

The Effect of Green Algae (*Cladophora crispata* L.) Extract on Salinity Stress Tolerance of (*Zea mays* L.) Plant



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Abstract

This study conducted in 2017 at the Plant Protection Department (PPD), Agriculture college - University of Misan, Iraq and laboratory of breeding plant department, Agriculture college - University of Tehran ,Iran in order to evaluate the effect of green algae (*Cladophora crispata* L.) extract on salinity stress tolerance of maize plant (*Zea mays* L.). Seeds were planted in plastic pots filled with sand-peat moss (1:1) and irrigated with different sea water concentrations (0, 5, 10, 20 ,30%) with algal extract (0, 0.5, 1, 1.5) m/l and without algal extract separately. After one month of cultivation, the plants sprayed with algal extract one time every ten days. The results of the study showed that increasing the concentration of seawater in the irrigation water leads to a significant reduction in the percentage of seed germination, length of the vegetative and root parts, the number of roots and fresh weight of the vegetative and root mass. However, spraying maize plants with green algae extract lead to a significant increase in all above traits and positive effects was directly proportional to the increasing concentration of algal extract. The study also showed that raising the concentration of seawater in irrigation water leads to high concentration of iron, copper and zinc and decreased the concentration of manganese in the maize leaf tissues. The application of algal extract in irrigation water and as foliar spray showed a significant increase in some micro minerals which consider as an indicator of improving plant resistance to salinity stress.

Keywords: *Zea mays* L., *Cladophora crispata* L., Salinity Stress, Algal Extract.

Introduction

Maize plant (*Zea mays* L.) belongs to Poaceae family which consider one of the most worldwide grain crop, where it is the second crop after wheat and followed by rice. Apart from the usage of corn in food products, it is used as a fodder and other products like oil extraction, starch, medical products and recently it used to produce bio-fuel, therefore, it called the king of crops [18].

Maize is bisexual flowered, monoecious plant, it has an imperfect flower where the male flowers forming an inflorescence called Tassel on the top of stem, while female inflorescence emerged from leaf armpit forming the Ear.

In Iraq, maize crop cultivated in central and northern regions in two seasons, spring and autumn, in spite of the importance of this crop, its production has been decreased in Iraq for several reasons, especially water scarcity and salinity, as it amounted to 182.3 thousand tons for the autumn season 2015 [6].

Water stress is one of the environmental limiting factors that controlling the agricultural production in arid and semi-arid regions, where salinity decreases plant productivity through its poisoning impacts and osmotic effects. The poisoning effects of salinity comes from absorption of large quantity of harmful elements by plant roots like sodium and chlorine due to increase its concentrations in saline soils, as the plant absorbs these elements by active absorption process for the purpose of accumulating the concentration of these elements within the tissue of the roots in order to increase the osmotic concentration and thus reducing the water potential to enable the roots to draw up water from soil solution into root tissues, as well the salinity affects the area of plant leaves where it causes leaf shrinkage and reducing leaves area which in turn leads to reducing photosynthesis process [24].

Many researches and studies refer to the effectiveness of green algae extracts in resistance of salinity stress of certain plants. The role of green algae extract in improving salinity stress resistance has proved by [23] in rice plant through increasing the activity of antioxidant enzymes and reducing free roots production, also [20] explained that the usage of extracts of blue green algae with nutrition media prepared to produce some flowering plants (beets, peas and tobacco) by tissue culture technique leads to improve the growth and development of callus of all plants. Algae is an important source of some substantial chemical materials such as proteins, vitamins and carbohydrates [22]. The results of spraying wheat plants in the nutrient media irrigated with different concentrations of sea water with the extract of green algae leads to increase the tolerance of plant to salt stress and increased the growth and yield of wheat plant treated with extract compared to non-treated plant [1]. Likewise, [16] found that the addition of green algae extract *Cladophora cryspata* to nutrition media contains several concentrations of sea water prepared to produce callus for developing embryos and formation of seedlings for two varieties of rice plant cultivated in vitro leads to increase the plant resistance of salinity stress and all other studied traits.

Many researches and studies also indicated that the concentration of micro-elements in the tissues of plants exposed to saline stress is an indicative of the resistance of these plants to stress, where [10] refer to increasing the concentration of iron, copper and zinc ions in the roots, leaves and shoots of wheat plant treated with sodium chloride at a concentration of 150 mmol. In the same context, [8] refer to the significant increase in the concentration of trace metal elements like iron, copper, zinc and manganese in all parts of wheat plant when spraying the vegetative parts with sodium chloride solution (0, 1, 2 and 5%). However, the observation of [4] was a significant increase in the concentration of zinc and a significant decrease in the concentration of manganese in rice, barley and sugarcane plants exposed to salt stress.

