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EFFECT OF ALGAE EXTRACT FOLIAR APPLICATION ON YIELD AND QUALITY TRAITS OF SOYBEAN (*GLYCINE MAX* L.) GROWN ON CALCAREOUS SOIL UNDER IRRIGATION WATER REGIME

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Abstract

The present study was conducted during two summer seasons 2017 and 2018 at the farm of El-Nubaria Agricultural Research Station, Ministry of Agriculture and land Reclamation to evaluate the responses of Algae extract foliar application on yield and quality traits of soybean (*Glycine max* L.) grown on calcareous soil conditions under irrigation water regime. Experimental design was a spilt plot design in a complete randomized blocks arrangement with three replications used to conduct all trials, four treatments of algae extractions under two irrigation treatments 75% and 100% of the ETc water stress treatments. Algae extracts in form of foliar application treatments (control, Algae extracts 1 = 1ml/l, Algae extracts 2 = 2ml/l and Algae extracts 4 = 4ml/l). Foliar application of Algae extracts liquid fertilizers are useful for achieving higher agricultural production, because act as plant growth stimulants is improved due to plant growth, protein, carbohydrate production and chlorophyll production. These beneficial effects are most noticeable when the plant is under stress. Algae extracts increased growth, grain yield and oil % of soybean under water stress (75% ETc water of irrigation treatment). These results suggest that foliar application of Algae extracts at the rate of 4 ml/l under water stress is effective strategy to improve soybean productivity under stress. Drought tolerance efficiency % was the highest values at application of 4 ml/l algae foliar spray treatment. The Water Utilization efficiency WUE increased with increasing water stress, means that the algae foliar spray application lead to an increase in plant efficiency to produce higher yield with lower water.

Key words: Algae extracts; foliar application; irrigation water regime; soybean.

Introduction

Soybean (*Glycine max* L.) is one of the most important oil seeds around the world and according to last USDA world statistics the cultivating area of soybean was almost 125.81 Million hectares with an average yield of 2.76 Metric tons per hectare in 2017/2018 (Foreign Agricultural Service/USDA 2018 Office of Global Analysis). As compared to other oil seed crops, soybean collectively occupies around 6% of the world's land under cultivation (Goldsmith, 2008).

The soybean is a member of family Leguminosae, sub-family papilionaceae. It is one of the most important protein and oilseed crops throughout the world. Its oil is the largest component of the world's edible oils. It has emerged as one of the important commercial crops in many countries. Soybean is also known as the "Golden bean" because of its multiple uses (SOPA, 2013).

Soybean is the most widely grown oilseed in the world, with 80 % of its production concentrated in the United States, Brazil and Argentina (USDA 2013). In Egypt, soybean is grown on an area of 15233 ha and its production is 43342 tons with an average seed yield of 2.84 tons ha^{-1} (FAO, 2010). Its seeds consist of 40% protein, 20% oil, 35% carbohydrate and ~5% ash (Jooyandeh, 2011). It can provide oils and vegetable protein suitable for feeding humans as well as animals. Soybean is the most important oil crop of world which

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contains about 18 to 22% cholesterol free oil with 85% unsaturated fatty acids and 38 to 42% protein (Ali *et al.*, 2009). Also, El Agroudy *et al.*, (2011) suggested that the Soybean oil is used directly in food and preventing blood pressure, also Arteriosclerosis. It also contains lot of the essential vitamins for the body, a basic source of protein in the poultry and animal feed.

The seaweed liquid fertilizers are useful for achieving higher agricultural production, because the extract contains growth promoting hormones like Auxins, Gibberellins, Cytokinin, Abscisic acid, Ethylene, Betaine and Polyamines other than the trace elements, vitamins, amino acids, antibiotics and micronutrients (Panda *et al*., 2012). Aziz *et al*., (2011) found that Irrigated plants with 1000 ppm saline water and spraying with 2.5 cm^3/L seaweed extracts gave the highest values of fresh and dry weight of inflorescences. It can be concluded that seaweed extracts application enhances plant tolerance against salinity, where results indicated that application of seaweed extracts at 2.5 and 3.0 cm^3/L and saline water at 1000 ppm had a favorable effect on vegetative growth, and chemicals constituents of plants. Also, Ramya *et al*., (2011) stated that the seaweed liquid extracts of marine algae at lower concentration (1.5%) was found to have maximum influence on growth parameters, shoot length, fresh weight, dry weight, and moisture content. Moreover, biochemical parameters such as photosynthetic pigments, protein content, and sugars, were also enhanced when compared to untreated seedlings. There was also noticeable increase in pod weight, pod length and number of seeds per pod. While, higher concentrations (above 1.5%) were found to show inhibitive effect. Seaweed extracts act as plant growth stimulants; their effectiveness may be influenced by the species included and the manufacturing technique used. Overall crop performance is improved due to plant growth, protein, carbohydrate production and chlorophyll production and photosynthesis. These beneficial effects are most noticeable when the plant is under stress (www.chaseseorganics.co.uk).

El-Sheekh *et al*., (2000) noticed that all the crude extracts of seaweed increased protein content in root and shoot systems, total soluble sugars and chlorophyll content in Vicia faba leaves. Kumar and Sahoo (2011) observed the effect of seaweed liquid extraction growth and yield of *Triticum estivum* var. Application of a lower concentration (20%) of seaweed liquid extract enhanced the percentage of growth and yield, as measured by kernel number and seed dry weight. Also, Khan *et al*., (2009) found that Marine algal seaweed species are often regarded as an underutilized bioresource; many have been used as a source of food, industrial raw materials, and in therapeutic and botanical applications for centuries. In addition to seaweed used as amendments in crop production systems due to the presence of a number of plant growth-stimulating compounds.

Tarraf *et al*., (2015) indicated that foliar application of algae extracts to fenugreek plants significantly increased plant height, number of leaves, number of branches and fresh and dry weights of plant at vegetative growth stage, especially in plants treated with 5 g/L algae extract. Also, plants with algae extract markedly increased nitrogen, phosphorous and potassium contents. In addition, Kasim *et al*., (2015) found that drought treatment (40% and 20% field capacity) resulted in a significant decrease in some growth criteria, photosynthetic pigments through providing hormones and micro nutrients essential for wheat growth. Farooq *et al*., (2009) descripted that drought stress reduces leaf size, and reduces water-use efficiency. Among the nutrients, potassium ions help in osmotic adjustment. Low-molecular-weight osmolytes, including proline and other amino acids, are crucial to sustain cellular functions under drought. Also, Bleakley and Hayes, (2017) algae as a valuable source of nutrition. However, there are inconclusive reports about the digestibility of algae and the bioavailability of the proteins within. Algae offer many advantages as biofuel sources including: high growth rates, high lipid content, the ability to grow on non-agricultural land, and the genetic versatility to improve strains rapidly and produce co-products (Kightlinger *et al*., 2014). Water treatments showed a significant influence on the N utilization rate with average values of 76% for well-watered and 27% for stressed soybeans (Warter *et al*., 2018). In addition, Mohanty *et al.*, (2013) demonstrated that Seaweeds or marine macro-algae are important renewable plant resources. Application of liquid extract from these organisms as foliar spray or seed treatment showed positive result on enhancement of vegetative growth and yield of several crops. Also, they increase the biochemical constituents of plants and possess environmental stress mitigating potential. Amendment of seaweed liquid fertilizer to soils improves the soil health by enhancing the micronutrient quantity and quality, and microbial activity. The objective of this study was mainly to investigate effect of Algae extract foliar application as bio-stimulant on yield and quality traits of soybean grown in calcareous soil under irrigation water regime.

