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Influence of foliar spray with seaweed extract on growth, yield and its quality, profile of protein pattern and anatomical structure of chickpea plant (*Cicer arietinum* L.)

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ABSTRACT

This work was carried out during the two successive winter seasons of 2013/ 2014 and 2014/ 2015 at Met Rabia village, (Private Farm) Bilbas, Sharkia Governorate, Egypt, to study the effect of foliar spray with seaweed extract on growth, yield, quality, profile of protein pattern as well as anatomical structure of stem and leaf of chickpea cv. Giza 195. Seaweed extract was sprayed at concentrations of 0, 0.25, 0.50, 0.75 and 1.00 ml / L. The obtained results indicated that foliar application with seaweed at 0.25 ml / L showed no effect on some studied characters of vegetative growth and yield components as well as on seed quality and profile of protein pattern of chickpea cv. Giza 195. Whereas, foliar application with the other sprayed concentrations especially 0.75 and 1.00 ml seaweed extract / L induced significant promoting effects on vegetative growth and yield characters as well as induced favorable changes in seed quality and profile of protein pattern of chickpea cv. Giza 195. The maximum significant promotion was obtained when plants of chickpea cv. Giza 195 were sprayed twice with 1.00 ml seaweed extract / L. This treatment elicited beneficial changes in both vegetative and reproductive characters, which resulted in higher yield of seeds per plant. Also, significant increases in mineral content of chickpea seeds were found by using 1 ml seaweed extract /L.

Foliar application with 1 ml seaweed extract /L. induced favorable changes in anatomical structure of stem and leaves of chickpea cv. Giza 195. Such treatment induced prominent increase in stem diameter due mainly to the marked increase in thickness of cortex, phloem and xylem tissues. Moreover, xylem vessels had wider cavities which amounted to more total active conducting area to cope with vigorous growth resulting from treatment with 1 ml seaweed extract /L. Likewise such treatment increased thickness of both midvein and lamina of leaflet blades of chickpea cv. Giza 195. The thicker lamina induced by seaweed extract was mainly due to increase induced in thickness of both palisade and spongy tissues. In addition the vascular bundle of midvein was increased in size as a result of spraying seaweed extract.

Key words: Chickpea, Seaweed extract, Growth, Photosynthetic pigments, Yield, Seed quality, Profile of protein, Stem and leaf anatomy

Introduction

The family Fabaceae (Papilionaceae) consists of about 440 genera and 12000 species. It ranks second only to the poaceae (Gramineae) in agricultural importance (Cronquist, 1981). Chickpea (*Cicer arietinum* L.) commonly known as gram or bengal gram is the most important pulse crop. Chickpea finds many uses. It is mainly used for human consumption. Green leaves are used as nutritious and palatable and pot herb or leafy vegetable. Unripe seeds are eaten raw, boiled and spiced eatable and cooked vegetable. Sprouted, spiced and salted seeds are eaten with great relish. Crushed seeds are used various sweet preparation and handmade bread. Roasted seeds as such or with papped rice are commonly eaten. Water soaked seeds, its husk and broken bits form a nutritious animal feed. Gram plants and dried stokls form excellent fodder for cattle. The crop is also used for green manuring and soil fertility improvement and as a cover crop against soil erosion (Majumdar, 2011).

Seaweed extract is a new generation of natural organic fertilizer highly nutritious and encourage faster germination of seeds and enhance yield and resistant ability of several crops (Dhargalkar and Pereria, 2005) . Seaweed are rich in macro and micro nutrients (Chapman and Chapman, 1980). The seaweed resources are intensively to increase harvest quantity and quality in agriculture and horticulture. The beneficial effects of seaweed products on the cultured plants are well documents. The using of seaweed products improve seeds germination, seedlings development, increase plant tolerance to environmental stresses (Zhang and Ervin, 2004, 2008) and improve plant growth and yield (Hong *et al*., 2007; Zodape *et al*., 2008; Khan *et al*., 2009; Kumari and Sahoo, 2011 and Craigie, 2011). The influence is explained by content of plant growth promoting

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substances such as cytokinins, auxin, gibberellins, abscisic acid, ethylene, polyamines and betaines in algal extract (Crouch and Van Staden, 1993; Stirk *et al*., 2003; Yokoya *et al*., 2010; Blunden *et al*., 2010 and Prasad *et al*., 2010). Seaweed extract contains growth promoting hormones (IAA and IBA), cytokinins, trace elements (Fe, Cu, Zn, Co, Mo, Mn, Ni), vitamins and amino acids (Challen and Hemingway, 1965). It was also reported that seaweed manure is rich in potassium but poor in nitrogen and phosphorus (Kingman and Moore, 1982).

Thus, the present investigation is an attempt to bring to light more information about the effect of spraying different concentrations of seaweed extract on morphology, anatomy and productivity of chickpea cv. Giza 195.

Materials and Methods

This work was carried out during the two successive winter seasons of 2013/ 2014 and 2014/ 2015 at Met Rabia village, (Private Farm) Bilbas, Sharkia Governorate, Egypt, to study the effect of foliar spray with seaweed extract on growth, chemical composition and yield as well as anatomy of stem and leaves of chickpea cv. Giza 195 grown in clay loam soil. The physical and chemical analysis of the experimental soil are presented in Table 1.

Table 1. Physical and chemical properties of the experimental soil (season 2013/ 2014).

Properties					
Mechanical		Chemical (mg/100 g soil)			
Sand %	28.64	pH	7.11	Ca++	0.05
Silt%	32.45	EC mmohs/cm	1.45	Mg++	0.03
				Na+	0.27
Clay%	38.91	Total N%	0.04	$CO3^-$	-
Soil texture	Clay loam	Total P%	0.06	HCO3	0.12
				Cl^-	0.13
F.C%*	32.28	Total K%	0.031	$SO4^-$	0.11

* *field capacity*

Seaweed extract was sprayed at concentrations of 0, 0.25, 0.50, 0.75 and 1.00 ml / L. The control plants were sprayed with tap water. These treatments were arranged in a randomized complete block design with three replicates.

The seeds of chickpea cv. Giza 195 were sown on 10th November in both growing seasons, after inoculation with root nodules bacteria (*Rhizobium leguminosarum*). Plot area was four rows with 4 m length, 60 cm apart and hills were spaced at 50 cm distance, two seeds were sown in each hill, then thinned to one plant/ hill. One row was left between each two experimental units as a gourd row to avoid the overlapping of spraying solution of seaweed. One row was used for samples to measure vegetative growth and the other three rows were used for yield determination.

The source of chickpea cv. Giza 195 was Legume Research Department, Field Crop Institute, Agric. Res. Center, Egypt. The source of root nodule bacteria was the General Organization for Agriculture Equalization Fund (G.O.A.E.F.), Ministry of Agriculture, Egypt. Seaweed extract (CTA stimulant-4) obtained from Meristem Company, Spain. Chickpea plants were sprayed with solution of seaweed extract two times for each used concentration at 30 and 60 days after sowing. Each plot received 1.5 liters in the first application and 2.25 liters in the second one. This volume was adequate to wet plants of the plot thoroughly, and excess solution was dripping. Tween 20 at 0.5% was used as welting agent.

