, technical length, length of fruitin zone and diameter of the main stem.

Results presented in Table (1) clearly show that all sprayed concentrations of ascorbic acid, except the relatively low used one of 150 ppm, promoted significantly all investigated morphological characters. The maximum promotion was obtained when flax plants were sprayed twice with 450 ppm ascorbic acid. Such treatment induced significant increases of 23.2, 24.1, 21.4 and 20.7% over the control for plant height, technical length, length of fruiting zone and diameter of the main stem at its median portion; respectively.

Table 1: Average values of investigated morphological characters of flax cv. Sakha- 1, at harvest time, as affected by foliar spray with different concentrations of Ascorbic acid (Average of the two seasons, 2013/2014 and 2014/2015 combined)

Treatments	Concentrations (ppm)	characters			
		Plant height (cm)	Technical length (cm)	Length of fruiting zone (cm)	Diameter of the main stem (mm)
Control	0	107.3 D	86.4 C	20.1 C	2.13 D
Ascorbic acid	150	110.5 CD	87.7 C	21.9 BC	2.17 CD
	300	121.8 B	96.9 B	23.8 AB	2.36 B
	450	132.2 A	107.2 A	24.4 A	2.57 A
	600	119.8 BC	95.5 B	23.2 AB	2.33 BC
L.S.D. (0.05)		9.95	7.67	2.26	0.18

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

The present results are in agreement with those of Emam *et al.* (2011). They stated that ascorbic acid increased plant height and technical length of flax plants as compared with those of the control plants. Worthy to note that plant height which is the outcome of technical length and length of fruiting zone of flax plant and represent one of the most effective factors used for measured plant growth showed positive significant response to foliar application with ascorbic acid especially at the high median used concentration of 450 ppm which gave maximum promotive effect. The obtained results are generally in harmony with those reported by Mahmoud (1994) on wheat and faba bean using 500 ppm ascorbic, Abdel-Aziz (1999) on lentil using 500 ppm ascorbic acid, Anton *et al.* (1999) and Abdo and El-Moselhy (2004) on barley using 500 ppm ascorbic acid, El-Kobisy *et al.* (2005) on pea using 400 ppm ascorbic acid, Nassar and Abdo (2009) on Egyptian lupine using 400 and 600 ppm ascorbic acid and Nassar (2013) on mungbean using 450 and 600 ppm ascorbic acid.

II- Stem anatomy

Microscopical measurements of certain histological characters of the main stem, at its median portion, of flax cv. Sakha-1 sprayed with 450 ppm ascorbic acid and those of control are given in Table (2). Likewise, microphotographs depict these treatments are shown in Figure (1).

Table 2: Measurements in micrometers (µm) of certain histological features in transverse sections through the median portion of the main stem of flax cv. Sakha-1, aged90 days, as affected by spraying with 450 ppm ascorbic acid (Means of three sections from three specimens)

Histological characters (µm)	Treatments		
	Control	450 ppm ascorbic acid	± % to control
Stem diameter	2129	2557	+ 20.1
Epidermis thickness	32.3	39.5	+ 22.3
Cortex thickness	46.7	71.8	+ 53.7
Fibrous region thickness	38.2	53.9	+ 41.1
Average diameter of fibrous cell	21.5	32.3	+ 50.2
Secondary phloem thickness	83.8	107.7	+ 28.5
Xylem tissue thickness	96.9	125.6	+ 29.6
Pith diameter	1531	1769	+ 15.5

It is realized from Table (2) and Figure (1) that foliar spray with ascorbic acid at concentration of 450 ppm increased the diameter of the main stem of flax cv. Sakha 1 by 20.1% more than that of the control. This increase could be attributed to the prominent increase in all included tissues. The thickness of epidermis, cortex, fibrous region, secondary phloem and xylem tissue as well as diameter of the pith were 22.3, 53.7, 41.1, 28.5, 29.6 and 15.5% more than those of the control; respectively. Moreover, average diameter of fibrous cell was also increased in treated plants by 50.2% more than those of untreated ones.

The present findings are generally in accordance with those reported by El-Kobisy *et al*. (2005) using 400 ppm ascorbic acid on pea plants as well as by Nassar and Abdo (2009) using the same concentration, 400 ppm ascorbic acid, on Egyptian lupine plants. Also, Nassar (2013) reached to similar conclusion using 450 ppm ascorbic acid on mungbean plants. They recorded favorable anatomical changes in stem anatomy due to the effect of ascorbic acid which induced prominent increases in most of included tissues for investigated species.