Materials and Methods

Experimental site and conditions

The present study was conducted during two summer seasons 2017 and 2018 at the farm of El-Nubaria

Agricultural Research Station, Ministry of Agriculture and land Reclamation, 30º 54´ N, 29° 52´ E, with altitude of 25 m above sea level. The soil is calcareous (total $CaCO_3$ is 27.2%) and belongs to the order Aridisols, Typic Calciorthids. Soil texture in the experimental site was loamy sand with ECe 1.6 dSm^{-1} and pH 8.3. In addition to organic matter content 0.6%. The irrigation requirements were calculated using CROPWAT 8.0 using the climatic data of Nubaria Research Station weather data. The average values of standard evapotranspiration are shown in Table 1.

Soil samples were collected to determine main soil physical and chemical properties: particle size distribution (sand, silt and clay percentages and soil texture class) were determined according to the FAO (1970). The measured soil chemical parameters were: electrical conductivity (EC), soil reaction (pH), organic matter percentage (OM %), and total calcium carbonate contents were determined according to Page *et al*., (1982). Total Nitrogen in soil was determined by (Bremner and Mulvaney 1982), the amount of available phosphorus in soil was described by Olsen *et al*., (1954) and the concentration of P was measured colorimetrically using the ascorbic acid method (Olsen and Watanabe, 1965), and the concentration of K and Na were measured by flame photometer (Black, 1965). The amounts of available Iron, Manganese and Zinc were determined by extracting the soil with DTPA solution according to (Lindsay and Norverll, 1978). The physical and chemical analysis of soil site is shown in Table 2.

Experiment layout

A spilt plot design in a complete randomized blocks arrangement with three replications was used to conduct all trials (two levels of irrigation water regime, and four treatments of algae extractions). The total numbers of experimental plots were 24 plots. The plants were treated with algae extracts as a foliar application and it is effects on yield and quality traits of soybean (*Glycine max* L.) cultivars under irrigation water regime grown in calcareous soil were studied.

Main Treatments: included Applying 100% ETc of Soybean crop based on ETc at Nubaria region (T1), and Applying of 75% ETc of Soybean crop (T2). The value of ETc was calculated according to the data obtained from reference evapotranspiration and crop factor (ETc = ETo * Kc). The Sub- main treatments included four treatments of algae extracts at rates of 0 (control), 1, 2, and 4 ml/l. These treatments were foliar application twice/ season (June and July), and the chemical composition of algae extract shown in Tables (3 and 4).

Plant-water and irrigation-water measurements

Crop-water relation:

Crop Evapotranspiration (ET_o):

The ET_o was calculated using the climatic data of El-Nubaria Agricultural Research Station, Ministry of Agriculture and land Reclamation, Egypt using CROPWAT 8. (Doorenbos and Kassam, 1986) and the crop evapotranspiration by using the following equation:

$$Etc = ETo \times Kc$$

Where: ET_c: crop evapotranspiration (mm d^{-1}), ETo: reference evapotranspiration (mm d^{-1}) and Kc: Crop coefficient (dimension less)

Applied Irrigation Water: The mount of applied irrigation water was calculated according to the following equation:

$$AIW = ETc / Ea\ (1- LR)$$

Where: AIW: applied irrigation water (mm d^{-1}), ETc: evapotranspiration values (mm d^{-1}) and Ea: irrigation application efficiency (dimension less) and LR: leaching requirements (assumed 10% of the calculated applied irrigation water was additionally applied per irrigation during the growing season for leaching purposes).

Drought Tolerance Efficiency (DTE): was estimated by using the formula given by Fisher and Wood (1981) as follows:

$$DTE(\%) = \frac{Yield\ under\ stress}{Yield\ under\ non\ stress} \times 100$$

Water utilization efficiency values: were estimated according to Iskandar and David (2004) as follows:

Water use efficiency (kgm^{-3}) =

$$\frac{Seed\ Yield\ (kg)}{Water\ applied\ (m^{-3})}$$

Table 1: The monthly average values of reference evapotranspiration of experimental site (Nubaria Research Station) for both planting seasons.

Planting season	April	May	June	July	August	September	October
2017	3.5	4.6	6.1	7.6	6.8	6.2	5.3
2018	4.3	6.1	6.6	7.6	6.4	5.4	4.9

Table 2: Soil physical and chemical analysis at the experimental site.

Texture class	EC dSm^{-1}	pH	$CaCO_3$ %	OM %	Soil available elements, ppm							
					N	P	K	Ca	Na	Fe	Mn	Zn
Loamy Sand	1.6	8.3	27.2	0.6	21	7.8	200	310	400	5.0	1.23	2.9

Table 3: Chemical composition and mineral of algae extract and HPLC chromatogram hormones of algae extract.

	%						ppm			
Element	**N**	**P**	**K**	**Mg**	**Na**	**Ca**	**Fe**	**Zn**	**Mn**	**Cu**
Conc.	13.30	2.22	2.13	0.22	0.01	0.33	1936.00	68.00	21.00	18.00

HPLC chromatogram hormones of algae extract sample, $mg.g^{-1}$		
Indole acetic acid	**Indole butyric acid**	**Gibberellic acid**
13.66	3.25	1.19

Crop

Soybean cultivar (*cv.* Giza 111) was obtained from Food Legumes Research Section, Agricultural Research Center, Giza, Egypt. Soybean cultivar was sow on the 3th may, and was harvested on the 3th of September. N fertilizer was ammonium sluphate (20.5% N, 100 kg/fed), P fertilizer was superphosphate (15.5 % P_2O_5, 300 kg/fed), and K fertilizer was Potassium sulphate (48 % K_2O, 50 kg/fed) which were added according to the recommendation of Ministry of Agriculture and Land Reclamation, Egypt. The plots were irrigated using surface irrigation method and plant development, up to 3 weeks before final harvest. All agronomic practices were keeping normal and uniform for all the treatments.

Crop measurements:

At maturity, a sample of plants was taken from 1 meter/plot that are devoted to determine the following characteristics: Plant length (cm), number of branches / plants, fresh weight/ plant (gm), and dry weight / plant (g). The chlorophyll content was measured in fresh leaves using Chlorophyll meter Spad 502 at 9 Am according to Wood *et al.*, (1992). The result is expressed as chlorophyll index and leaf free proline content determination was done according to Bates *et al.*, (1973). At harvesting time, the yield and yield components were estimated as follows: Number of pods / plants, 100-seed weight (g) and yield (kg/fed). Carbohydrates % was determine in grains according to (DuBois *et al.*, 1956). Seed oil percentage was estimated according to A.O.A.C. (1990). Oil content was calculated as follow: Oil content (%) = (weight of the flask + oil - empty the flask weight/ weight of sample) × 100. Also at harvest samples from leaves and grain were taken for determination of nutrients by methods in by Cottenee, *et al.*, (1982). Statistically analysis were done (Snedecor and Cochran (1990) and Steel and Torrie (1980) for data of two seasons by using the least differences (L.S.D) to compare the means.