The chemical analysis of seaweed extract according to Meristem Company, Spain are as follows.:

Ascophyllium nodosum seaweed extract	15 %	Appearance- Colour- Solubility	Liquid-dark brown 100 %
Total nitrogen	5.6 %	PH	7
Organic nitrogen	0.6%	Density	1.2 g/ ml
Ureic nitrogen	5%		
Water soluble magnesium (MgO)	0.2%	Magnesium Oxide (MgO) chelated by EDTA	0.2 %
Water soluble iron (Fe)	1%	Iron (Fe) chelated by EDTA	1%
Water soluble manganese (Mn)	0.5%	Manganese (Mn) chelated by EDTA	0.5%
Water soluble zinc (Zn)	0.5%	zinc (Zn) chelated by EDTA	0.5%

The source of nitrogen, phosphours and potassium were ammonium sulphate (20.5%N), calcium superphosphate (15.5% P_2O_5) and potassium sulphate (48% K_2O), respectively. Fertilizer application and cultural operations followed the normal practices of chickpea cultivation.

The data recorded were as following:

I-Morphological characters

A random sample of six plants was randomly taken from each plot at 105 days after sowing and the following data were recorded:
1- Plant height (cm), measured from the cotyledonary node up to the upper most point of the plant.
2- Number of branches / plant.
3- Number of leaves / plant.
4- Fresh weight of shoot (g)/plant.

II-Photosynthetic Pigments

Disk samples from the ninth upper leaf were taken at 90 days from sowing date of the second growing season of 2014/2015 to determine chlorophyll a and b as well as carotenoids according to the method described by Wettestein (1957).

III-Yield and its Components

A random sample of twelve plants was randomly taken from each plot at 170 days after sowing and the following data were recorded:
1. Average number of pods/ plant.
2. Average number of seeds/ plant.
3. Weight of 100 seeds (g).
4. Yield of seeds (g)/ plant.

IV. Chemical analysis in seeds (seed quality)

a-Mineral elements content in seeds

A known weight (0.2 g) of well mature dried seeds from each treatment in each replicate of plants grown in the second season of 2014/2015 was digested in H_2SO_4 (concentrated), H_2O_2 (5:1) for chemical analysis of minerals: nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sodium (Na), iron (Fe), manganese (Mn) , zinc (Zn) and copper (Cu) according to A.O.A.C. (1999). The concentration of the N, P, K, Ca, Na elements were expressed in %, whereas the elements Fe, Zn, Mn and Cu were expressed in ppm. Nitrogen content of seeds was multiplied by 6.25 to calculate the crude protein content (A.O.A.C., 1999).

b- Determination of amino acids in seeds

Amino acids were extracted according to the methods described by Csomos and Simon-Sarkadi (2002) and Shalabia (2011) and measured using the Automatic Amino Acid Analyzer (AAA 400 INGOS Ltd). The concentration of amino acids were calculated as g A.A./100 g protein.

V- Electrophoretic determination of protein pattern in seeds

The protein profiles were determined as described previously by Laemmli (1970). Briefly, 250 mg grain powder was homogenized by pestle and mortar in 1 ml from 20% sucrose. The homogenized material was centrifugated at 12.000 rpm for 25 min. The supernatant was removed to new eppendorf tubes and stored at -20 until use. Prior to use, thawed frozen solubilized samples were incubated at 95ºC for 5 min. SDS-Polyacrylamide gel electrophoresis (SDS-PAGE) was performed as described by Laemmli (1970). Each well was loaded with 10 µl protein on 12 % SDS-PAGE under constant current at 25 mA. After electrophoresis gel was transferred to plastic cassette and stained with Coomassie Brilliant Blue for two hours and distaining for several times to visualize the protein bands .

VI - Anatomical studies:

A comparative microscopical examination was performed on plant material for treatment which showed remarkable response. In addition to the control, tested materials included the main stem at its median portion and the lamina of the terminal leaflet of the corresponding compound leaf. Specimens were taken throughout the second growing season of 2014/2015 at the age of 90 days from sowing date. Specimens from control and chosen treatment, including stems and leaves, were killed and fixed for at least 48 hrs. 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 56ºC, sectioned to a thickness of 20 micrometer (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 from application of seaweed and photomicrographed

Statistical analysis

The obtained Data (morphological characters, photosynthetic pigments, yield and seed quality) were subjected to conventional methods of analysis of variance according to Snedecor and Cochran (1982) with using the program COSTAT 6.311. The least significant difference (L.S.D.) for each character was calculated at 0.05 level of probability.

Results and Disscussion

I- Morphological characters of vegetative growth

Data on morphological characters of vegetative growth of chickpea cv. Giza 195 as affected by foliar application with different concentrations of seaweed extract in two successive winter growing seasons are presented in Table (2). The investigated morphological characters included plant height (cm), number of branches per plant, total number of compound leaves developed per plant and fresh weight of shoot (g) per plant.

Table 2: Morphological characters of vegetative growth of chickpea cv. Giza 195,aged 105 days, as affected by foliar application with different concentrations of seaweed extract in two successive growing seasons of 2013/2014 and 2014/2015

Treatments	Conc. (ml/L.)	Morphological characters							
		Plant height (cm)		No. of branches / plant		No. of leaves / plant		Fresh weight of shoot (g) / plant	
		First season	Second season	First season	Second season	First season	Second season	First season	Second season
Control	0.00	52.6	50.9	2.3	2.6	142.3	181.6	53.63	55.97
Seaweed extract	0.25	67.3	69.5	2.9	3.3	195.9	251.3	58.82	62.46
	0.50	72.1	70.3	3.6	3.9	260.5	282.3	67.79	73.18
	0.75	73.5	71.3	3.9	4.3	273.6	295.9	69.53	77.62
	1.00	74.3	72.6	4.9	5.6	348.5	381.3	121.42	130.35
L.S.D. (0.05)		8.36	7.24	0.66	0.85	25.9	32.2	7.48	8.12

1-Plant height:

It is realized from Table (2) that all sprayed concentrations of seaweed extract induced significant increase in plant height of chickpea cv. Giza 195 in both studied seasons without significant differences among all tested concentrations. The maximum height was recorded at treatment of 1 ml seaweed extract/L., being 41.3 and 42.6% more than height of untreated plants in the first and second season; respectively.

2-Number of primary branches / plant:

Results presented in Table (2) clearly show that the relatively low used concentration of 0.25 ml seaweed extract / L. had no significant effect on number of primary branches / plant of chickpea cv. Giza 195 in both studied seasons. By contrast, all other assigned concentrations of seaweed extract induced significant increase in this respect. The maximum significant increase in number of primary branches per plant of chickpea cv. 195 was detected at high used concentration of 1 ml seaweed extract/L., being 113.0% more than the control in the first season and it was 115.4% more than the control in the second one.

3-Number of leaves/plant:

Data given in Table (2) reveal that all sprayed concentrations increased significantly number of compound leaves developed per plant of chickpea cv. Giza 195 in both studied seasons. The highest number was recorded at treatment of 1 ml seaweed extract/L., being 144.2 and 110.0% more than number of leaves developed per untreated plant in the first and second season; respectively.

