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Effect of Chemical, Organic and Bio Fertilizers on photosynthetic pigments, carbohydrates and minerals of Wheat (*Triticum aestivum*. L) Irrigated with Sea Water

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Abstract

The present study was conducted to investigate the effect of chemical, organic and bio-fertilizers on photosynthetic pigments, soluble sugars, non-soluble sugars, total carbohydrates and mineral elements in wheat (*Triticum aestivum* L.) plants grown under different concentrations of sea water (0%, 20% and 40%). Chemical fertilizer was used at concentrations of 0, 250 and 500 kg/ha; Rhizobium and Azotobacter were used as Biofertilizers; and Humic acid in concentrations of (0, 5 and 10 kg/ha) was used as organic fertilizer. The obtained results showed that photosynthetic pigments, carbohydrates and nutrient elements were markedly reduced at the high levels of sea water particularly 40% ratio. While, fertilizer treatments had an observed promotion effects on those constituents, particularly Bio and organic fertilizers that were more effective than chemical fertilizers even at high concentrations of sea water. This may be because of the potential effect of organic and bio- fertilizers on providing the nutrient elements needed by plants besides some other beneficial compounds that help plants to withstand high salt stress conditions.

Keywords: Sea water, fertilizers, wheat, pigments, carbohydrates, minerals.

Introduction

Wheat (*Triticum aestivum*, L.) is one of the most important crops in most countries of the world including the Kingdom of Saudi Arabia. Therefore, increasing wheat production is a national target to fill the gap between production and consumption. Saudi Arabia needs sustained agricultural development to cope with the social and economic obligations that are the normal consequences of the continued high rates of population growth. This urgent need requires continuous scientifically based implementation of effective agricultural practices. Production of wheat should be increased through extending cultivated area such as selecting high yield cultivars, using appropriate agronomic practices; among which fertilization and water management are the most important. Water and salt stress are the most important limiting factors in wheat production in Kingdom of Saudi Arabia as well as other arid and semi-arid regions all over the world (Almaghrabi, 2012). In addition, one of the major concerns in using chemical fertilizers to increase the crop production is the pollution resulted from the contamination of water and soil. Unfortunately, in the last few years, the area of wheat cultivation was substantially reduced and hence, the production was significantly decreased. This adverse trend in the area, grain production and yield per hectare was due mainly to the lack of available water used for irrigation and the increase of salt concentration in water and soil.

Organic farming has emerged as an important priority area globally in view of the growing demand for safe and healthy food and long term sustainability and concerns on environmental pollution associated with

indiscriminate use of chemical fertilizers. Though the use of chemical inputs in agriculture is inevitable to meet the growing demand for food in world, there are opportunities in selected crops and niche areas where organic production can be encouraged to tape the domestic export market (Karmakar *et al*., 2007). Biofertilizers are important not only for the reduction in quantity of chemical fertilizers but also for getting better yield in sustainable agriculture. Organic agriculture is a holistic production management system which promotes and enhances agro ecosystem, health, including biodiversity, biological cycles, and soil biological activity (Samman *et al*., 2008). Use of soil microorganisms which can either fix atmospheric nitrogen, solubilize phosphate, synthesis of growth promoting substances or by enhancing the decomposition of plant residues to release vital nutrients and increase humic content of soils, will be environmentally begin approach for nutrient management and ecosystem function (Wu *et al*., 2005) Application of biofertilizer is considered today to limit the use of mineral fertilizers and supports an effective tool for desert development under less polluted environments, decreasing agricultural costs, maximizing crop yield due to providing them with an available nutritive elements and growth promoting substances (Metin *et al*., 2010). Soil microorganisms are important components in the natural soil subecosystem because not only can they contribute to nutrient availability in the soil, but also bind soil particles into stable aggregates, which improve soil structure and reduce erosion potential (Muhammed et al., 2013). There have been positive effects of inoculating wheat seed with various biofertilizer sources on the crop yields (Bahrani et al., 2010). In this regard, Ahmed *et al.* (2011) indicated that all the growth characters were significantly affected by inoculation of wheat grain with bio-organic fertilizers. The applications of biofertilizers in agriculture are suggested as a sustainable way of increasing crop yields and economize their production as well (Wali Asal, 2010). Bio-fertilization is very safe for human, animal and environment to get lower pollution and saving fertilization cost. In addition, their application in soil improves soil biota and minimizes the sole use of chemical fertilizers (Sabashini *et al.*, 2007). The use of organic fertilizer such as humic acid, can meet the nutrient requirement of sustainable wheat production under desert soil conditions. Such appropriate management of organic and biofertilizers reduces the potential disadvantages in comparison to the mineral fertilizers (Ahmed *et al.* 2011). Therefore, in the development and implementation of sustainable agriculture techniques, biofertilization has great importance in alleviating environmental pollution and deterioration of nature (Jalilian *et al.*, 2012; Mehran *et al.*, 2011). Existence of microbial communities like *Azotobacter, Rhizobium* and *Azospirillum* in the soil or rhizosphere promotes the growth of the plant through the cycling and availability of nutrients, increasing the health of the roots during the growth stage by increasing the absorption of nutrients (Vessey, 2003). Moreover, the application of humic acid is observed to be a significant soil organic matter which improves plant growth and crop production (Abdel-Razzak and El-Sharkawy, 2013). The present study was carried out to investigate the effect of Bio-organic fertilizers on the chemical constituents of wheat crop irrigated with different ratios of seawater.