Table 4: Amino acids content of the used algae extract.

Amino acid	Appre-viation	concen-tration	Amino acid	Appre-viation	conce-ntration
Aspartic	ASP	1.85	Threonine	THR	0.83
Serine	SER	0.70	Glutamic	GLU	2.24
Proline	PRO	0.67	Glycine	GLY	1.07
Alanine	ALA	1.55	Valine	VAL	1.11
Methionine	MET	0.33	Isoleucine	ISOL	0.71
Leucine	LEU	0.29	Tyrosine	TYR	0.53
Phenylalanine	PHE	0.87	Histidine	HIS	0.24
Lysine	LYS	0.70	Arginine	ARG	0.98
Cysteine	CYC	0.22			
Total amino acids					15.89

Results and Discussion

Applied Irrigation Water (AIW)

The applied irrigation water was calculated using equation No. 2, the obtained results showed that the AIW was approximately the same and the slightly difference between the two seasons resulted from the difference in weather conditions between the two seasons, the irrigation process was happened according to the irrigation rotation in the experimental site, the AIW was calculated from the sum of irrigation requirements per day and the number of days between the two irrigation times. The AIW quantities for the two planting seasons were showed in Table 5a and measured using a water gauge. Also, Table 5b showed that average applied irrigation water during 2017 and 2018 season was 2674.98 and 2006.23 m^3/fed under100% of the ETc and 75% ETc respectively. This may be to results showed that 75% ETc is a suitable treatment for achieving efficient for using water, it can provide about 25 % of water requirements in two seasons.

Growth Characteristics

The results demonstrates in Table 6 showed that the vegetative growth characteristics of soybean cultivar grown under the effect of two irrigation treatments (100% ETc and 75% ETc) and applied Algae extract spray. The data indicated that there were no significant difference between the two irrigation treatment with plant length, fresh weight, number of pods/plant and number of branches/plant. While dry weight was increased significantly by increasing irrigation at 100%ETc.

Results in Table 6 showed that foliar application of Algae extract treatments was significantly with Algae 2 and Algae 4 at all vegetative growth characteristics of soybean compere to control. While, Algae 2 cleared that there was a high significantly with fresh weight, number of pods/plant and number of branches/ plant.

The interaction effect between two irrigation treatments (100% ETc and 75% ETc) and applied

Algae extract (Algae ml/l, Algae2 ml/l and Algae4 ml/l) on growth characteristics.

Plant height: The obtained results showed that plant height was affected by different treatments of algae extract which was added as foliar application under different irrigation water regimes. The results showed that the plant height increased significantly under 100% ETc between Control group and the treated plants with Algae extract with different concentrations. Also, the results showed that no significant difference between 1 and 2 ml/l and it was significantly higher under 4 ml/l. Under 75% ETc, the results cleared that plant height under foliar spray of algae- extract was significantly higher than control and the increase of plant height was significant between 1 and 4 ml/l and not significant between 1 and 2 ml/l. Moreover the difference between plants under different irrigation regimes was not significant between plants subjected to the same concentration of Algae extract Table 6. These findings are in agreement with Mahajan (2014) noted that plant height was varied significantly due to the soybean treatments at maturity under foliar spray of seaweed extract. Also, Anisimov and Chaikina (2014) showed that the aqueous extracts of algae in varying degrees have a positive effect on the length of the roots of soybean. The highest stimulatory effect showed extracts of the green alga at concentrations of 10^{-5} gSW mL^{-1}, and the roots on 18.0% were longer than the control roots. Highest and lowest height had shown in 70 and 30% FC treatments (Rezaei *et al*., 2012) and (da Costa *et al.,* 2017).

Fresh weight g/ plant: The results showed that fresh weight increased significantly between Control and other plants subjected to different concentrations of Algae extract and the increase in plant fresh weight increased significantly with increasing algae concentration. The same trend was found for plants grown under 75% ETc regime. Also, the results showed that plant fresh weight increased significantly between plants grown under 100% and 75% ETc except under 1 and 2 ml/l concentrations the increase was not significant (Table 6).

Dry weight / plant: The plant dry weight data showed that the values under 100% ETc wasn't significant between control and 1 ml/l algae extract. The results also cleared that the increase in using algae extract showed a significant difference between control, 1ml/l and the other concentrations of Algae extract (2, 4 ml/l). Under 75% ETc, the obtained results showed that the values of plant dry weight (g/plant) increased significantly between control and other treatments and wasn't significant between 2 and 4 ml/l algae extract. the results also showed no significant difference between algae treatments under 100% and 75% ETc, that means algae extract foliar application for plants decrease the effect of water stress and this was clear with control where; the difference was significant with the algae treatments Table 6. These results go in line with Shaaban *et al*., (2010) observed that dry matter accumulation in the wheat shoots were determined 66 days after sowing. The highest values were recorded with 1.0 g/L algal extract + 1.0 g/L micronutrient fertilizer. As previously mentioned, this treatment was the best to realize the nearest nutrient ratios to the optimums.

Number of pods / plants: The results showed that the number of pods per plant showed a significant difference between control and different algae treatments except between 1 and 4 ml/l. under water stress treatment 75% ETc, the results showed that there was no significant difference between control and algae treatments except between control and 4 ml/l concentration, this might be attributed to the good effect of Algae extract on plant growth Table 6. These results go in line with Mahajan (2014) found that significantly highest number of pods per plant was observed in Seaweed granules + seaweed foliar spray (37.14) while poorest number of pods per plant was noted in control (20.85). Also, Hasanah *et al*., (2017) found that increased drought stress (80% - 40% of FC) tends to decrease number of filled pods. The number of pods per plant decreased sharply in the severe stress treatment, as compared to the moderate stress and recommended levels (Bourgault *et al.,* 2007 and El-Nwehy *et al.,* 2018).

Number of branches / plant: The results indicated showed that under 100% ETc treatment there was a significant difference between control plants and plants treated with seaweed algae extract except under 1 ml/l concentration there was no significant effect but when the concentration of algae extract, this lead to a significant difference, this means that algae extract had a good effect on plant growth and its branching. Under 75% ETc treatments, there was a significant difference between control plants and the algae extract plants treatment. In addition to that, there wasn't difference between the algae extract treatments, this means that foliar spray of algae decreased the effect of water stress on plant branching Table 6. These results go in line with Mahajan (2014) showed that significantly maximum number of branches per plant was obtained in Seaweed granules + seaweed foliar spray (4.74) and minimum number of branches was observed in control (4.21). Drought stress reduced the number of branches in Soybean (Rezaei *et al.,* 2012).

Yield and yield component:

Data listed in Table 7 illustrated that there were no

Table 5a: Amount of applied irrigation water in field experiment during 2017 and 2018 growing seasons.