4-Fresh weight of shoot/plant:

It is obvious from Table (2) that all adopted concentrations except that of 0.25 ml seaweed extract/L. promoted significantly fresh weight of shoot per plant of chickpea cv. Giza 195 in both studied seasons. The maximum significant increase in fresh weight of shoot per plant was achieved when plants of chickpea were sprayed with 1 ml seaweed extract/L., being 126.4 and 132.9% more than fresh weight of shoot per untreated plant in the first and second season; respectively.

From the abovementioned results about the effect of seaweed extract on morphological characters of vegetative growth of chickpea cv. Giza 195, it could be stated that most of the sprayed concentrations promoted significantly morphological characters (plant height, number of primary branches / plant, number of leaves /

plant and fresh weight of shoot / plant) and the maximum promotion was achieved at 1.00 ml seaweed extract / L. These results are in accordance with those reported by Sivasankari *et al*. (2006) working with *Vigna sinensis* showed that the seeds soaked with aqueous extract of seaweeds (*Sargassum wightii and Caulerpa chemnitzia*) promoted the seedling growth including the parameters of shoot length, root length, fresh weight and dry weight. Likewise, Blunden (1971) found that extracts of seaweed when applied to seeds or when added to the soil, enhanced growth of the plants. Thirumaran *et al*. (2009) on *Abelmoschus esculentus* found that seed germination, shoot length, root length, number of lateral roots reached its maximum at 20% seaweed liquid with or without chemical fertilizer. In this respect, perhaps the increase in morphological characters due to the extract of seaweed contains growth promoting hormones (IAA and IBA), cytokinins, trace elements (Fe, Cu, Zn, Co, Mo, Mn, Ni), vitamins and amino acids (Challen and Hemingway, 1965 and Crouch and Van Staden, 1993). Anisimov *et al.*, (2013) showed that water extracts of red algae *Neorhodomela larix*, *Tichocarpus crinitus,* of brown algae *Saccharina japonica*, *Sargassum pallidum*, and green algae *Ulva fenestrata* and *Codium fragile* increase the length of seedling roots of buckwheat (*Fagopyrum esculentum*). Hamed (2012) reported that spraying plants of *Phaseolus vulgaris* with seaweed extract at 750 ppm significantly increased plant height , number of leaves per plant , leaf area , shoot fresh and dry weights per plant.

II- Photosynthetic pigments:

It is clear from Table (3) that sprayed plants with 0.25 or 0. 50 ml / L seaweed showed no significant effect on photosynthetic pigments (chl a , chl b, total chl and carotenoids) concentration of chickpea cv. Giza 195 leaves. By contract, any of the other sprayed concentrations of seaweed induced significant increase in this respect. The best value in photosynthetic pigments was recorded at 1.00 ml / L, being 31.1 , 30.9 , 31.1 and 31.7 % over the control for chl a , chl b, total chl and carotenoids; respectively. No marked changes were observed in the ratios of chl a / chl b and total chl / carotenoids by application of seaweed extract at all levels. These results are in accordance with those reported by Sivasankari *et al*.(2006) working with *Vigna sinensis* showed that the seeds soaked with aqueous extract of seaweeds (*Sargassum wightii and Caulerpa chemnitzia*) increase chlorophyll, carotenoids and protein content. Seaweed applied of *Ascophyllum nodosum* extract to soil or on foliage of tomatoes produced leaves with higher chlorophyll content than those of untreated controls. This increase in chlorophyll content was a result of reduction in chlorophyll degradation, which might be caused in part by betaines in the seaweed extract (Whapham and *et al*., 1993).

Table 3: Photosynthetic pigments of chickpea cv. Giza 195, aged 90 days, as affected by foliar application with different concentrations of seaweed extract in the second growing season of 2014/2015

Treatments	Conc. (ml/L.)	Photosynthetic pigments (mg/g FW)					
		Chlorophyll a (Chl a)	Chlorophyll b (Chl b)	Total chlorophyll (Chl)	Carotenoids	Chl a / Chl b	Total Chl / carotenoids
Control	0.00	1.51	0.71	2.22	0.63	2.13	3.52
Seaweed extract	0.25	1.57	0.75	2.32	0.65	2.09	3.57
	0.50	1.67	0.77	2.44	0.71	2.17	3.44
	0.75	1.91	0.87	2.78	0.78	2.20	3.56
	1.00	1.98	0.93	2.91	0.83	2.13	3.51
L.S.D. (0.05)		0.16	0.10	0.34	0.09	—	—

III- Yield characters:

The mean values of yield characters of ckickpea cv. Giza 195 as affected by foliar application with different concentrations of seaweed extract in two successive winter growing seasons are given in Table (4). The investigated characters included number of pods/plant, number of seeds/plant, weight of 100 seeds (g) and yield of seeds (g)/plant.

1- Number of pods/plant

Data presented in Table (4) clearly show that the relatively low used concentration of 0.25 ml seaweed extract/L. had no significant effect on number of pods/plant of chickpea cv. 195 in both studied seasons. Whereas, all other sprayed concentrations of seaweed extract induced significant increase in number of pods/plant in the two studied seasons. The maximum significant increase in pod number was achieved at high used concentration of 1 ml seaweed extract/l., being 81.3% more than the control in the first season and it was 80.7% more than the control in the second one.

Table 4: Yield characters of chickpea cv. Giza 195 as affected by foliar application with different concentrations of seaweed extract in two successive growing seasons of 2013/2014 and 2014/2015

Treatments	Conc. (ml/L.)	Yield characters							
		No. of pods / plant		No. of seeds / plant		Weight of 100 seeds (g)		Seed yield (g) / plant	
		First season	Second season	First season	Second season	First season	Second season	First season	Second season
Control	0	49.6	53.3	57.2	61.3	19.97	20.52	11.42	12.58
Seaweed extract	0.25	52.7	57.6	61.5	74.9	19.62	20.71	12.07	15.51
	0.50	63.3	68.9	81.9	89.6	19.44	21.05	15.92	18.86
	0.75	72.5	81.0	93.6	105.3	20.17	20.62	18.83	21.71
	1.00	89.9	96.3	117.2	121.4	19.28	19.53	22.60	23.71
L.S.D. (0.05)		6.92	8.13	7.57	8.91	N.S.	N.S.	2.54	2.62

2-Number of seeds/plant

It is obvious from Table (4) that all tested concentrations of seaweed extract, except that of low used one (0.25 ml seaweed extract/L.) only in the first season, increased significantly number of seeds/plant of chickpea cv. Giza 195 in both studied seasons. The highest number was recorded at treatment of 1 ml seaweed extract / L., being 104.9 and 98.0% more than number of seeds per untreated plant in the first and second season; respectively.

3-Specific weight of seeds (average weight of 100 seeds)

It is clear from Table (4) that all assigned concentrations of seaweed extract had no significant effect on specific weight of seeds of chickpea cv. Giza 195 in both studied seasons.