Cladophora genus includes branched green algae, regularly or irregularly, filamentous thallus with basal-distal differentiation, attached when young but in some taxa becoming free-floating; forming feathery bundles on substrates; especially in flowing water [14]. Fresh water *Cladophora* exist in a variety of morphological shapes that vary in cell dimensions and branching pattern [9]. However, only a few studies have been carried out on freshwater *Cladophora* taxa and their use as a natural source of bioactive compounds [5].

Cladophora crispata is a yellowish-green thallus, branched filament growing apically and intercalary, the branches consist of long and cylindrical cells, gradually attenuated in the branches to slightly narrowed but rounded apices. The chitin, pectose and cellulose of *C. crispata* structure provide functional groups, capable of binding various heavy metals, therefore, *C. crispata* is generally considered as the proper bio-indicator of heavy metals in water bodies [2].

Due to the lack of studies related to the effect of green algae and examine of the concentration of micro mineral elements in the resistance of saline stress and to find an eco-friendly alternatives to increase the resistance of maize (*Zea mays* L.) to salt stress, this study was conducted.

Materials and Methods

This research was carried out in the Plant Protection Department (PPD), Agriculture college - University of Misan, Iraq and laboratory of breeding plant department, Agriculture college - University of Tehran ,Iran. The seeds of maize (Baghdad A variety) were brought from the Seed Inspection and Certification Department, Ministry of Agriculture, Abu Ghraib, Baghdad. The percentage of seed germination were examined after sterilizing process using ethyl alcohol 70% and the commercial bleach 50% concentration containing sodium hypochlorite 5% then seeds were washed with pure distilled water several times.

1. Preparation of different concentrations of seawater

The seawater was brought from Mina al-Bakr in the Arabian Gulf and the concentrations (0, 5, 10, 20 and 30) were prepared. The concentration 5% prepared by adding 50 ml of sea water in a 1 liter glass container to 950 ml of distilled water, 900

ml of distilled water added to 100 ml of seawater and 800 ml of distilled water to 200 ml of sea water to represent 10% and 20% respectively, however, concentration of 30% prepared by adding 700 ml of distilled water to 300 ml of sea water, and 0% concentration referred to only distilled water.

2. Preparation of green algae extract and seed planting

The green algae were collected from rivers and watercourses near the site of the College of Agriculture - University of Misan and were washed by distilled water thoroughly to remove all impurities and then dried by spreading them on a piece of cloth in room temperature, the dried algae grinded by an electric grinder, then preserved in a glass bottle until the process of extraction. The extraction process was carried out by dissolving 50 g of algae powder in a liter of ethyl alcohol 70% and placed on a Magnetic Stirrer device for 24 hours, the filtration was then carried out using 0.54 μ m filter paper and the leachate left in the Petri dishes exposed to air in room temperature to allow the alcohol to evaporate and collect the precipitate which represents the green algae extract and kept in the refrigerator until the preparation of concentrations of the extract.

Four concentrations of green algae extract were prepared by taking three weights of green algae extract (0.5, 1.0 and 1.5 g) and dissolving each weight in a liter of distilled water to obtain three concentrations 0.5 g/L, 1 g/L and 1.5 g/L respectively, in addition to the control treatment of 0% which represents distilled water only, these concentrations were used in the spraying process. As for the concentrations used in the irrigation process, the weights of the extract mentioned dissolved in the concentrations of seawater and used in irrigation. The seeds were grown in 20 cm long plastic pots after filling them with peat-moss and sand 1:1 (v:v), 4 seeds each.

The pots were irrigated with different concentrations of sea water and green algae extract. The spraying process was carried out with the algae extract on the vegetative parts of growing plants by means of a manual sprayer of one liter five times, the first spray after ten days of germination and between the spray and other were ten days. The spray extract concentration was on the plants irrigated by seawater which contains algae extract in same concentrations.

3. Measurements

After two months of planting, the measurements were taken. The plants were removed and the roots were cleaned from the soil. The number of roots, the length of the roots and the vegetative length were calculated by the ruler. The leaves were dried using oven apparatus for 24 hours and 72 °C until complete drying. The dry leaves were grinded by an electric grinder and the powder was collected in glass containers and preserved in the refrigerator until measurements of micro-elements concentration were made.