Date of irrig-ation	No. days Between irrig-ation	Quantity of applied irrigation water (m^3/fed)/irrigation interval			
		2017		2018	
		100% ETc	75% ETc	100% ETc	75% ETc
5/6	11	177.1	132.8	226.5	169.87
16/6	14	394.55	295.9	426.89	320.16
30/6	14	491.56	368.67	236.5	177.37
14/7	11	551.76	413.82	702.24	526.68
25/7	10	336.3	252.22	316.01	237.00
4/8	11	345.57	259.17	371.44	278.58
15/8	13	233.4	175.05	168.96	126.72
28/8	10	168.43	126.32	202.75	152.06
Total		2698.67	2024.0	2651.29	1988.46

Table 5b: Average applied irrigation water during 2017 and 2018 season.

Seasons	Quantity of applied irrigation water (m^3/fed)	
	100% of the ETc	75% of the ETc
2017	2698.67	2024.0
2018	2651.29	1988.46
average	2674.98	2006.23

significant difference between the two irrigation treatments for yield and yield component. This due to irrigation under 75% ETc treatment gave good values with Yield (ton/ha), 100-seed weight and Oil (%). On the other side, treatments of seaweed algae extract showed that yield and yield component had significant higher at Algae2 ml/l and Algae4 ml/l compere with control. Also, Algae 2 ml/l achieved significant higher with 100-seed weight.

These results are due to the interaction effect between two irrigation treatments (100% ETc and 75% ETc) and applied Algae extract foliar spray (Algae ml/l, Algae2 ml/l and Algae4 ml/l) on yield and yield component.

Yield (ton/ha): The indicated results confirmed that the algae extract had a significant increase on plant yield, where a significance difference was found between the control plants and treated plants with algae foliar spray, moreover no significant difference was found between the algae treatments where the foliar spray of algae increased yield with increasing the rate of application of algae application but not with a significant effect table 7. Under 75% ETc, the same trend was found exception there wasn't a significant effect between 1 and 2 ml/l treatments and between 1 and 4 ml/l treatments. These results cleared that the application of algae foliar spray enhanced the productivity of soybean yield. Bourgault et *al*., (2007) suggested that regulated deficit irrigation decreased yields of bean, but increased yields of green gram. Alternate furrow irrigation did not reduce yields but green gram yields were higher than those of common bean.

100-seed weight: For 100-seed weight, the water stress reduced its weight where; under 100% ETc (no stress), results showed that the same trend of total yield was found where, the 100-seed weight increased with increasing the application rate of algae extract and this increase was significant with control plants and there was no significant difference between algae treatments. Under 75% ETc treatment (water stress) the algae foliar application increased 100-seed treatment but this increase no significant effect was found between whole treatments Table 7. These findings are in agreement with those found by Mahajan (2014) indicated that seed yield per plant was varied significantly due to the soybean treatments under foliar spray of seaweed extract. Drought stress reduced the number of seeds per plant in soybean. Grain weight of 1000 was decreased with increasing the drought stress (Rezaei *et al*., 2012 and El-Nasharty *et al*., 2017). Moreover, Hasanah *et al*., (2017) showed that increased drought stress (80% - 40% of FC) tends to decrease 100-dry seeds weight. Seeds of green gram are much smaller than those of bean: 5 to 6 g per 100 seeds compared to 35-43 g (Bourgault *et al*., 2007). Oil (%): The obtained results confirmed that water stress had no significant effect with the control and treated plants with 1 ml/l algae extract and the difference was significant with 2 and 4 ml/l treatments Table 7. In addition to that it was clear that seaweed algae extract decreased the impact of water stress on grown plants. These findings are in agreement agree with those found by Mahajan (2014) found that the maximum oil (%) was (18.56) While control (17.90) had the lowest value as compared to all others. Also, Ku *et al*., (2013) the results confirmed both the negative correlation between seed protein and seed oil contents as well as the effect of drought on seed protein and seed oil contents. Mertz-Henning *et al*., (2017) noted that the effect of water deficit at vegetative stress and reproductive stress on oil contents of grains in different soybean. So, oil content ranged from 20.63 to 22.57%.

Biochemical characteristics:

Table 8 cleared that irrigation under 75% ETc treatments had significantly effect on the proline content compere to 100% ETc this may be due to the proline increase under water stress. While, the chlorophyll content, carbohydrates, Nitrogen and Protein content (%) were no significant effect between two irrigation treatment (100% ETc and 75% ETc), In addition, comprising between the application rate of algae foliar extract showed that there significant with Algae 2 ml/l and Algae 4 ml/l for all parameters. Moreover, foliar application of Algae 2 ml/l was the best treatment of chlorophyll content. In this respect the interaction effects of water irrigation regime and algae extract treatments were illustrated in tables 8.

Proline contents: showed that the water stress increased significantly the proline content, where it was found that under 100% ETc there was no significant effect between control plants and 1 ml/l treatment and the proline content increased significantly with increasing the application rate of algae foliar extract. Under 75% ETc treatment the same trend was found. This means that water stress increasing the content of amino acids in plant tissues Table 8. Similar results were obtained by Dehnavi and Sheshbahre (2017) noted that leaf proline content increased in response to drought stress. Mafakher *et al*., (2010) indicated that the proline content of the leaf increased at both growth stages in all varieties of chickpea in response to drought.

Chlorophyll content: The results showed that the chlorophyll content decreased with increasing water stress level and the rate of algae extract application rate where; under 100% ETc treatment, the results cleared that there wasn't a significant effect between control and 1 ml/l treatment but with increasing the rate of algae extract application the chlorophyll increased. In addition to that there was a significant effect between control and the algae foliar application treatments with all it levels and that means, algae foliar extract can decrease the effect of water stress on plant chlorophyll content Table 8. These results go in line with Chojnacka *et al.,* (2012) and Mahajan (2014) found that significantly maximum chlorophyll content was noted in Seaweed granules + seaweed foliar spray (33.84) and minimum value was noted in Control (30.68). Ku *et al.,* (2013) obtained that the degree of chlorophyll reduction in soybean leaves with the strength of drought treatments. Mafakher *et al.,* (2010) showed that drought stress imposed at the vegetative stage, significantly decreased content and total chlorophyll. Also, Hasanah *et al.,* (2017) indicated that increased drought stress (80% - 40% of FC) tends to decrease chlorophyll total content. Godlewska *et al.*, (2016) indicated that the content of chlorophyll was 2.5 times higher in 0.5% algae extract than in the control. Extracts showed the slight impact on the morphology of plants.

Carbohydrates: As the indicated results showed, the water stress increased the carbohydrate content in plant tissues but this increase wasn't significant under 100% ETc treatment except with increasing the application rate of algae spray to 4 ml/l, it was significant with the control plants. Under water stress treatment (75% ETc) results showed that the algae spray treatments increased the carbohydrates content significantly when compared with the control plants but there was no significantly affect with increasing the rate of algae spray Table 8. These findings are in agreement w-ith those found by Dehnavi and Sheshbahre (2017) noted that

Table 6: Effect of Algae extract foliar spray on growth characteristics of soybean cultivar grown on calcareous soil under irrigation water regime (combined analysis of two successive seasons).