4- Yield of seeds / plant

It is realized from Table (4) that the effect of foliar application with seaweed extract on yield of seeds/plant of chickpea cv. Giza 195 showed the same trend that previously mentioned about the effect of seaweed extract on number of seeds/plant. All sprayed concentrations of seaweed extract, except that of low used one of 0.25 ml seaweed extract/L. in the first season, increased significantly yield of seeds/plant of chickpea cv. Giza 195 in both studied seasons. The maximum increase was detected at 1 ml seaweed extract/L., being 97.9 and 88.5% more than seed yield per untreated plant in the first and second season; respectively.

Referring to the aforementioned results concerning the effect of seaweed extract on yield characters of chickpea cv. Giza 195, it could be stated that number of pods/plant, number of seeds/plant and seed yield/plant showed high response to most of the investigated treatments especially when seaweed extract was foliarly sprayed with concentration of 0.75 or 1.0 ml/L. It is noted that seaweed extract at 1.0 ml/L. gave a maximum significant promotive effect on yield characters.

Similar results were also reported by Rama Rao (1991) on *Zizyphus mauritiana* as well as by Kumari *et al.* (2011) on tomato and by Hamed (2012) on *Phaseolus vulgaris*.

IV. Mineral Content in chickpea seeds

Data presented in Table (5), indicate that the concentrations of some macro-elements (nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg)) and some microelements (Iron (Fe), Zinc (Zn), Copper (Cu), manganese (Mn) and sodium (Na) in chickpea seeds were significantly affected by spraying of 0.75 and 1 ml seaweed extract, whereas the concentrations of these elements were not significantly affected at the 0.25 and 0.50 ml seaweed extract levels. Application of 1 ml/L seaweed extract gave the highest increases in the concentrations of N, P, K, Ca, Mg, Na, Fe, Zn, Mn and Cu . The increases reached 38.2% in N, 19.2% in P, 25.7% in K, 75.9% in Ca, 69.2% in Mg, 21.1% in Na, 43.9% in Fe, 50% in Zn, 100% in Mn and 58.3% in Cu, over the untreated control plants. Similar results were observed by Zodapea *et al.* (2009), on *Triticum aestivum,* using 1% spray from an extract of *Kappaphycus alvarezii,* found that wheat grains contained higher concentrations of K, P, Ca, Mg, Zn, Mn and Mo, whereas the Cu content did not change, if compared with the control. Crouch *et al.* (1990) noted increased uptake of Mg, K and Ca in lettuce with seaweed concentrate application. Also, Turan and Köse (2004), Mancuso *et al.* (2006) and Rathore *et al.* (2009) observed increased uptake of N, P, K and Mg in grapevines, cucumber and soybean with application of seaweed extract. Sosnowski *et al.* (2014) found that application of the seaweed extract led to an increase in P, K, Zn and Mn in alfalfa aerial biomass, meanwhile the content of Mg, Ca, Cu and Mo did not change significantly.

The increase of macro- and microelements in chickpea seeds resulted from the application of seaweed extract may be attributed to the beneficial effects of seaweed extract as natural regulators or as an organic biostimulator, which improve plant vigor (Galbiattia *et al*. 2007). The seaweed extract contains many ingredients *;i.e.,* auxins, cytokinins, mineral elements, vitamins which induce many processes connected with

cytological and histological aspects of plant (as shown from the obtained results of anatomical changes in stem and leaf of chickpea illustrated in Tables (8 & 9) and Figs (3& 4) or due to having an influence on the content of some macronutrients (Wierzbowska and Bowszys, 2008) and increase the nutrient uptake from soil (Verkleij, 1992; Turan and Köse, 2004). Also, It was found that the presence of bioactive substances in seaweed extract improved stomata uptake efficiency in the treated plants compared the untreated plants (Mancuso *et al.,* 2006).
Also, it can be observed from the results calculated in the same table that the ratio of Ca:P was sharply increased while the ratio of K:(Ca+Mg) was decreased in chickpea seeds of plants treated with seaweed extract. The highest value of Ca:P (7.74) was recorded by the level 0.75 ml extract whereas the lowest one (4.83) was recorded by 0.25 ml extract. As for the K:(Ca+Mg) ratio, the highest ratio (1.67) was recorded at 0.25 ml whereas the lowest one (1.06) at 0.75 ml extract. In this respect, Sosnowski *et al.* (2014) found that the Ca:P and K:(Ca + Mg) ratios changed significantly in alfalfa as a result of the *Ecklonia maxima* extract application. The Ca / P ratio was reduced by 10%, while that of K /to Ca + Mg increased by 16%. Many investigators reported that it is important to determine the ratios of Ca:P and K:(Ca+Mg). The ratios of Ca:P and K:(Ca+Mg) were determined in seeds due to the importance of qualitative ratios between minerals as a quality parameter in the nutritional value (Staniak, 2004; Wierzbowska and Bowszys, 2008).

Table 5: The concentrations of some macro-and microelements and crude protein percentage in seeds of chickpea cv. Giza 195 as affected by different levels of seaweed extract in the second growing season of 2014/2015

Treatments	Conc. (ml/L.)	Minerals concentrations										Ratio of some elements		Crude protein %
		N	P	K	Ca	Mg	Na	Fe	Zn	Mn	Cu	Ca: P	K:Ca +Mg	
		%						ppm						
Control	0.0	4.28	0.203	1.87	0.99	0.133	0.19	18.0	12.0	5.0	12.0	4.90	1.66	26.8
Seaweed extract	0.25	4.39	0.209	1.91	1.01	0.134	0.19	19.0	14.0	6.0	13.0	4.83	1.67	27.4
	0.75	4.41	0.206	1.91	1.05	0.138	0.20	19.0	13.0	6.0	14.0	5.10	1.61	27.6
	0.75	5.26	0.226	2.09	1.75	0.220	0.21	19.7	18.0	6.0	12.0	7.74	1.06	32.9
	1.00	5.49	0.242	2.35	1.75	0.225	0.23	25.9	18.0	10.0	19.0	7.23	1.19	34.3
L.S.D. (0.05)		0.34	0.035	0.06	0.03	0.017	0.01	1.566	1.684	0.97	1.70	-	-	2.17

V- Amino acids Content in chickpea seeds

Analysis of amino acids in chickpea seeds using Amino Acid Analyzer detected 15 amino acids*;i.e.,* Aspartic acid (Asp), Therionine (Thr), Serine (Ser), Glutamic acid (Glu), Proline (Pro), Glycine (Gly), Alanine (Ala), Valine (Val), Metionine (Met), Isoleucine (Ile), Leucine (Le), Tyrosine (Tyr), Phenylalanine (Phe), Histidine (His) and Lysine (Lys) as shown in Figs (1, A & B) and Table (6).
Data presented in Table (6) show that chickpea seeds contained higher concentration in Lys, Glu, Asp, Gly, Leu, Phe, His, Ala, moderate concentration in Val, Ser, Ile, Thr, Tyr, and lower concentration in Met and Pro. Amino acids were divided into essential amino acids i.e. Thr, Val, Met, Ile, Leu, Tyr, Phe, His, Lys, and non essential amino acids *;i.e.,* Asp, Ser, Glu, Pro, Gly and Ala (Table 6).