Materials and Methods

The present study was carried out in open field at the King Abdulaziz University, Saudi Arabia to determine the effect of chemical fertilizer, bio fertilizer and organic fertilizer on photosynthetic pigments, carbohydrate contents and mineral nutrients in wheat (*Triticum aestivum* L.) plants irrigated with different ratios of seawater.

Wheat seeds were sown in 40 cm cimater pots filled with sandy soils mixed with pellet and peat moss in the ratio of 2:1:1 and irrigated with tap water. The pots were divided into three groups each of which was treated with different kind of fertilizers as follows: a) Chemical fertilizer "Urea" was used in the rates of 100, 250 and 500 kg/ha. b) Biofertilizer was used in the form of *Rhizobium* or *Azotobacter*, inoculation of the grains with the bio-fertilizer containing *Rhizobium* or *Azotobacter* was done just before sowing, using Arabic gum (4%) as adhesive material. c) Organic fertilizer was used in the form of Humic acid (HA) in the rate of 5, 10 and 20 kg/ha, 7days before sowing and incorporated through soil preparation. Each treatment was performed in three replicates with the recommended dose and was irrigated with 0%, 20% or 40% of seawater. Six weeks after germination the following parameters were measured:

Photosynthetic pigments

Chlorophyll a, b and carotenoids were determined spectrophotometrically according to Metzner *et al.*, (1965). Briefly, 0.5 grams of fresh leaves were taken and ground in pestle and morter using 10 cm^3 acetone 85% with some clear sand, then centrifuged at 3000 r.p.m. The supernatant was removed to 50 cm^3 conical through filter paper whatman No. 1, then the flasc was completed with action 85% up to the 50 ml.

The absorbance was measured at 663, 644 and 452 nm wave lengths to determine Chl a, Chl b and carotenoids respectively. Then pigment concentrations were calculated by µg/ml according to the following equations:

Chl a (µg /ml) = 10.3 E663 – 0.918 E644
Chl b (µg/ml) = 19.7 E644 – 3.87 E663
Carotenoids (µg/ml) = 4.2 E542.5 – (0.0264 chl a + 0.4260 chl b)

Carbohydrate content

Soluble and total carbohydrates of wheat plants were determined using the anthrone sulphuric acid method (Badour, 1959) as following: 0.05 gm dry weight of plant tissue powder was heated in water bath at 100C for 60 minutes in 2N HCl under condenser .The solution was then cooled and filtered through centered glass funnel. 5 ml of anthrone reagent was added to one ml of the tested solution in a clean dried test tube. The mixture was heated in a water bath at 100C^{o} for 15 minutes ,then placed in a cold water bath. The developed green colour was read at 630 nm against a blank containing only water and anthrone reagent using a Spectrophotometer (UV-1800). A calibration curve was constiueted using pure glucose .Taking into account the dilution and the original weight of the sample, the total carbohydrate content was calculated as mg g $^{-1}$ dry weight.

Non-soluble carbohydrates were determined as a difference between values of total and soluble carbohydrates.

Mineral elements

Nitrogen concentration

Total N was determined by Micro-Kjeldahl digestion method as indicated in FAO guide to laboratory establishment for plant nutrient analysis (FAO, 2008). Plant samples were collected and were washed with distilled water, oven dried at 70 °C to a constant weight and the dry weight measured using an electronic balance. The samples were ground by a rotor mill and allowed to pass through a 0.5 mm sieve. For the digestion with H_2SO_4 (0.1 N) containing digestion mixture (10 parts potassium sulphate and 1 part copper sulphate), 1 g of each treatment's ground sample were used.