Treatment	Algae (ml/l)	Plant length (cm)	Fresh weight (gm/ plant)	Dry weight (gm/ plant)	Number of pods/plant	Number of branchs/plant
100% of the ETc (2674.98m^3/fed)	C	66.7 de	176.4 e	141.3 cd	23 d	3.1 c
	Algae ml/l	77.7 bc	205 cd	148.9 cd	29.2 b	3.7bc
	Algae2 ml/l	82.7 bc	266.7 b	173.3 b	36.7 a	4.7 a
	Algae4 ml/l	91.7a	297.3 a	208.3 a	30 b	4.7 a
75 % of the ETc (2006.23m^3/fed)	C	61.7 e	145.7 f	92 e	22.7 d	3.3 c
	Algae ml/l	74.5 cd	181.2 de	132.3 d	24.7 cd	4.3 ab
	Algae2 ml/l	80.3 bc	265.6 b	160.2 bc	26.3 bcd	4.7 a
	Algae4 ml/l	86 ab	222.9 c	156.7 bc	28.3 bc	4.7 a
Mean values of (Irr)	100%ETc	79.7	236.36	167.97	29.7	4.03
	75%ETc	75.6	203.83	135.31	25.5	4.25
Mean values of (Algae)	C	64.2	161.1	116.7	22.8	3.2
	Algae	76.1	193.1	140.6	27	4
	Algae2	81.5	266.1	166.8	31.5	4.7
	Algae4	88.8	260.1	182.5	29.2	4.7
LSD 5%	Irr	8.816	42.391	25.426	3.678	0.657
	Algae	6.249	31.754	28.355	4.484	0.601
	Irr x Algae	8.968	24.932	22.744	3.910	0.890

carbohydrate contents increased in response to drought stress.

Nitrogen and Protein content (%): The results showed that there was no significantly affect for water stress neither algae spray application treatments on nitrogen or protein content in plant tissues Table 8. In generally, algae extracts act as plant growth stimulants plant growth for protein and carbohydrate production prolonged chlorophyll production and photosynthesis.

Element content in leaves of soybean:

Mean values of Irrigation water regime at both 100% ETc and 75% ETc treatments were significant different effect in K, Zn and Cu content in leaves of soybean Table 9 and Fig. 1. On contrast, the data of results indicated that element content in leaves of soybean concerning Ca content, Na, Mg, Fe and Mn content these elements weren't a significant difference under two irrigation regime. In addition, results in Table 9 cleared that there were significant difference between all treatments and control with element content in leaves of soybean concerning application of Algae foliar spray. It is also clear that Algae 4 ml/l followed by Algae 2 ml/l were the best treatment effect for element content in leaves of soybean.

On the same distance, the interaction of the results between irrigation water regime and treatments of application of Algae foliar spray in soybean cleared that there wasn't a significant difference in potassium content under water treatments, 100% ETc and 75% ETc (water stress) decreased the potassium content but not significantly except control and plants treated with 1 ml/l algae extract Table 9 and Fig. 1. Moreover, the calcium concentration in plant tissues showed that there was a significant difference between control and algae spray application treatments except between control and 1 ml/l concentration for 100% ETc, under water stress (75% ETc) the results cleared that there was not a significant difference between control and 1 ml/l and between 1 and 2 ml/l algae algae foliar spray, but the high application rate of algae 4ml/l resulted in a significant increase in calcium concentration compared with control and other algae treatments, this might be attributed to, the algae high concentration makes the plant more healthy under the conditions of water stress Table 9 and Fig. 1. In addition, to that the results of sodium content showed that there was a significance difference between control and treated plants with algae foliar application under both 100% and 75% ETc. Also, there was a significant difference between algae treatments under 100% ETc and the same trend was found with 75% ETc except between 1 and 2m/l concentrations Table 9 and Fig. 1. Magnesium content results showed that there was a significant difference between control and different algae concentrations under 100% ETc irrigation treatment and the same trend was found with 75% ETc treatment except between 1 and 2 ml/l concentrations.

Fe concentration (ppm) in plant tissues showed that there was a significant difference between control and different algae spray treatments under both 100% and 75% ETc Table 9 and Fig. 1. Mn concentration showed that there was a significant difference between control and algae treatments under both 100% and 75% ETc irrigation treatments but between the algae treatments there wasn't a significant difference between 2 and 4 ml/l at 100% ETc treatment, and between 1 and 2 ml/l treatments under 75% treatments Table 9 and Fig. 1. Zn concentrations showed that there wasn't a significant difference between control and 1 ml/l algae treatment but it was significant for the other treatments. At 75% ETc treatment there was a significant difference between control and algae spray treatments Table 9 and Fig. 1. Cu concentration (ppm) cleared that water stress decreased the concentration of Cu but the increase in algae- foliar spray enhanced the increase in Cu, in addition to that there was a significant difference between control and different algae treatments under 100% ETc treatment. At 75% ETc treatment

Table 7: Effect of Algae extract foliar spray on yield component of soybean cultivar grown on calcareous soil under irrigation water regime (combined analysis of two successive seasons).

Treatment	Algae (ml/l)	Yield (ton/ha)	Weight of 100 grain (gm)	Oil %
100% of the ETc ($2674.98\ m^3$/fed)	C	2.73 c	32.5 c	19 d
	Algae ml/l	4.09 ab	40 ab	19.8 cd
	Algae 2 ml/l	4.31 ab	42.5 a	19.9 cd
	Algae 4 ml/l	4.57 a	42.5 a	22.1 a
75 % of the ETc ($2006.23\ m^3$/fed)	C	2.41 c	31.7 c	19 d
	Algae ml/l	3.93 ab	34.2 c	19.7 cd
	Algae 2 ml/l	3.65 b	36.7 bc	21.2 ab
	Algae 4 ml/l	4.39 a	35.8 bc	20.4 bc
Mean values of (Irr)	100% ETc	3.92	39.4	20.2
	75% ETc	3.59	34.6	20.1
Mean values of (Algae)	C	2.57	32.1	19
	Algae ml/l	4.01	37.1	19.8
	Algae 2 ml/l	3.98	39.6	20.6
	Algae 4 ml/l	4.48	39.2	21.3
LSD 5%	Irr.	0.70	3.608	0.976
	Algae	0.52	4.847	0.944
	Irr. x Algae	0.72	5.373	1.033

there was a significant difference between control and 4 ml/l algae treatment plants and not significant with 1, 2 ml/l algae treatments Table 9 and Fig. 1. These findings are in agreement with those found by Shaaban *et al*., (2010) noted that the most increment in K concentration in shoot tissues was obtained by the extra algal extract treatment. This may due to the reasonable percentage of K in the algal extract where it reached more than 1.5% on dry weight basis in different green algae species (El-Fouly *et al*., 1992) as well as the used alga that contain more than 0.5% on dry weight basis. Magnesium concentrations were superior with the 1.0 g/L algal extract as well as 2.0 g/L micronutrients fertilizer. Magnesium found also in a high percentage in the green micro-algae (more than 1.0% on dry weight basis, however, micronutrients fertilizer may consider the cause that led to more Mg absorbance Shaaban *et al*., 2010. the highest N, P, K and Mg-uptakes were recorded with the modified algal extract. Godlewska *et al*., (2016) indicated that the content of elements Zn and Na in the group treated with 10% algae extract was higher by 76%, 48%, 31%, and 59% than in the control, respectively.