Regarding to the effect of seaweed extract on the amino acids content in chickpea seeds, the obtained results illustrated in Table (6) show that foliar spraying with 1 ml/l seaweed extract caused a marked increase in the concentration of all measured essential and non essential amino acids comparing with the untreated control plants. The increases in the essential amino acids content reached about 22.8% in Thr, 62.5% in Val, 487.2% in Met, 39.4% in ILe, 57.1% in Leu, 42.7% in Tyr, 55.4% in Phe, 51.9% in His, and 20.5% in Lys. The increases in the non essential amino acids were about 57.1% in Asp, 64.8% in Ser, 51.9% in Glu, 355.9% in Pro, 51% in Gly, and 54.4% in Ala. Also, The increase in the total essential amino acid reached about 31.03%, whereas it was about 55.21% in the non essential amino acids if compared with the control. It can be observed that the highest increase in the essential amino acids was found in Met (487.2%), whereas the lowest one was found in Lys (20.5%) and Thr (22.8%). As for the non essential amino acids, the highest increase was observed in Pro (355.9%) whereas the lowest one was observed in Gly (51%) and Glu (51.9%). In this respect, Pise and Sable (2010) indicated that the liquid extract of *Ulva fasciata*, *Sargassum ilicifolium* and *Gracilaria corticata* affected the content of free amino acids in *Trigonella foenumgraecum*. Kalidass *et al.*, (2010) found that the liquid extract of *Ulva lacuta, Caulerpa scalpelliformis, Padina tetrastromatica* and *Sargassum linearifolium* in different concentrations increased the amount of amino acids of *Brassica nigra*. Lingakumar *et al.*, (2004, 2006) reported that the liquid extract from *Ulva lcuta* and *Sargassum sp*. when added to the soil bed promoted amino acid content of *Phaseolus mungo, Zea mays* and *Cyamopsis tetragonoloba*. Also, Lingakumar *et al.*, (2002) on *Zea mays and Phaseolus mungo*, observed that application of seaweed extract of *Gracilaria edulis* to soil bed showed positive response on the total amino acid content. The positive effect of seaweed extract in increasing the amino acids content in chickpea seeds may be due to presence of some growth promoting substances, *;i.e.,* IAA, IBA, Gibberellins, Cytokinins, micronutrients, vitamins and amino acids (Challen and Hemingway, 1965).

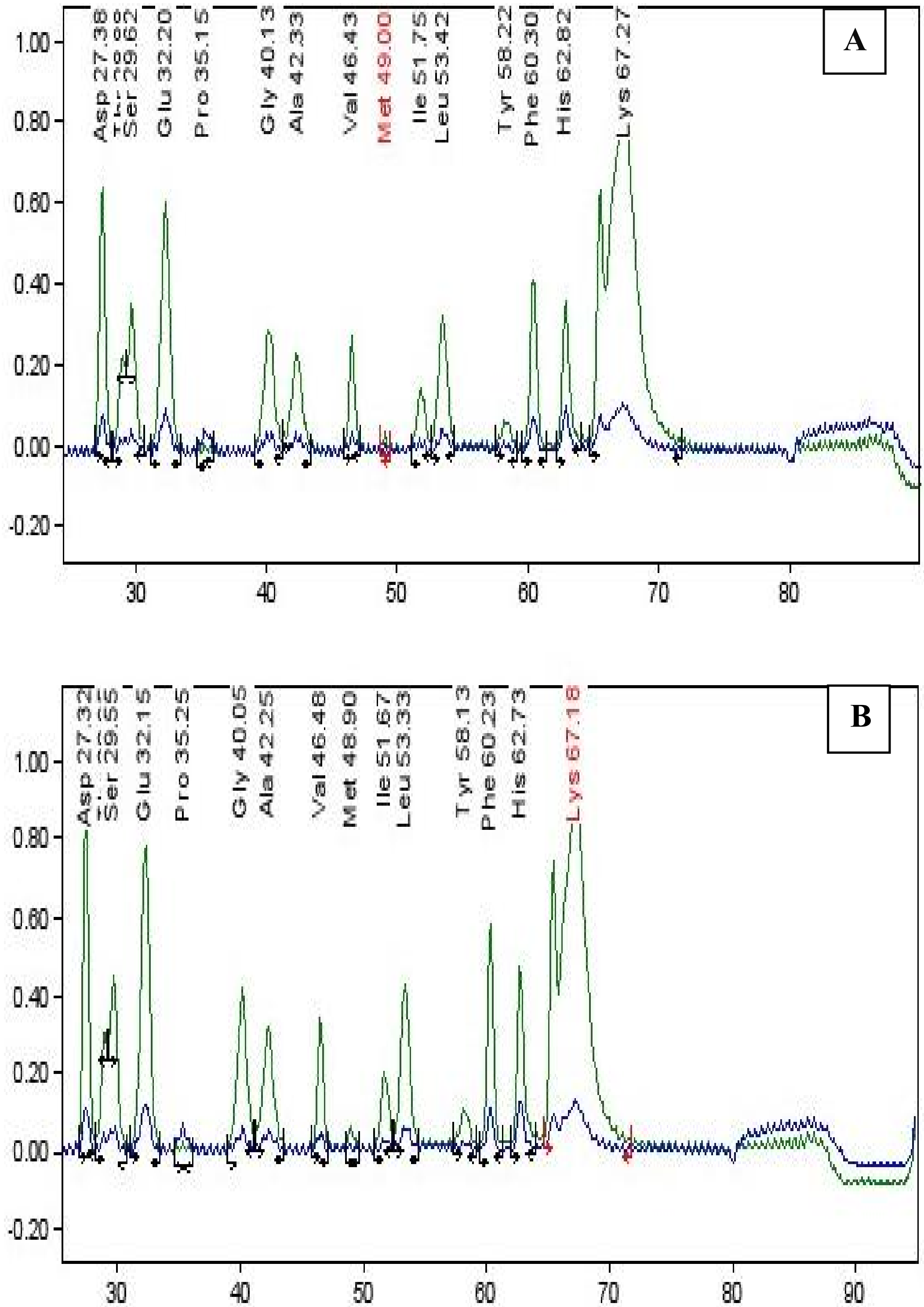

Fig. 1: Amino acids detected in chickpea seeds by Automatic Amino Acid Analyzer (AAA 400 INGOS Ltd)- A: Control plants, B: Plants treated with 1ml seaweed extract/ L.

Table 6: The concentrations of amino acid in seeds of chickpea cv. Giza 195 as affected by seaweed extract in the growing season of 2014/2015.