Other elements

Plant samples were taken from each treatment for determination of metallic elements according to Humphries (1956) method. The samples were dried, crushed into very fine powder, and 0.25 gm of this powder was placed into a digestion tube and 1 ml of concentrated H_2SO_4 was added and then placed on a sand heater inside the hood for15-20 minutes till it became dark in color. Then lifted, cooled and 1 ml of a mixture of perchloric acid and concentrated H_2SO_4 was added, and again heated for 30-50 minutes till the sample color changed to transparent water color. Samples were then lifted out and distilled water was added up to 100 ml. From this material the followings were determined (P, K, Ca, Mg, Na, Fe, Mn, Zn and Cu) using spectrophotometer - ICP-OES-Oplima8000

Statistical analysis

All data of the present investigation were subjected to analysis of variance and significant difference among means were determined according to (Snedecor and Cochran, 1980) with the aid of SPSS software. In addition significant difference among mean were distinguished according to the Duncans, multiple test range (Duncan, 1955) and differences between means were compared at 5%.

Results and Discussion

Photosynthetic Pigments

Data recorded in Fig. (1) showed clearly that high salinity stress caused an observed decrease in chlorophyll a (Chl. a), chlorophyll b (Chl. b) and carotenoids in non-fertilized plants, while the low level of salinity (20% of seawater) seems to increase slightly chlorophyll a concentration. It is clear that under salt stress, increased chl. A, Chl. B and carotenoids decreased to reach their lowest values at 40% level of salinity, at which Chl. a, Chl. b and carotenoids decreased by about 35.5%, 44.4% and 39.7% respectively, as compared with salt untreated control plants. On the other side, fertilization enhanced the formation of the pigments and caused an increase in their concentrations either in salt stressed or unstressed plants. In this regard Chem.1 and Chem.2 treatments of the chemical fertilizer resulted in an increase of about 50% and 67% in Chl. a and about 14.8% and 42.6% in Chl. b of the salt unstressed plants as compared with unfertilized control plants, while Chem.3 treatments did not cause any significant effect on Chl. a or Chl. b. Carotenoids also showed an increase in the concentration with fertilization treatments, in this concern Chem.1 and Chem.2 treatments increased carotenoids by about 23.5% and 67%, respectively, as compared with unfertilized control plants. Again Chem.3 treatments didn't cause any change in carotenoid concentration. Negative correlation (R^2 = 89%) between seawater concentration and pigment content was obtained.