Element content in grain of soybean:

Table 10 and Fig. 2 illustrated that effect of Algae extract foliar spray on nutrients content in grain soybean cultivar grown on calcareous soil under irrigation water regime. The results showed that there significant differences in element content in grain of soybean but Mn content in grain no significant effect under two irrigation water regime. In side that, application of Algae extract foliar spray on nutrients content in grain of soybean proved that the addition of Algae 4 ml/l was significant effect compere with control of K, Na, Fe, Zn, Mn, Cu content followed by Algae 2 ml/l with Na, Fe, Zn, Mn content. While, Ca and Mg content noted that there wasn't significant between treatment and control of application of Algae extract foliar.

Interaction between irrigation and algae treatments of Potassium content results showed that the water stress resulted in a significant decrease in grain potassium, also the results cleared that, for 100% ETc there was a significant increase with increasing the quantity of applied algae extract except at 1 ml/l the difference with control wasn't significant. In addition to that, under 75% ETc treatment the difference with control wasn't significant except with 4 ml/l algae treatment, the results showed that it was significant, this might be attributed to that the high application rate of algae extract resulted in increasing the potassium content Table 10 and Fig. 2. For calcium content the results showed that, water stress had a significant effect on calcium content in soybean grains where; a significant decrease was found in Ca content with increasing water stress. Under 100% ETc treatments, there was a significant effect of algae extract with all its treatments on calcium content comparing with control. For 75% ETc treated plants, the result showed that, there wasn't a significant effect for algae treatment on calcium content except for 2 ml/l treatment the difference was significant Table 10 and Fig. 2. Na (%) readings showed that water stress didn't affect sodium concentration significantly. For 100% ETc treated plants,

Table 8: Effect of Algae extract foliar spray on Bio chemical characteristics of soybean cultivar grown on calcareous soil under irrigation water regime (combined analysis of two successive seasons).

Treatment	Algae (ml/l)	Proline (μg/g)	Chlorophyll index	Carbohydrates % in grains	N% in grains	Protien % in grains
100% of the ETc (2674.98 m^3/fed)	C	16.5 e	35 d	45.6 bc	6.03 ab	37.7 ab
	Algae ml/l	25.5 e	37.7bcd	46.7 abc	6.13 ab	38.3 ab
	Algae 2 ml/l	42 d	41.5 a	46.1 abc	6.19 ab	38.7 ab
	Algae 4 ml/l	53.1 c	40.8 a	47.4 a	6.25 a	39 a
75 % of the ETc (2006.23 m^3/fed)	C	58.3 c	31.3 e	45 c	5.99 b	37.4 b
	Algae ml/l	62.3 c	37 cd	46.5 abc	6.04 ab	37.8 ab
	Algae 2 ml/l	91.5 b	40.3ab	46.6 abc	6.17 ab	38.6 ab
	Algae 4 ml/l	113.7 a	39.5abc	47.3 ab	6.13 ab	38.3 ab
Mean values of (Irr)	100% ETc	34.2	38.8	46.5	6.2	38.5
	75% ETc	81.5	37	46.4	6.1	38
Mean values of (Algae)	C	37.4	33.2	45.3	6	37.6
	Algae ml/l	43.9	37.3	46.6	6.1	38.1
	Algae 2 ml/l	66.7	40.9	46.4	6.2	38.7
	Algae 4 ml/l	83.4	40.2	47.3	6.2	38.7
LSD 5%	Irr	17.14	2.962	0.986	0.118	0.738
	Algae	32.214	2.177	1.127	0.154	0.962
	Irr x Algae	9.208	2.705	1.761	0.233	1.456

Table 9: Effect of Algae extract foliar spray on nutrients content in leaves of soybean cultivar grown on calcareous soil under irrigation water regime (combined analysis of two successive seasons).

Treatment	Algae ml/l	K%	Ca%	Na%	Mg%	Fe (ppm)	Mn (ppm)	Zn (ppm)	Cu (ppm)
100% of the ETc (2674.98 m^3/fed)	C	0.68 b	2.4 de	1.33 e	0.30 d	368 e	149 d	30.5 cd	18.5 e
	Algae ml/l	0.73 ab	2.6 cd	1.70 cd	0.34 c	417 d	175.5 c	32 bc	26.5 c
	Algae2 ml/l	0.74 a	2.7 bc	2.15 a	0.39 b	480.5 bc	252.5 a	39 a	32.5 b
	Algae4 ml/l	0.75 a	3.1 a	1.95 b	0.42 a	525.5 a	268 a	41 a	41.7 a
75 % of the ETc (2006.23 m^3/fed)	C	0.52 d	2.3 e	1.26 e	0.28 d	376 e	97.7 e	28.3 d	17 e
	Algae ml/l	0.59 c	2.4 de	1.65 d	0.33 c	426.3 d	181 c	32 bc	18 e
	Algae 2 ml/l	0.72 ab	2.6 cd	1.75 cd	0.35 c	456.3 c	189 c	32.3 bc	21 de
	Algae 4 ml/l	0.73 ab	2.9 ab	1.85 bc	0.41 ab	497 b	213 b	33.5 b	23 cd
Mean values of (Irr)	100%ETc	0.72	2.7	1.8	0.36	447.8	211.3	35.6	29.8
	75%ETc	0.64	2.5	1.6	0.34	438.9	170.2	31.5	19.8
Mean values of (Algae)	C	0.6	2.4	1.3	0.29	372	123.3	29.4	17.8
	Algae ml/l	0.66	2.5	1.7	0.34	421.7	178.3	32	22.3
	Algae 2 ml/l	0.73	2.6	2	0.37	468.4	220.8	35.6	26.8
	Algae 4 ml/l	0.74	3	1.9	0.41	511.3	240.5	37.3	32.3
LSD 5%	Irr	0.062	0.245	0.245	0.042	47.696	42.136	3.261	5.761
	Algae	0.077	0.194	0.171	0.025	19.949	34.233	3.833	8.181
	Irr x Algae	0.056	0.261	0.161	0.032	24.349	17.177	2.831	4.109