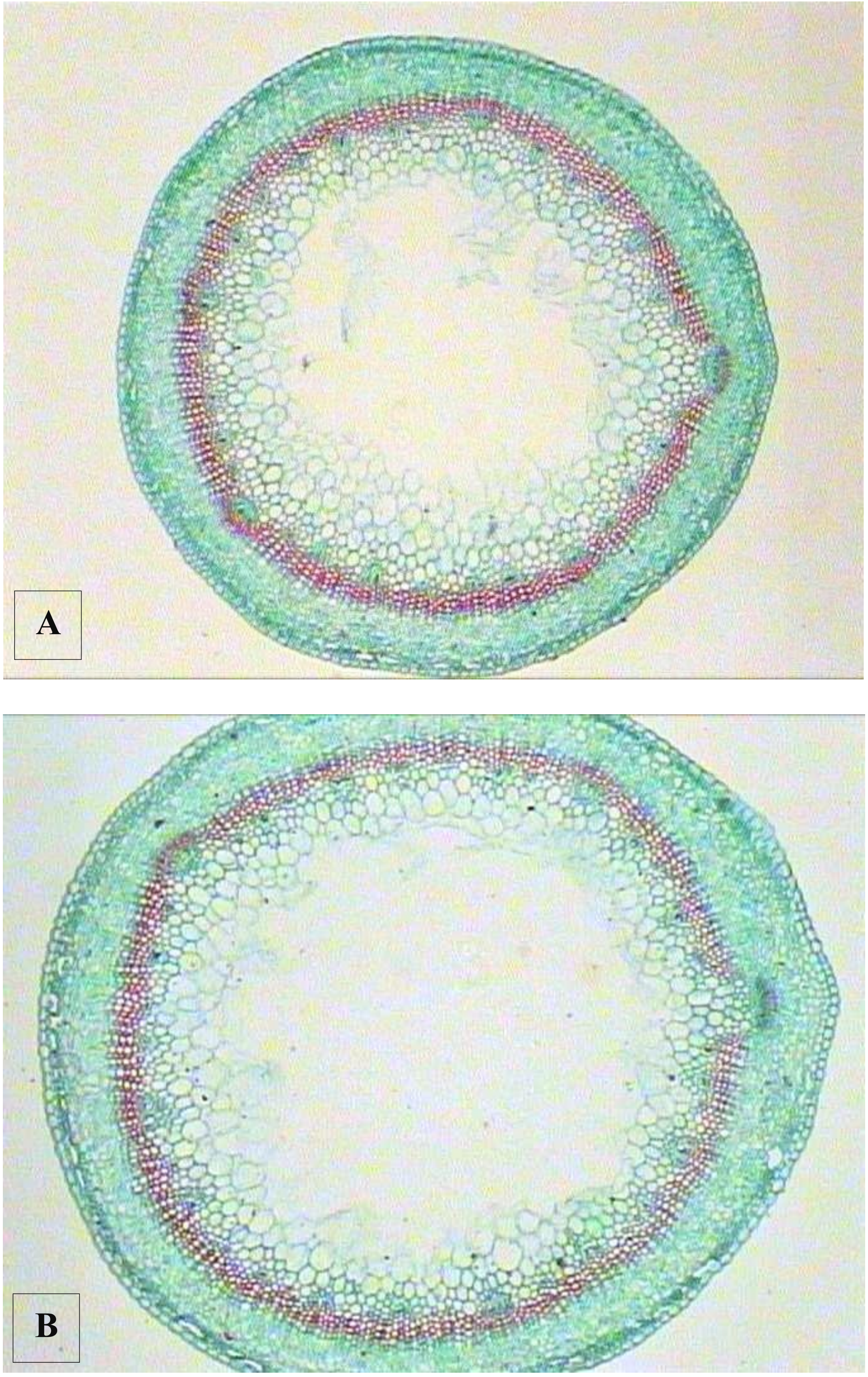

Fig. 1: Transverse sections through the median portion of the main stem of flax cv. Sakha-1, aged 90 days, as affected by foliar application with ascorbic acid.
A- Whole section from control plant. (X 40)
B- Whole section from plant treated with 450 ppm ascorbic acid. (X 40)

Cont.