4. Estimation of Micro Elements:

The micro elements (iron, zinc, copper, and manganese) were estimated by digesting 0.5 g of dry and powdered tissues with a mixture of 70% pyrochloric acid and

concentrated nitric acid [11]. The elements were estimated using the Shimadzu atomic absorption spectrometer ($\mu\text{g.}^{-1}\text{g dry weight}$).

5. Statistical Analysis

The experiment was designed as a Completely Randomized Design (CRD). The Factorial experiment was with two factors: seawater concentration x green algae extract concentration. The experiment consisted of 60 replicates, three replicates per concentration (4 X 5 X 3). Data collected from experiment were statically analyzed, After obtaining the variance analysis table, the mean differences was compared with the use of the Revised Least Significant Difference test (R.L.S.D.) with probability level of 0.05 [19].

Results and Discussion

Results:

Table (1) Effect of sea water and green algae extract on germination percentage of maize seeds (%)

Sea Water Con. Green Algae Con.	0	5	10	20	30	Green Algae Mean
0	94.10	90.32	87.55	80.43	60.73	82.62
0.5	95.56	91.75	88.11	81.79	63.81	84.20
1.0	97.34	92.06	90.68	82.32	64.72	85.42
1.5	98.65	96.43	93.51	85.73	66.03	88.07
Sea Water Mean	96.41	92.64	89.69	82.56	63.82	

R.L.S.D. Sea water con. 3.1 Green algae extract con. 1.7 Interference 4.8

As shown in table (1) the increase of seawater concentration added to the soil solution resulted in a significant reduction in germination percentage of maize seeds with the lowest rate at 30%, which reached 63.82% compared to the highest rate at the control treatment, which reached 96.41%, however, at the concentrations (5, 10 and 20%), the germination were 92.64, 89.69 and 82.56%, respectively. The effect of the green algae extract was significant with the highest germination percentage at 1.5 g / L, which was 88.07% compared to the lowest percentage of germination in control treatment (82.62%). However, the effect of the concentrations 0.5 and 1.0 g / L was 84.20% and 85.42% respectively. The effect of the interaction between the seawater and the green algae extract was also significant, where the highest percentage of germination in the treatment of seawater at 0% concentration interacted with green algae extract at concentration of 1.5 g / l which was 98.65%, whereas the germination of the treatment

of the seawater at 30% concentration interacted with green algae extract at 0% concentration reached 60.73%.

Table (2) Effect of sea water and green algae extract in the total vegetative length of maize plant (cm) after two months of planting.

Sea Water Con. Green Algae Con.	0	5	10	20	30	Green Algae Mean
0	54.34	48.46	42.32	33.49	26.87	41.17
0.5	63.76	60.23	47.65	38.04	28.98	47.73
1.0	68.98	63.12	52.34	40.39	30.17	51.00
1.5	74.56	68.17	59.38	43.19	32.56	55.99
Sea Water Mean	65.41	60.0	50.45	38.78	29.65	

R.L.S.D. Sea water con. 3.14 Green algae extract con. 2.02 Interference 5.16

The results of Table (2) show that the treatment of seawater has affected the reduction of the total length of the maize plant significantly. The lowest length was treated with seawater at 30% concentration of 29.65 cm compared to the highest length of maize plant at the control treatment which reached 65 cm. The green algae extract increased the length of the total vegetative, where the highest length of the plants was treated with the green algae extract with a concentration of 1.5 g / l, which reached 55.99 cm compared to the lowest length of plants in control treatment which was 41.17 cm. The effect of the interaction was also significant with the highest length of the plants in the treatment of the interaction between free water at the concentration 0% and green algae extract at the concentration of 1.5 g / l, which was 74.56 cm.

Table (3) Effect of sea water and green algae extract in the root length of maize plant (cm) after two months of planting.