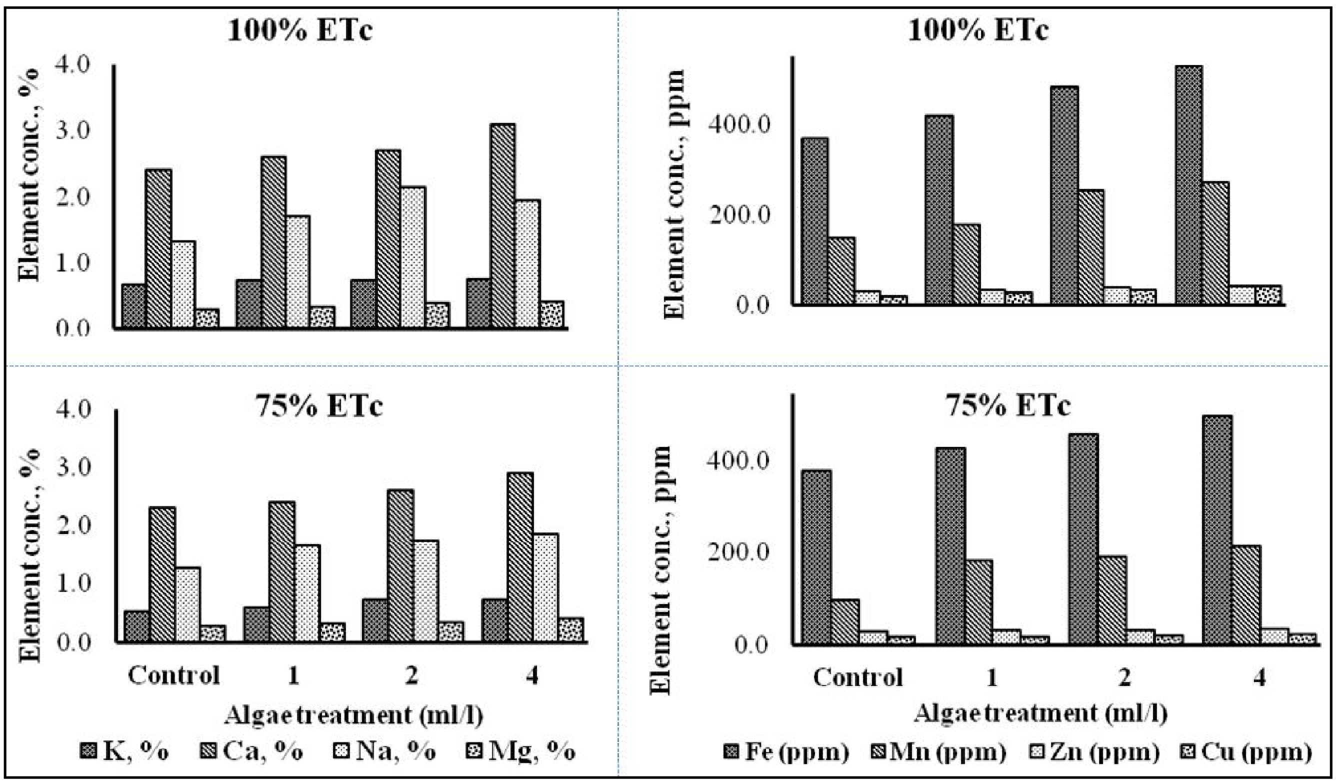

Fig. 1: Effect of Algae extract foliar spray on nutrients content in leaves of soybean cultivar grown on calcareous soil under irrigation water regime (combined analysis of two successive seasons).

the results explained that algae extract had a significant effect on Na concentration compared with control except at 1 ml/l treatment. The analysis of 75% ETc treated plants showed that only 4 ml/l concentration had a significant effect compared with control Table 10 and Fig. 2. Mg concentration results (%) showed that water stress decreased significantly the concentration of Mg, where; under the circumstance of application of 100% ETc, there was a significant increase comparing with control, in addition to there wasn't found a significant difference between 1 and 2 ml/l algae extract treatments. For 75% treatment, the results showed that there wasn't

Table 10: Effect of Algae extract foliar spray on nutrients content in grains of soybean cultivar grown on calcareous soil under irrigation water regime (combined analysis of two successive seasons).

Treatment	Algae ml/l	K%	Ca%	Na%	Mg%	Fe (ppm)	Mn (ppm)	Zn (ppm)	Cu (ppm)
100% of the ETc (2674.98 m^3/fed)	C	1.95 bc	0.38 b	2.25 bc	0.067 c	99 cd	29.5 e	34.5 c	27.7 b
	Algae ml/l	2.05 ab	0.40 a	2.35 ab	0.069 bc	106 c	34.3 cd	38.5 b	32 a
	Algae2 ml/l	2.15 a	0.41 a	2.45 a	0.079 a	128 b	44 b	39 b	33 a
	Algae4 ml/l	2.10 a	0.41 a	2.47 a	0.075 ab	164 a	48 a	41.5 a	33.5 a
75 % of the ETc (2006.23 m^3/fed)	C	1.80 d	0.36 c	2.13 c	0.051 e	84.5 e	25.5 f	32.5 c	24 c
	Algae ml/l	1.85 cd	0.36 c	2.23 bc	0.052 de	93 de	32.3 d	33 c	25 bc
	Algae2 ml/l	1.87 cd	0.38 b	2.25 bc	0.055 de	102 cd	36 c	33 c	25 bc
	Algae4 ml/l	2.05 ab	0.37 bc	2.40 a	0.059 d	135.5 b	41.7 b	34 c	27.3 b
Mean values of (Irr)	100%ETc	2.06	0.40	2.38	0.07	124.3	39	38.4	31.5
	75%ETc	1.89	0.36	2.25	0.05	103.8	33.9	33.1	25.3
Mean values of (Algae)	C	1.9	0.37	2.2	0.059	91.8	27.5	33.5	25.8
	Algae	2	0.38	2.3	0.06	99.5	33.3	35.8	28.5
	Algae 2 ml/l	2	0.39	2.4	0.067	115	40	36	29
	Algae 4 ml/l	2.1	0.39	2.4	0.067	149.8	44.8	37.8	30.4
LSD 5%	Irr	0.089	0.011	0.095	0.005	20.356	6.009	1.851	2.069
	Algae	0.145	0.024	0.117	0.013	15.545	4.059	3.956	4.637
	Irr x Algae	0.116	0.014	0.122	0.008	9.971	2.771	2.207	3.115

Table 11: Drought Tolerance Efficiency (DTE) as affected by irrigation treatment and foliar application of Algae extract in two seasons.

(DTE) %	Foliar application
88.2399	Control
96.013	1 ml/l of algae
84.76664	2 ml/l of algae
96.05901	4 ml/l of algae
91.26976	Mean

Table 12: Water utilization efficiency (kg /m^3 water) as affected by irrigation treatment and foliar application of Algae extract in two seasons.

Irrigation treatment		Foliar
75 % of the ETc	100 % of the ETc	application
0.50	0.42	Control
0.82	0.64	1 ml/l of algae
0.76	0.67	2 ml/l of algae
0.91	0.71	4 ml/l of algae
0.75	0.61	Mean

a significant effect for algae treatments comparing with control except at 4 ml/l algae extracts concentration Table 10 and Fig. 2.