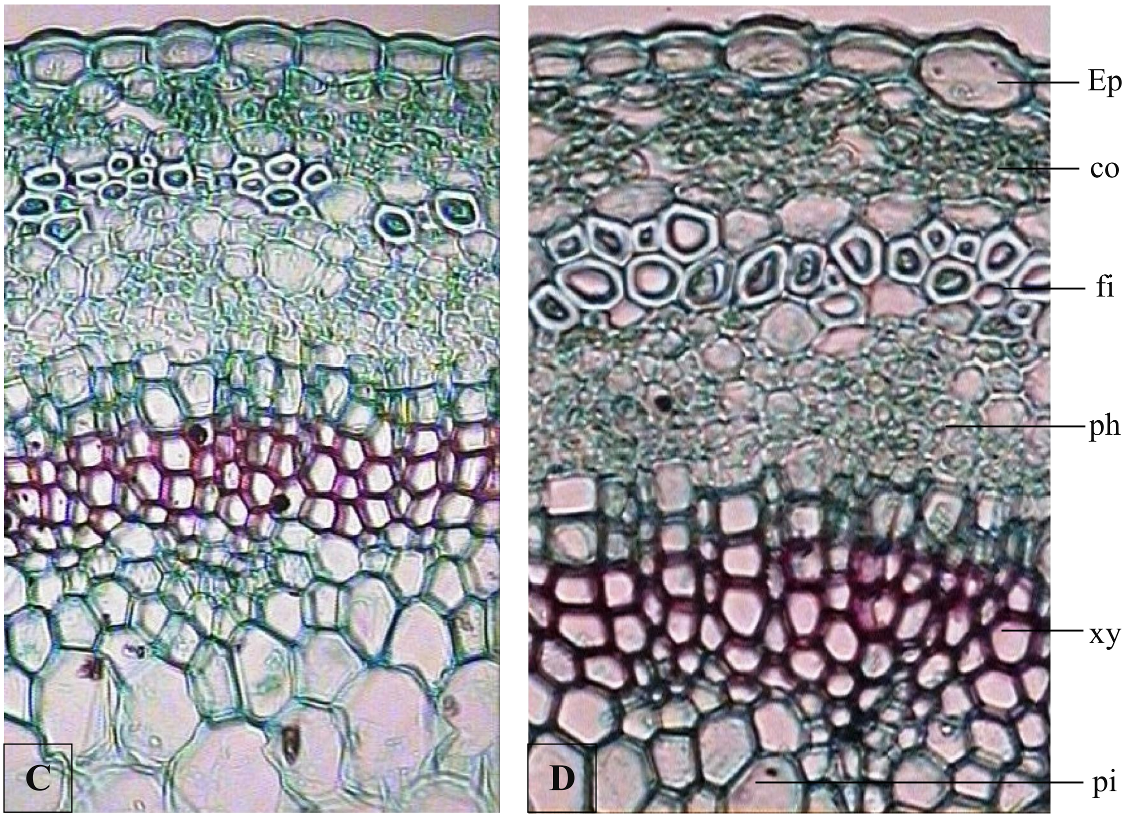

Fig. 1: Cont.
C- Magnified portion of A. (X 320)
D- Magnified portion of B. (X 320)
Details: co, cortex; ep, epidermis; fi, fibers; ph, phloem; pi, pith and xy, xylem.

III- Seed yield and its related characters

The average values of seed yield and its related characters of flax cv. Sakha-1 as affected by foliar spray with different concentrations of ascorbic acid in two growing seasons are presented in Table (3). The investigated characters included number of capsules/plant, number of seeds/capsule, weight of 1000 seeds, seed yield/plant, seed yield/feddan, seed oil percentage and seed oil yield/feddan.

It is obvious from Table (3) that foliar application with the relatively low tested concentration of 150 ppm ascorbic acid had no significant effect on all studied characters. By contrast, foliar spray with any of the other used concentrations of 300, 450 and 600 ppm ascorbic acid induced significant promotive effects on all studied characters except that of specific seed weight (weight of 1000 seeds) where the differences proved insignificant. Worthy to mention that the maximum significant increase was detected at 450 ppm ascorbic acid, being 21.8, 14.7, 39.9, 32.4, 9.24 and 44.6% more than those of the control for number of capsules/plant, number of seeds/capsule, seed yield/plant, seed yield/feddan, seed oil percentage and seed oil yield/feddan; respectively.

The obtained results are generally in accordance with those reported by Emam *et al.* (2011). They sprayed flax plants with ascorbic acid and recorded increases in number of capsules/plant, number of seeds/capsule, seed yield/plant, seed yield/feddan, seed oil percentage and seed oil yield/feddan.

IV- Fatty acids

Percentages of fatty acid methyl esters of seed oil of flax cv. Sakha-1 as affected by foliar application with the most effective concentration of 450 ppm ascorbic acid are given in Table (4).

It is realized from Table (4) that ascorbic acid showed no appreciable effect either on the components of fatty acids or on their percentages, although a negligible increase or decrease in the percentage of each component was observed.

These results are in contradiction with those reported by Emam *et al.* (2011). They stated that foliar application with ascorbic acid induced decrements in saturated fatty acids (Palmitic and stearic acid) in flax seed oil. On the other hand, they found that ascorbic acid treatment increased the percentage of linolenic acid and decreased the percentages of oleic and linoleic acids (unsaturated ones).