Amino Acids	Control	Seaweed 1ml/l	+ %
	g AA/100 g Protein		
Essential AA			
Thr	0.280	0.344	22.802
Val	0.831	1.350	62.472
Met	0.037	0.219	487.149
ILe	0.688	0.959	39.403
Leu	1.634	2.567	57.099
Tyr	0.283	0.404	42.707
Phe	1.622	2.520	55.389
His	1.379	2.094	51.874
Lys	15.217	18.331	20.463
Total E.A.A.	21.971	28.789	31.029
Non essential AA			
Asp	2.197	3.453	57.134
Ser	0.598	0.985	64.776
Glu	3.318	5.040	51.890
Pro	0.031	0.142	355.904
Gly	1.740	2.627	51.004
Ala	1.270	1.961	54.406
Total N.E.A.A.	9.154	14.207	55.204
Total	31.125	42.996	38.139

VI- Profile of protein pattern in seeds

An electrophoretic separation of total soluble proteins in seeds, yielded from untreated and treated plants of chickpea cv. Giza 195 with different concentrations of seaweed extract, using SDA-PAGE was performed. Data of SDS-PAGE of total soluble proteins in seeds of treated and untreated plants of chickpea cv. Giza 195 are shown in Figure (2) and Table (7).

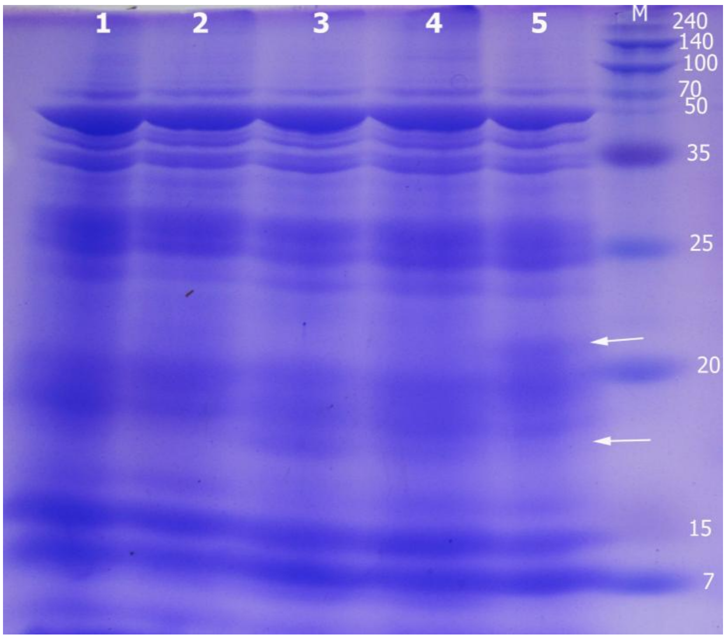

Fig. 2: SDS-PAGE separation of total soluble protein in seed yielded from chickpea plants cv. Giza 195 as affected by foliar spray with different concentrations of seaweed extract.

1- From plants sprayed with tap water (control).
2- From plants sprayed with 0.25 ml seaweed extract/L.
3- From plants sprayed with 0.50 ml seaweed extract/L.
4- From plants sprayed with 0.75 ml seaweed extract/L.
5- From plants sprayed with 1.0 ml seaweed extract/L.
6- Low molecular weight protein marker.

It is realized from Figure (2) and Table (7) that seaweed extract induced slight changes in the protein profile of chickpea seeds. It is evident that of 21 bands, protein of seeds yielded from control plants, from plants treated with 0.25 ml seaweed extract/L., from plants treated with 0.50 ml seaweed extract/L., from plants treated with 0.75 ml seaweed extract/L. and from plants treated with 1.0 ml seaweed extract/L. recorded 17, 17, 19, 19 and 16 bands respectively. It is clear that the control and four levels of seaweed extract shared 14 bands at the molecular weights of 91, 70, 47, 39, 35, 34, 30, 27, 25, 23,16,12, 6 and 3 KDa.

Results in Figure (2) and Table (7) indicate that the relatively low used concentration of 0.25 ml seaweed extract/L. had no effect on profile of protein pattern of chickpea seeds. Such treatment recorded 17 bands similar to those recorded in control treatment. By contrast, the other three tested concentrations of seaweed extract induced slight changes in profile of protein pattern in seeds of chickpea cv. Giza 195. The treatment of 0.50 ml seaweed extract/L. recorded 19 bands showing two new bands over the control at molecular weights of 19 and 18 KDa. Likewise, the treatment of 0.75 ml seaweed extract/L. recorded 19 bands showing three new bands over the control at molecular weights of 121, 19 and 18 KDa and showed disappearance of one band less than the control at molecular weight of 20 KDa. At the same time, the treatment of 1.0 ml seaweed extract/L. recorded 16 bands showing disappearance of three bands below the control at molecular weights of 32, 20and 2 KDa, such treatment showed two new bands more than the control at molecular weights of 21 and 19 KDa. As far as the authors are aware, previous information about the effect of seaweed extract on profile of protein patterns in seeds of chickpea or other related plants are not available.

Table 7: Densitometer analysis of total soluble proteins (SDS-PAGE) in seeds yielded from plants of chickpea cv. Giza 195 sprayed with different concentrations of seaweed extract. Showing band number and molecular weight (MW)

Band No.	MW (KDa)	Treatments				
		Control	Seaweed extract (ml/L.)			
			0.25	0.50	0.75	1.00
1	121	0	0	0	1	0
2	91	1	1	1	1	1
3	70	1	1	1	1	1
4	47	1	1	1	1	1
5	39	1	1	1	1	1
6	35	1	1	1	1	1
7	34	1	1	1	1	1
8	32	1	1	1	1	0
9	30	1	1	1	1	1
10	27	1	1	1	1	1
11	25	1	1	1	1	1
12	23	1	1	1	1	1
13	21	0	0	0	0	1
14	20	1	1	1	0	0
15	19	0	0	1	1	1
16	18	0	0	1	1	0
17	16	1	1	1	1	1
18	12	1	1	1	1	1
19	6	1	1	1	1	1
20	3	1	1	1	1	1
21	2	1	1	1	1	0

VII- Anatomical Studies

1-Stem anatomy

Microscopical measurements of certain histological characters in transverse sections through the median portion of the main stem of chickpea cv. Giza 195 treated with 1 ml seaweed extract/L. and those of control are presented in Table (8). Also, microphotographs depict these treatments are shown in Figure (3).

Table 8: Measurements in micrometers (μ m) of certain histological features in transverse sections through the median portion of the main stem of chickpea cv. Giza 195, aged 90 days, as affected by foliar spray with 1 ml seaweed extract/L. (Means of three sections from three specimens)

Histological characters (μ m)	Treatments		
	Control	Seaweed extract (1 ml/L.)	± % to control
Stem diameter	2769	3174	+ 14.6
Cortex thickness	174	213	+ 22.4
Phloem tissue thickness	102	136	+ 33.3
Xylem tissue thickness	418	622	+ 48.8
Vessel diameter	53	69	+ 30.2
Pith diameter	1406	1218	- 13.4