Sea Water Con. Green Algae Con.	0	5	10	20	30	Green Algae Mean
0	18.36	16.47	11.43	8.32	6.76	12.27
0.5	19.12	17.72	12.39	9.32	7.56	13.22
1.0	21.03	18.46	13.07	9.69	7.03	13.85

1.5	22.63	20.03	14.12	9.91	7.89	14.92
Sea Water Mean	20.28	18.17	12.73	9.13	7.29	

R.L.S.D. Sea water con. 1.12 Green algae extract con. 0.20 Interference 1.31

The data of above table (3) show that the increase in the concentration of seawater in irrigation water has significantly reduced the roots length of plants. Where the lowest rate for roots length recorded when treated with sea water concentrate 30%, which was

7.29 cm compared with the control treatment with roots length of 20.28 cm. The effect of the green algae extract was also significant, where the longest length of the roots was treated with the green algae extract at 1.5 g / L, which was 14.92 cm compared to the control treatment where the root length was 12.27 cm. furthermore, the effect of the interaction was also significant, where the mean length of the roots was highest in the treatment of sea water at 0% concentration interacted with green algae extract at 1.5 g

/ L, which was 22.63 cm however, the maize roots under interaction between the treatment of seawater 30% and algae extract 0% concentration reached up to 6.76 cm.

Table (4) Effect of sea water and green algae extract in the root number of maize plant after two months of planting.

Sea Water Con. Green Algae Con.	0	5	10	20	30	Green Algae Mean
0	8.65	8.12	7.53	6.28	4.41	6.99
0.5	9.62	9.05	8.11	6.89	4.79	7.69
1.0	10.83	10.04	8.65	6.21	5.07	8.16
1.5	12.32	10.73	9.25	6.98	5.76	9.00
Sea Water Mean	7.61	9.49	8.37	6.59	5.01	

R.L.S.D. Sea water con. 0.14 Green algae extract con. 0.11 Interference 0.26

It is clear from the results of Table (4) that the increase of seawater concentration in the irrigation water has affected the number of roots negatively. The lowest number of roots recorded when plants treated with seawater at the concentration of 30% which reached 5.01 root compared with the highest average number of roots at control treatment which was 7.61 root. The effect of the green algae extract was also significant, with the highest rate of roots number of plants that treated with the green algae extract at 1.5 g / L

concentration which reached 9 root compared with the lowest root number of the treatment with green algae extract at 0% which was 4.79 root.

Likewise, the effect of the interaction was also significant, with the highest number of roots in the treatment of seawater 0% interacted with green algae extract at 1.5 g / L, which was 12.32 root, compared to the lowest number of roots when treated with sea water concentration 30% and algae extract at 0% concentration, which reached 4.79 root.

Table (5) Effect of sea water and green algae extract in the fresh weight of plant leaves (g) after two months of planting.

Sea Water Con. Green Algae Con.	0	5	10	20	30	Green Algae Mean
0	3.56	3.12	2.57	2.02	1.81	2.61
0.5	4.42	3.81	3.05	2.23	1.93	3.09
1.0	5.05	4.73	3.86	2.90	2.12	3.73
1.5	5.76	5.09	4.43	3.12	3.34	4.35
Sea Water Mean	4.69	4.19	3.48	2.57	2.30	

R.L.S.D. Sea water con. 0.13 Green algae extract con. 0.12 Interference 0.25

The data of Table (5) showed that the sea water had a significant effect on the reduction of the fresh weight of the plant leaves. The lowest weight of leaves recorded in the treatment of sea water 30% concentration which was 2.30 g compared to the highest weight (4.69 g) of the control treatment. The effect of green algae extract was also significant, where the fresh weight of plant leaves treated with green algae extract of

1.5 g, was 4.35 g compared to the lowest weight (2.61g) of control treatment.

Furthermore, the effect of the interaction between sea water and green algae extract was also significant, where the highest weight of leaves recorded in interaction treatment between sea water at 0% concentration and the green algae extract at 1.5 g / L which was 5.76 g of compared to the lowest weight in the treatment of sea water of 30% concentration and green algae extract with 0% which was 1.93 g.

Table (6) Effect of sea water and green algae extract in the fresh weight of plant roots (g) after two months of planting.

Sea Water Con. Green Algae Con.	0	5	10	20	30	Green Algae Mean
0	0.44	0.37	0.28	0.22	0.18	0.29
0.5	0.67	0.47	0.35	0.29	0.21	0.42

1.0	0.84	0.63	0.41	0.33	0.26	0.57
1.5	0.94	0.83	0.64	0.41	0.29	0.62
Sea Water Mean	0.72	0.58	0.42	0.31	0.24	

R.L.S.D. Sea water con. 0.03 Green algae extract con. 0.02 Interference 0.05

The results of Table (6) show a significant effect of sea water on reducing the fresh weight of the root mass. The lowest weight of the root mass caused by the treatment of sea water at 30% concentration which reached 0.24 g compared to the highest weight at the control treatment (0.72 g). The data of the same table showed that the green algae extract had a significant effect on the weight of root mass and that the increase was directly proportional to the concentration of the extract. The highest weight of the roots was of plants that treated with the green algae extract at 1.5 g / L, which was 0.62 g, where the weight of roots of the control treatment amounted to 0.29g.