Table 3: Seed yield and its related characters of flax cv. Sakha-1 as affected by foliar spray with different concentrations of ascorbic acid (Average of the two seasons, 2013/2014 and 2014/2015 combined)

Treatments	Conc. (ppm)	Characters						
		No. of capsules / plant	No. of seeds / capsule	Weight of 1000 seeds (g)	Seed yield (g) / plant	Seed yield (kg)/feddan	Seed oil %	Seed oil yield (kg) feddan
Control	0	14.15 C	7.96 C	8.31	0.936 C	549.22 C	37.9 C	208.15 C
Ascorbic acid	150	14.55 BC	8.11 BC	8.46	0.998 C	580.53 C	38.2 BC	221.76 C
	300	15.68 B	8.79 A	8.29	1.142 B	653.17 B	40.6 AB	265.19 B
	450	17.23 A	9.13 A	8.32	1.309 A	726.94 A	41.4 A	300.95 A
	600	15.59 B	8.68 AB	8.41	1.137 B	641.83 B	39.8 ABC	255.45 B
L.S.D. (0.05)		1.27	0.64	N.S.	0.135	57.29	2.47	25.83

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

Table 4: Percentage of fatty acid methyl esters of seed oil of flax cv. Sakha-1 as affected by foliar spray with 450 ppm ascorbic acid in the second growing season of 2014/2015

Fatty acids composition (%)	Treatments	
	Control	Ascorbic acid (450 ppm)
Palmitic ($C_{16:0}$)	8.53	8.74
Stearic ($C_{18:0}$)	6.39	6.52
Oleic ($C_{18:1}$)	21.77	21.89
Linoleic ($C_{18:2}$)	18.95	19.05
Linolenic ($C_{18:3}$)	44.31	43.78

It is clear that oil extracted from seeds of untreated plants comprised five identified fatty acid methyl esters belong to two main groups. The first group namely unsaturated fatty acids which comprised about 85.0% of the total fatty acids and consists of three fatty acids which of linolenic ($C_{18:3}$) came on top, being about 44.3% of the total fatty acids of the seed, followed by oleic acid ($C_{18:1}$) which comprised about 21.8% and by linoleic acid ($C_{18:2}$) which comprised about 19.0% of the total fatty acids of the flax seed oil. The second group namely saturated fatty acids which composed of about 15.0 % of the total fatty acids of flax seed oil and consists of two fatty acids namely palmitic acid ($C_{16:0}$, comprised about 8.5%) and stearic acid ($C_{18:0}$, comprised about 6.4%). The increase in unsaturated fatty acids percentage (85%) beside the decrease in saturated ones (about 15%) in flax seed oil is more preferable due to suitability of flax seed oil in painting industry. These results are generally in agrrement with those obtained by El-Kady (2010).

V- Straw and fiber yields and fiber length

Average values of straw and fiber yields per plant and per feddan as well as of fiber length which is represent fiber quality of flax cv. Sakha-1 as affected by foliar application with different concentrations of ascorbic acid are given in Table (5).

Table 5: Straw yield, fiber yield and fiber length of flax cv. Sakha-1 as affected by foliar spray with different concentrations of ascorbic acid (Average of the two seasons, 2013/2014 and 2014/2015 combined)

Treatments	Conc. (ppm)	Characters				
		Straw yield (g)/plant	Straw yield (ton)/ feddan	Fiber yield (g)/plant	Fiber yield (kg)/ feddan	Fiber length (cm)
Control	0	2.297 C	1.3411 C	0.468 C	273.34 C	85.2 D
Ascorbic acid	150	2.415 BC	1.3719 BC	0.493 BC	280.06 BC	87.4 CD
	300	2.573 AB	1.4716 AB	0.535 AB	305.99 A	95.8 AB
	450	2.628 A	1.4953 A	0.547 A	313.86 A	103.3A
	600	2.496 AB	1.4589 AB	0.519 AB	303.35 AB	94.7 BC
L.S.D. (0.05)		0.164	0.1163	0.049	24.68	8.3

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

Data presented in Table (5) indicate that all tested concentrations of ascorbic acid, except the relatively low used concentration of 150 ppm, increased significantly straw and fiber yields per plant and per feddan as well as fiber length of flax cv. Sakha-1. The maximum increase was obtained at 450 ppm ascorbic acid, being 14.4, 11.5, 16.9, 14.8 and 21.2% more than the control for straw yield/plant, straw yield/feddan, fiber yield/plant, fiber yield/feddan and fiber length; respectively.

In this respect, Emam *et al.* (2011) found that straw yield/feddan of flax cv. Sakha-2 was significantly increased in response to foliar application with ascorbic acid, being in agreement with the present findings.

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