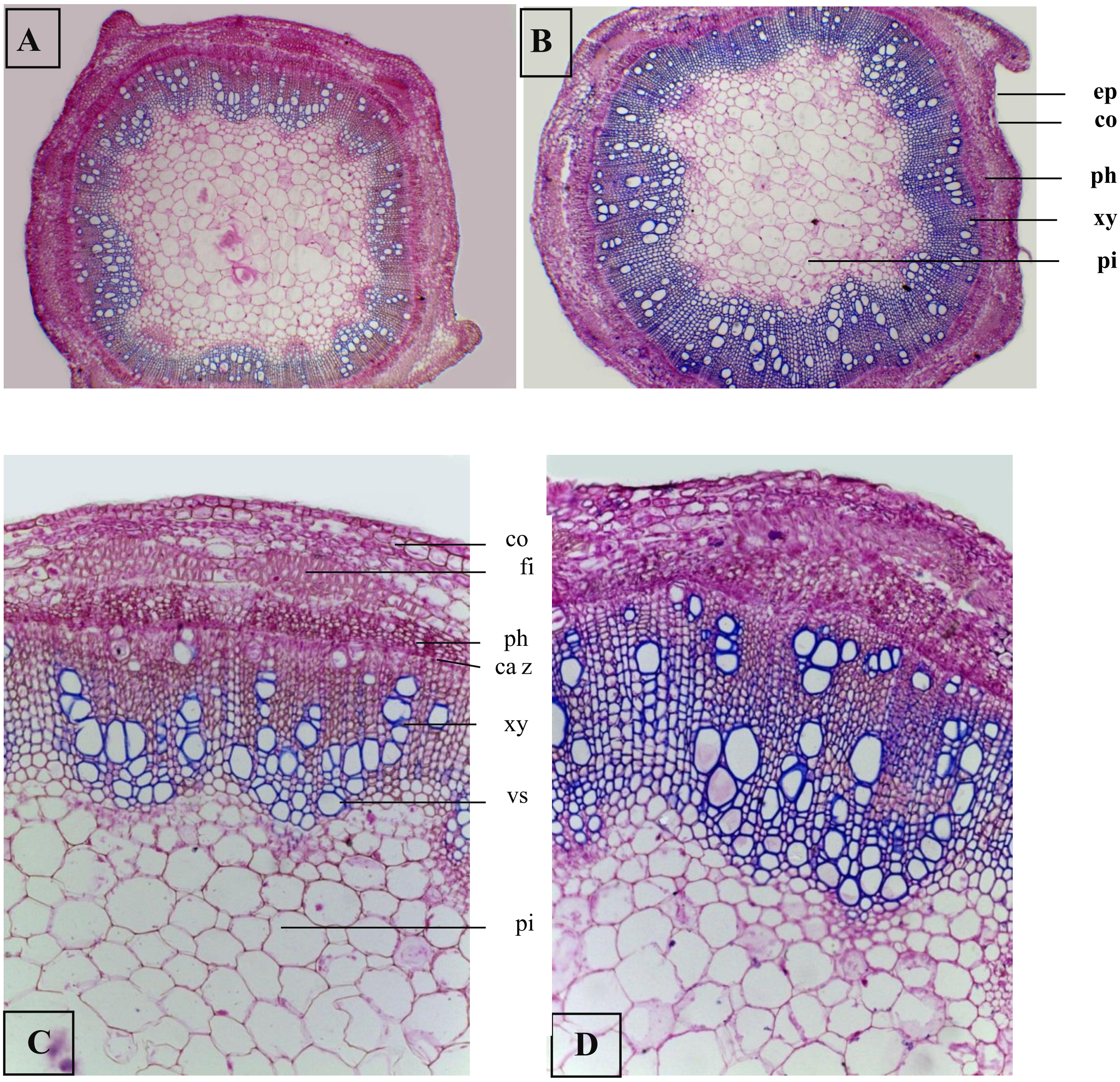

Fig. 3: Transverse sections through the median portion of the main stem of chickpea cv. Giza 195 at the age of 90 days as affected by foliar application with seaweed extract.
A- From untreated plant (control). (X 40)
B- From plant sprayed with 1 ml seaweed extract/L. (X 40)
C- Magnified portion of A. (X 100)
D- Magnified portion of B. (X 100)
Details: ca z, cambium zone; co, cortex; ep, epidermis; ph, phloem; fi, fibers; pi, pith and xy, xylem.

It is obvious from Table (8) and Figure (3) that foliar application with seaweed extract at concentration of 1 ml/L. increased the diameter of main stem of chickpea plant by 14.6% more than that of the control. Nevertheless, pith diameter in treated stems was decreased by 13.4% less than that of the control. Worthy to note that, the increase in stem diameter which was induced due to foliar application with seaweed extract could be attributed mainly to the prominent increases in most of the included tissues. Thickness of cortex, phloem tissue and xylem tissue were increased by 22.4, 33.3 and 48.8% more than those of the control; respectively. Moreover, vessel diameter was increased over that of the control by 30.2% due to foliar application with 1 ml seaweed extract/L.

2-Leaf anatomy

Microscopical counts and measurements of certain histological features in transverse sections through the blade of the terminal leaflet of the compound leaf developed on the median portion of the main stem of chickpea cv. Giza 195 as affected by foliar spray with 1ml seaweed extract/L. and those of control are given in Table (9). Likewise, microphotographs illustrating these treatments are shown in Figure (4).