The effect of the interaction was also significant with the highest root weight ratio in the treatment of sea water at 0% concentration interacted with green algae extract at 1.5 g / L, which was 0.94 g compared with the treatment of sea water 30% concentration and the green algae extract at 0% which was 0.18 g. It is clear from the above Table (7) that the treatments of seawater had a significant effect on the increase of the iron concentration in the leaves tissue of the maize plant, where the highest concentration of iron element was in the treatment with seawater 30% concentration which reached 583.2 µg.-1g dry weight compared with the lowest concentration at the control treatment , which amounted to 392.83 µg.-1g dry weight. The effect of the green algae extract was also significant, where the iron concentration of plant tissues when treated with green algae extract at 1.5 g / L reached up to 545.26 µg. -1g dry weight compared to its concentration at 435.16 µg. -1g dry weight.

Table (7) Effect of sea water and green algae extract in concentration of iron ion in µg. ⁻¹ g dry weight in maize leaves after 2 months of planting.

Sea Water Con. Green Algae Con.	0	5	10	20	30	Green Algae Mean
0	340.5	389.6	432.9	465.7	547.1	435.16
0.5	374.8	412.7	481.7	512.9	562.4	468.9
1.0	408.6	443.6	516.0	556.8	598.6	504.72

1.5	447.4	489.9	567.4	596.9	624.7	545.26
Sea Water Mean	392.83	433.95	499.5	533.08	583.2	

R.L.S.D. Sea water con. 32.17 Green algae extract con. 27.40 Interference 50.57

Likewise, the effect of the interaction between sea water and green algae extract was also significant, with the highest concentration of the iron component in the treatment of the interference between sea water of 30% concentration and green algae extract at the concentration of 1.5 g / L, which reached 624.7 $\mu\text{g.}^{-1}\text{g dry weight}$ compared with the control treatment of sea water interacted with control treatment of green algae extract which was 340.5 $\mu\text{g.}^{-1}\text{g dry weight}$.

Table (8) Effect of sea water and green algae extract in concentration of Copper ion in $\mu\text{g.}^{-1}\text{g}$ dry weight in maize leaves after 2 months of planting.

Sea Water Con. Green Algae Con.	0	5	10	20	30	Green Algae Mean
0	34.43	42.60	58.14	67.56	81.63	62.27
0.5	40.87	48.94	61.98	72.32	89.57	69.94
1.0	49.33	55.22	67.86	79.98	93.68	78.21
1.5	60.87	72.22	87.66	98.54	104.85	84.83
Sea Water Mean	46.38	54.75	68.91	84.10	92.43	

R.L.S.D. Sea water con. 4.31 Green algae extract con. 2.15 Interference 6.46

The results of Table (8) show that the addition of seawater to irrigation water led to a higher concentration of copper in leaves tissue of maize plant. The highest concentration of this element was 92.43 $\mu\text{g.}^{-1}\text{g dry weight}$ when the plants treated with seawater of 30% concentration, compared with control treatment (0% sea water) which was 46.38 $\mu\text{g.}^{-1}\text{g dry weight}$. The results of the same table showed that the spraying of the plants with the green algae extract increased the concentration of the copper element in the leaves significantly, where it reached the highest concentration (84.83 $\mu\text{g.}^{-1}\text{g dry weight}$) at the treatment of 1.5 g / l green algae extract, compared to the control treatment (62.27 $\mu\text{g.}^{-1}\text{g dry weight}$).

Furthermore, the effect of interaction was also significant, where the highest concentration of copper element (104.85 $\mu\text{g.}^{-1}\text{g dry weight}$) found in the leaves of maize plants under the interaction between sea water treatment of the concentration of 30% and the green algae extract treatment at the concentration of 1.5 g / l. However, the lowest concentration was in interaction between Sea water treatment of 0%

concentration and green algae extract treatment at 0 concentration, which amounted to 34.43 $\mu\text{g.}^{-1}\text{g dry weight}$.