The micro nutrients concentration under water stress and algae treatments showed that Fe (ppm) water stress decreased the concentration of Fe in grains where; under the circumstance of 100% ETc application, there was a significant increase in Fe concentration comparing with control except the lower concentration (1 ml/l algae) and the same trend was found at 75% ETc treatment except there wasn't a significant difference between 1 and 2 ml/l algae extract treatments Table 10 and Fig. 2. For Mn concentration (ppm) the results showed that water stress decreased its concentration significantly under different algae treatments. For 100% ETc treatment, the results showed that, there was a significant difference between control and algae treatments and the same trend was found at 75% ETc Table 10 and Fig. 2. Zn concentration (ppm) showed that water stress decreased the concentration of Zn significantly. Under the circumstance of 100% ETc treatment, the results showed that the concentration of Zn (ppm) increased significantly with increasing algae extrac-t concentration except between 1 and 2 ml/l treatments. At 75% ETc treatment the results showed that the increase in Zn (ppm) wasn't significant for different algae treatments Table 10 and Fig. 2. Cu (ppm) concentration in grains results showed that water stress decreased significantly Cu (ppm) concentration. The results also showed that, under 100% ETc treated plants there was a significant increase in plants treated with algae compared with control but the Cu (ppm) concentration didn't affect significantly with increasing algae extract concentration. Under 75% ETc Cu (ppm) was affected with the effect of water stress and algae extract together, where; there wasn't a significant effect between algae treatments comparing with control except at 4 ml/l treatment, in addition to that there wasn't a significant difference between different algae treatments Table 10 and Fig. 2. These results go in line with Mahajan (2014) observed that the seed nitrogen content (%) was significant variability due to soybean

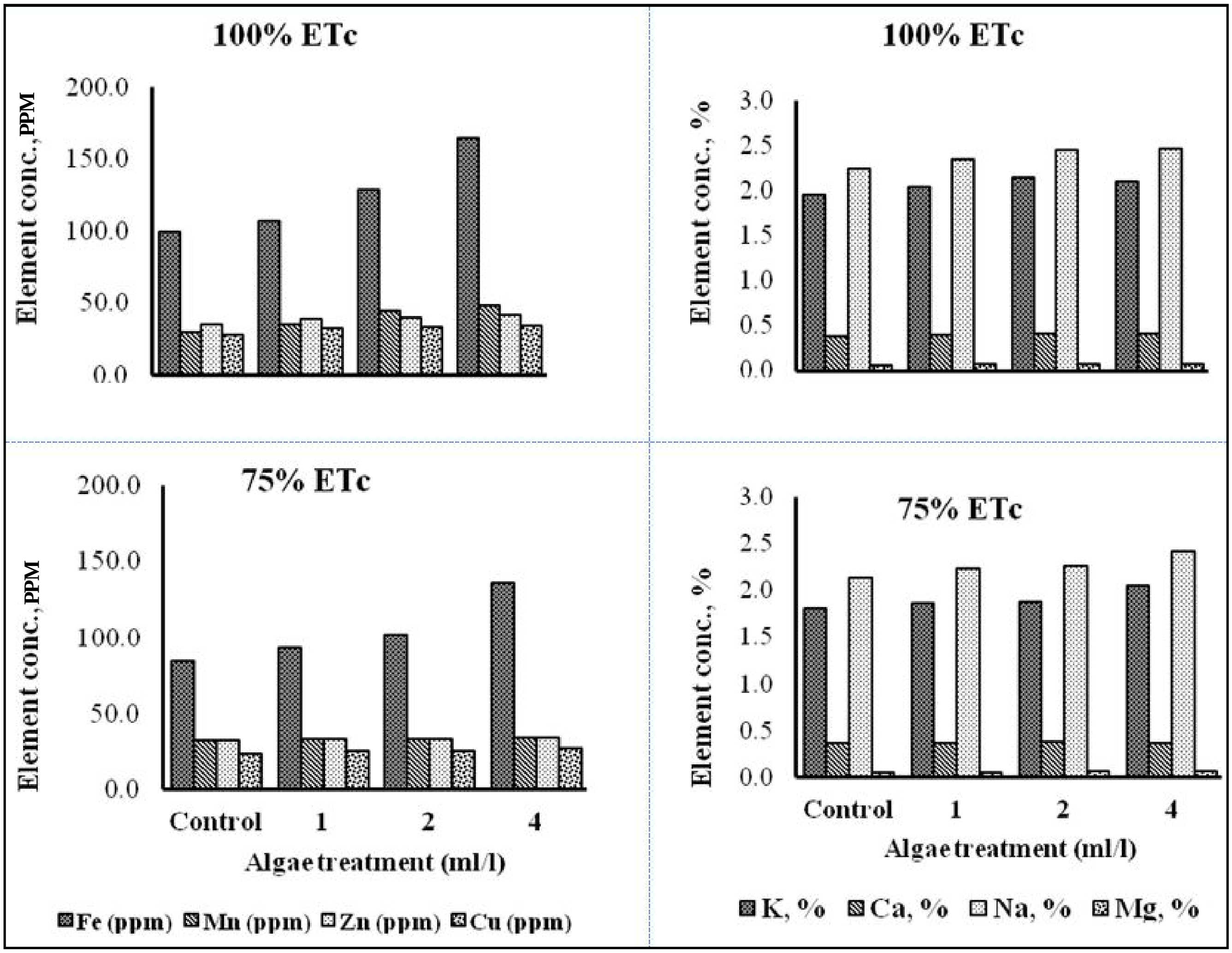

Fig. 2: Effect of Algae extract foliar spray on nutrients content in grains of soybean cultivar grown on calcareous soil under irrigation water regime (combined analysis of two successive seasons).

treatments under foliar spray of seaweed extract. Also, Shaaban *et al.*, (2010) found that nitrogen concentration was significantly increased as a response to algal extract at both treatments. This may attributed to the high protein content of the algal extract which split into natural plant amino acids involved directly in the metabolism (Shaaban, 2001a).

Drought Tolerance Efficiency (DTE %)

Data listed in Table 11 observed that there relationship between both of application Algae extract and drought Tolerance Efficiency. So results cleared that 4 ml/l of algae had ability under water stress is effective strategy to improve soybean productivity under stress and therefore drought tolerance efficiency % was the highest values at application of 4 ml/l algae foliar spray treatment.

Water utilization efficiency (WUE), (kg /m^3)

The indicated results showed that the WUE increased with increasing water stress, means that the algae foliar spray application lead to an increase in plant efficiency to produce higher yield with lower water (more crop per drop), where the measured yield was higher at 75% ETc treatment than 100% treatment for all algae treatments Table 12.

Conclusion

Application of extracts from algae has beneficial effects on growth and stress adaptation. Algae extracts have properties beyond basic nutrition, often enhancing growth and stress tolerance in soybean especially in 75% ETc water of irrigation treatment. 4 ml/l of algae had ability under water stress is effective strategy to improve soybean productivity under stress and therefore drought tolerance efficiency % was the highest values at application of 4 ml/l algae foliar spray treatment. The WUE increased with increasing water stress, means that the algae foliar spray application lead to an increase in plant efficiency to produce higher yield with lower water. In addition, some parameters of plant were significantly high effect this due to application of 2 ml/l algae foliar spray under water stress.

Conflict of Interest

The authors declared that present study was performed in absence of any conflict of interest.

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Author Contribution

All authors significantly contributed in all parts and aspects of paper.

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