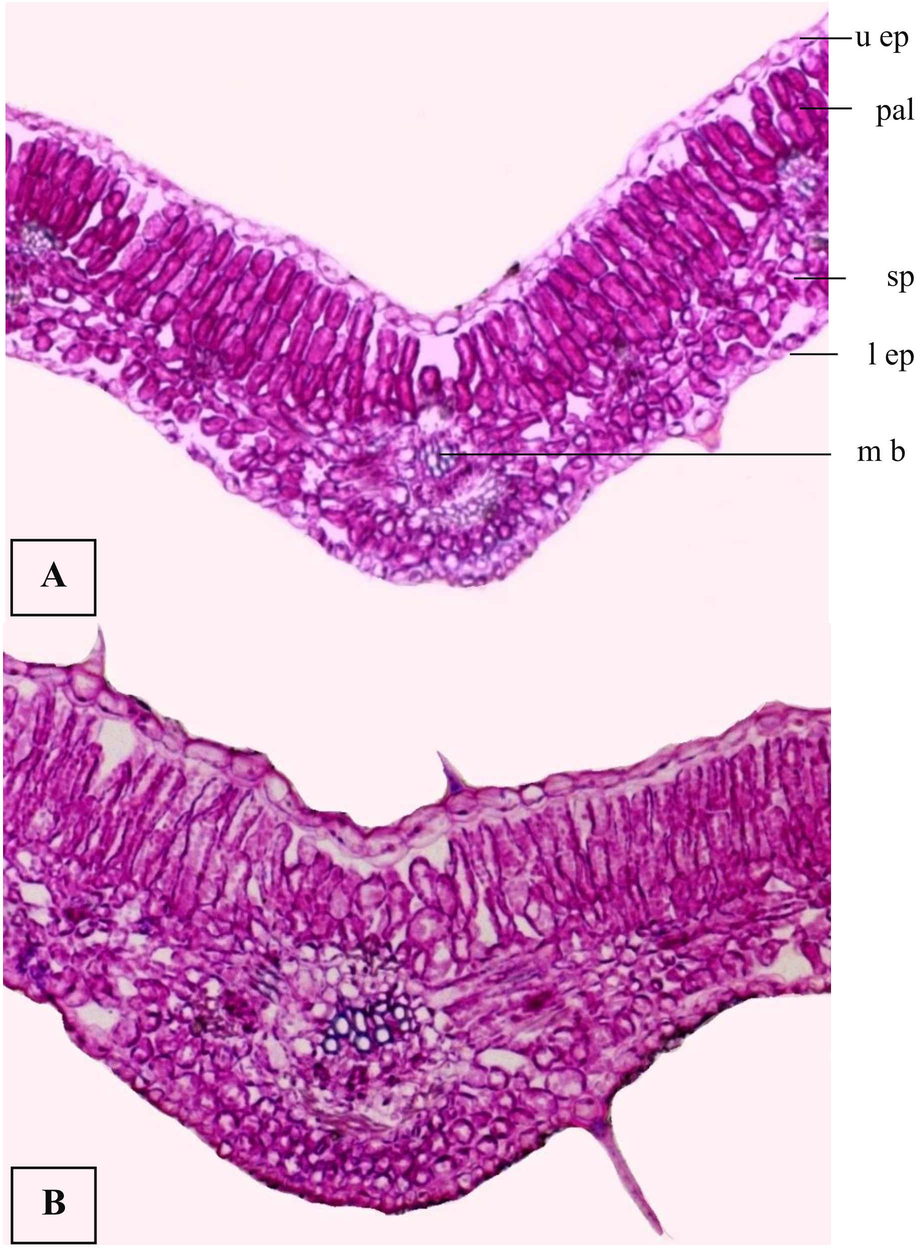

Fig. 4: Transverse sections through the blade of terminal leaflet of the compound leaf developed on the median portion of the main stem of chickpea cv. Giza 195 aged 90 days as affected by foliar application with seaweed extract.
A- From untreated plant (control). (X 100)
B- From plant sprayed with 1 ml seaweed extract/L. (X 100)

Cont.

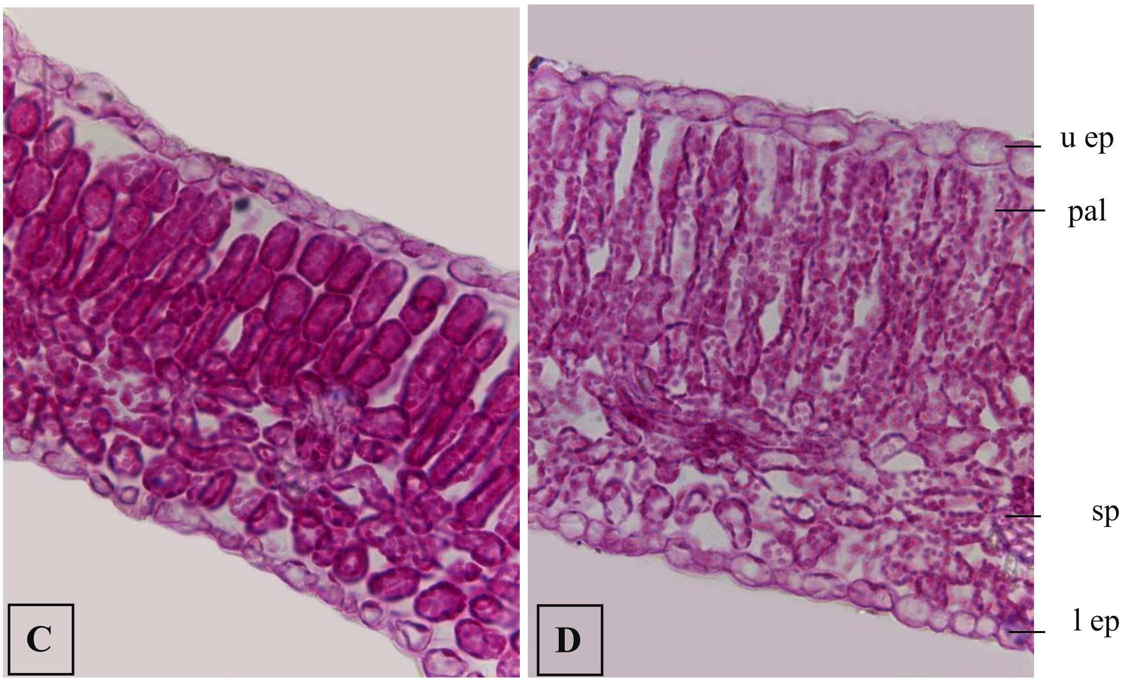

Fig. 4: Cont.
C- Magnified portion of A. (X 294)
D- Magnified portion of B. (X 294)
Details: l ep, lower epidermis; m b, midvein bundle; pal, palisade tissue; sp, spongy tissue and u ep, upper epidermis.

Table 9: Counts and measurements in micrometers (μm) of certain histological features in transverse sections through the blade of terminal leaflet of the compound leaf developed on the median portion of the main stem of chickpea cv. Giza 195, aged 90 days, as affected by foliar spray with seaweed extract (Means of three sections from three specimens)

Histological characters	Treatments		
	Control	Seaweed extract (1 ml/L.)	± % to control
Midvein thickness	252.8	360.5	+ 42.6
Lamina thickness	226.1	274.2	+ 21.3
Palisade tissue thickness	124.9	162.5	+ 30.1
Spongy tissue thickness	76.5	89.1	+16.5
Dimensions of the midvein bundle:			
Length	90.5	158.2	+74.8
Width	72.6	125.9	+73.4
Number of vessels / midvein bundle	10.2	14.1	+38.2
Vessel diameter	8.9	11.2	+25.8

It is realized from Table (9) and Figure (4) that spraying seaweed extract at concentration of 1 ml on chickpea cv. Giza 195 increased thickness of both midvein and lamina of leaflet blades by 42.6 and 21.3% more than those of the control; respectively. It is noted that the increase in lamina thickness which was induced due to spraying with seaweed extract was accompanied with 30.1 and 16.5% increments in thickness of palisade and spongy tissues compared to the control; respectively. Likewise, the vascular bundle of the midvein was increased in size as a result of spraying seaweed extract. The increment was 74.8% in length and it was 73.4% in width more than the control. Also, number of vessels/midvein bundle was increased by 38.2% more than the control. Moreover, xylem vessels increased in diameter, being 25.8% more than the control which amounted to more total active conducting area to cope with vigorous growth resulting from treatment with 1 ml seaweed extract/L.

As far as the authors are aware, previous information about the effect of spraying seaweed extract on anatomical structure of chickpea stems and leaves are not available in the literature. However, Salama and Yousef (2015) using 1.5 ml seaweed extract/L. on basil plant as well as Salama *et al*. (2016) using 1 ml seaweed extract/L. on stevia plant recorded favorable changes in anatomical structure of stems and leaves of investigated species due to treatment with seaweed extract. Stem diameter increased due mainly to the increase induced in thickness of cortex, phloem tissue, xylem tissue and parenchymatous area of the pith compared to the control.

Likewise, seaweed extract induced increase in thickness of both midvein and lamina due to the increase in the thickness of both palisade and spongy tissues as well as in the dimensions of the medvein bundle, being in harmony with the present investigation.

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