The data of following table (9) showed a significant effect of sea water in decreasing the concentration of manganese in the leaves tissue of maize plant. The lowest concentration of this element was in the treatment of 30% concentration of sea water with a concentration of 249.5 $\mu\text{g.}^{-1}\text{g dry weight}$ compared to the highest concentration of manganese in the plants treated with 0% concentration of sea water which amounted to 352.75 $\mu\text{g.}^{-1}\text{g dry weight}$. The effect of the green algae extract was also significant, where the treatment of the green algae extract resulted in increasing the concentration of manganese in the leaves tissues of maize with the highest concentration of this element (335.2 $\mu\text{g.}^{-1}\text{g dry weight}$) at the treatment of 1.5 g / l extract, compared to the lowest concentration at control treatment, which reached 259.4 $\mu\text{g.}^{-1}\text{g dry weight}$.

Table (9) Effect of sea water and green algae extract in concentration of Manganese ion in $\mu\text{g.}^{-1}\text{g dry weight}$ in maize leaves after 2 months of planting.

Sea Water Con. Green Algae Con.	0	5	10	20	30	Green Algae Mean
0	312	292	258	234	201	259.4
0.5	332	319	293	264	224	286.4
1.0	373	347	327	301	282	326.0
1.5	394	356	337	298	291	335.2
Sea Water Mean	352.75	328.5	312.75	274.25	249.5	

R.L.S.D. Sea water con. 13.17 Green algae extract con. 14.42 Interference 27.59

The effect of the interaction between sea water and green algae extract was also significant, where the highest concentration of manganese in leaves tissue was found in the treatment of sea water with concentration of 0% interacted with the green algae extract of concentration 1.5 g / L, which reached 352.75 $\mu\text{g.}^{-1}\text{g dry weight}$.

The data of below table (10) indicates that the sea water had a significant effect on the increase of the concentration of zinc in the vegetative tissue of the maize plant where the highest concentration of this element in the treatment of seawater of 30% concentration was 202.45 $\mu\text{g.}^{-1}\text{g dry weight}$ compared to control treatment with a concentration of 141.57 $\mu\text{g.}^{-1}\text{g dry weight}$. The effect of green algae extract was also significant with highest concentration of zinc component at the treatment with the green extract of 1.5 g / l which reached 158.95 $\mu\text{g.}^{-1}\text{g dry weight}$ compared to control treatment with a concentration of zinc element 124.76 $\mu\text{g.}^{-1}\text{g dry weight}$. The effect of the interaction was also significant, where the highest concentration of the zinc element was in the treatment of sea water of 30% concentration interacted with green algae

extract at 1.5 g / l which reached 232.77 $\mu\text{g.}^{-1}\text{g}$ dry weight compared with the lowest concentration of this element, which was found in the treatment of the interaction between sea water at the 0% concentration and the green algae extract at the concentration of 0%, which amounted to 124.76 $\mu\text{g.}^{-1}\text{g}$ dry weight. These findings are consistent with the findings of [4] in his study on rice plant, barley, and sugar cane.

Table (10) Effect of sea water and green algae extract in concentration of Zinc ion in $\mu\text{g.}^{-1}\text{g}$ dry weight in maize leaves after 2 months of planting.

Sea Water Con. Green Algae Con.	0	5	10	20	30	Green Algae Mean
0	124.76	136.89	148.68	163.43	182.76	151.30
0.5	139.05	144.98	153.38	186.94	191.54	163.27
1.0	143.53	155.76	173.95	197.09	202.74	174.56
1.5	158.95	169.92	183.98	206.22	232.77	190.29
Sea Water Mean	141.57	158.62	164.79	188.42	202.45	

R.L.S.D. Sea water con. 1.11

Green algae extract con. 6. 2

Interference 14.3

Discussion

Evidents from results of Tables 1-6 shown that the increase of seawater concentration in the irrigation water has led to a significant decrease in all characteristics of vegetative growth (percentage of germination, length of shoot, length of roots, number of roots and weight of the fresh shoots and roots). This effect due to the increase in the concentration of salinity in the soil solution, resulting in increased osmotic concentration and reduction of the water potential of the soil solution, which leads to a lack of water absorption by the roots. As water is the media of all vital bio-chemical activities within the plant, therefore, the decrease of water inside the plant affects most of the substantial processes, including the process of cellular division and thus weak growth.

The negative impacts of salinity on plant growth may be attributed to its role in the level of proteins within the plant, as it increases the level of protein degradation, or it reduces the process of proteins synthesis from amino acids, it also inhibits the important nitrogen absorption in the formation of amino acids and affects the effectiveness of some important enzymes such as analytical enzymes and oxidizing enzymes [3]. As the lack of water absorption by the roots and as a precautionary mechanism and in order to retain water, the plant is forced to close the stomata and this negatively affects the process of gas exchange and thus reduce the process of photosynthesis. Also, the salt stress reduces the production of Co_2 in the Calvin cycle and the oxidation of NADP-also reduces the compound of Ferredoxin in photosynthesis process because electrons

move from the first reaction center to the oxygen to form the roots of active oxygen ($-O_2$) by a process called Mehler reaction [7,13]. Also, the lack of water content within the plant reduces the turgor pressure of cells and shrinkage of the leaves, and reducing the water potential of the soil solution because of increased salinity may lead to the difficulty of absorption of mineral elements necessary for plant growth. In addition to the osmotic effect, salinity has a toxic effect on plant cells due to the high concentration of sodium and chlorine in the soil solution, which increases their uptake by plant roots through negative absorption [15].

Regarding the effects of green algae, the results shown in Tables (6-1) indicate the positive effect of increasing the vegetative growth of the maize plant (percentage of germination, length of shoot, length of roots, number of roots and weight of the fresh shoots and roots). This may be due to the fact that it is a rich source of nitrogen because of its high atmospheric nitrogen fixation as nitrogen one of the amino acid components necessary to create the protein. Or it may be due to the fact that green algae are the source of many plant hormones that stimulate the process of cell division and increase the number and prolongation of cells and thus increase the size of plant tissues and plant growth, such as Auxins, Cytokinins and Gibberellins (Tarakhovskaya, 2007). In the other hand, the role of algal extract in salt resistance may be attributed to its role in the bio-synthesis of the free amino acids and carotenoids, especially the amino acid Proline. This means increasing the in-cell osmosis potential, which increases the resistance of the plant to the salinity stress [1].

The results shown in Tables 7, 8 and 10 indicate a significant increase in concentration of Fe, Cu and Zn in leaf tissues when the concentration of seawater in irrigation water is raised. This may be due to the increased concentration of these elements in the soil solution by their presence in seawater so they are ready to be absorbed by the roots and then transfer them to the leaves through the stem. Whereas the results of Table 9 indicate a reduction in the concentration of Mn in the leaf tissues of the maize plant when the concentration of seawater is increased. This may be owing to the increase in the absorption of positively charged ions, especially the heavier charge, such as the iron ion (Fe^{+2}) compared to the charge of Mn^{+1} the weak charge. As for the impact of green algae, the results of tables (10-7) indicate that the concentration of these elements in leaf tissues maize plants sprayed with algal extract was increased proportionally with the increase of algae extract concentration, this may be due to the fact that the green algae have the ability to absorb these elements from the medium in which they live and thus may retain appropriate amounts of these elements in their cells, thereby, spraying the maize plants with the extract of algae may be the source of these elements which can be taken by sprayed leaves. Researches indicate that the increased concentration of micro-elements in the tissues of plants is an indication of increased resistance of these plants to salinity because the presence of these elements in the root cells reduces the absorption of the element of sodium as the metal ions that carry the same charge compete sodium to link with effective sites [17].

Conclusions

We conclude from this study the use of green algae extract *Cladophora crispata* L. has led to improve of some of the studied morphological traits and also increased the concentration of some micro-elements in the maize plant tissue, which is an indicator of improving the salinity stress resistance.

References

1. Abd-Elbaky, H.; Hhussein, M. and El-Baroty, S. (2008). Algal Extracts Improve Antioxidant Defense With Sea Water. African J.Bio Chem Res., 2(70); 151-158.
2. Abdul Jabbar, Ahmed Shaker (2009). Accumulation Effect of Pb and Cd on Green Alga *Cladophora crispata*. Al- Mustansiriya J. Sci., Vol. 21, No 4, 2010: 31-34.
3. Apse , M. P; Aharon, G. S.; Snedden, W. A. and Blumwald, E. (1999). Salt tolerance conferred by over expression of a vacuolar Na⁺ /Antiport in Arabidopsis SP. Plant .S.ci.,285:1256-1258.
4. Baxter, I.; Herman ,C. and Lahner, B.(2012). Biodiversity of mineral nutrient and trace element I Botany Vol53(3):247-257.
5. Beata Messyas, Boguslawa Leska, Joanna Fabrowska, Marta Pikosz, Edward Roj, Adam Cieslak, Grzegorz Schroeder (2015). Biomass of freshwater Cladophora as a raw material for agriculture and the cosmetic industry. Open Chem., 2015; 13: 1108.
6. Central Bureau of Statistics, 2015. Report on the production of cotton and maize, Directorate of Agricultural Statistics, Iraq (Arabic). [Online] Available: <http://www.cosit.gov.iq/ar/agri-stat/veg-prod>
7. Demiral, T and Turkan, I.(2005).Comparative lipid peroxidation , antioxidant defense and proline content in root of two rice cultivars differing in salt tolerance. Environmental and Experimental Botany. Volume 53, Issue 3, June 2005, Pages 247-257.
8. EL-Fouly, M.; Mobark, Z. M and Salama, z. A. (2011). Micronutrient (Fe, Mn, Zn). Foliar spray for increasing salinity tolerance in wheat (*triticum asctivum* L) African J.of Plant Sci., I (5): 314-322.
9. Graham, L.E, Wilcox, L.W. (2000). Algae. Wisconsin University Prentice-Hall, Inc. Upper Saddle River, NJ07458.
10. Hu,Y.; Tucher, S. and Schmidhalter, U. (2000).Spatial distribution and net deposition rates of Fe,Mn and Zn in the elongating leaves of weat under sdlin soil conditions. Aust .J.PlantPhysiol.,27:53-59.
11. Kacar,B.(1984).Plant nutrition practice Guide. Ankara Univ. Agricultural Fac. Pub: 900, Practice Guides:214. Ankara, Turkey.
12. License, S. A.(2008).Maize from new word encyclopedia .Organizing knowledge for happiness , prosperity and word peace. <http://WWW.New world encyclopedia .org/entry/Maize>.
13. Mandhian , S; Madan. S. and Sawhney, (2005).Antioxidant defense mechanism under salt stress in wheat seedling. Biol plant ,Vol227,pp227-231.
14. Moat War Dine Naw and Soe Soe Win (2011). Morphology and Nutritional Values of Green Alga Cladophora from Kachin State. Universities Research Journal., 2011; Vol. 4, No. 1: 101-102.
15. Munns. R. (1993).Physiological processes limiting plant growth in saline soil .Some dogmas and hypothesis .plant cell enviro.,16:15-24.

16. Obaed, A.R. (2015). The biological changes accompanying to salt stress and evaluating the role of green algae (*Chladophora crispata*) on rice (*Oryza sativa L.*) cultured in vitro. PhD Thesis, Agriculture Collage, university of Basrah, Iraq 167.pp.
17. Raza, S.H; Haabib, R.A. and Hameed, M. (2007). Glycine betaine induced modulation of antioxidant enzyme activates and ion accumulation in two wheat cultivars differing in salt tolerance .*in vitro* Experim. Bot .,3:368-376.
18. Sekhon RS, Lin H, Childs KL, Hansey CN, Buell CR, de Leon N, Kaeppler SM. Genome-wide atlas of transcription during maize development. Plant J. 2011; 66(4):553–563. doi: 10.1111/j.1365-313X.2011.04527.x.
19. Steel, R.G.D. and Torrie, J.H. (1980). Principles and Procedures of Statistics. A Biomaterial Approach. New York, USA, P: 633.
20. Stir, K. and Ordog ,V.(2002).Cytokinin and auxin-like activity in and cyanophyta microalgae.,J.Appl.phscal.14:215-221.
21. Tarakhovskaya, E.; Maslov, I.; Shishova, M.(2007). Phytohormones in algae . Russian. J. Plant Physiology .,54(2):86-194.
22. Thajuddin, N. and Subermanin, G. (2005). Cyanobacterial biodiversity and potential applications in biotechnology . Curreent Sci.,89(10):47-57.
23. Vaidyanathan, H.; Sivakumer, P.; Chakrabarty, R. and Thomas,(2003). Scavenhing of reactive oxygen species in NaCl stressed rice (orysa sativa L.) differentiation in salt tolerant and sensitive varieties. Plant Sci., 165:1411-1418.
24. Yordonova, R. and Popova, L.(2007).Effect of exogenous treatment with salicylic acid on photosynthetic activity and antioxidant capacity of chilled wheat plants. Gen. appl. plant phys.33:155-170.