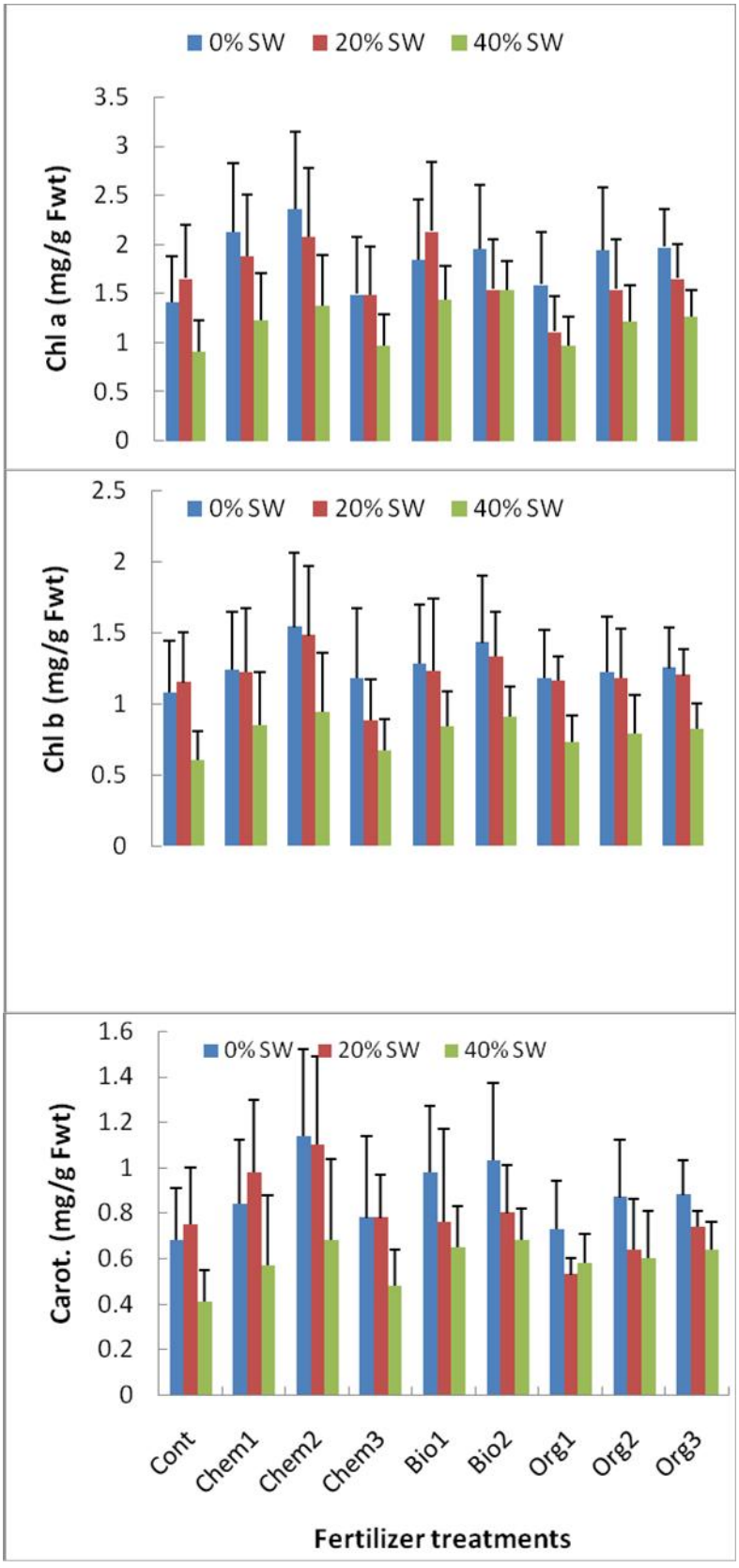

Figure 1: Effect of chemical fertilizer, biofertilizer and organic fertilizer on chl a, chl b and carotenoids of wheat plants grown under different concentrations of seawater (SW). (Cont = Control; vertical lines indicate SD values).

Biofertilizers, also, caused an increase in photosynthetic pigments of salt stressed or unstressed plants. In salt unstreassed control plants, the effect of Bio.2 treatment (*Azotobacter*) was more pronounced in increasing the pigments than the effect of Bio.1 treatment (*Rhizobium*). In this regard, Bio.1 treatment increased Chl. a, Chl. b and carotenoids by about 30.5%, 18.5% and 44%, respectively, as compared with unfertilized control plants; while the corresponding increases with Bio. 2 were about 38.3%, 32.4% and 51.5%, respectively. As for humic acid as an organic fertilizer, recorded data indicate clearly that as organic fertilizer concentration increased the pigment concentration increased. In this respect, Org.1, Org.2 and Org.3 treatments caused an increase of about 12.8%, 37.6% and 39.7%, respectively in Chl. a; and about 4.6%, 12.9% and 15.7%, respectively in Chl. b, as compared with unfertilized control plants.

Concerning effects of fertilizers on salt stressed plants, data recorded in the same figure also shows that chemical, Bio and organic fertilizers tended to alleviate the negative effect of salinity stress on the photosynthetic pigments. At 40% salinity level Chem.1 and Chem.2 treatments caused increase of about 26% and 33%, respectively, in Chl. a and about 41% and 56%, respectively, in Chl. b as compared to the unfertilized plants grown under the same level of salinity stress (40% sea water). Carotenoids also was increased in the fertilized plants under salt stress. In this regard Chem. 1 and Chem.2 treatments caused an increase in carotenoid concentration of about 39% and 65%, respectively, in the 40% salt stressed plants, as compared to unfertilized plants under same level of salt stress. While Chem.3 treatment didn't cause significant changes in carotenoid concentration in plants grown under salt stress.

Bio.1 and Bio.2 treatments resulted in an increase of about 36.8% and 40.5%, respectively, in Chl. a and about 40% and 52%, respectively, in Chl. b of the 40% salt stressed plants as compared with unfertilized plants grown under the same salinity stress level. Carotenoids at any case were also increased under salt stress when plants were treated with Biofertilizers. The increase in carotenoid concentration in the 40% sea water treated plants reached about 58% with Bio.1 treatment (*Rhizobium*) and about 66% with Bio.2 (*Azotobacter*) comparing with non-fertilized plants under 40% salinity stress.

Organic fertilizers showed also an enhancement effect on the photosynthetic pigments of salt stressed plants. In the 40% sea water salinity stressed plants, chlorophyll a was increased by about 7%, 33% and 39% with Org.1, Org.2 and Org.3 treatments, respectively; while Chl. b was increased by about 22%, 32% and 37%, respectively. Carotenoids were also increased with organic fertilizer treatments. The percent increases in carotenoids under 40% salt stress conditions reached nearly 42%, 46% and 56% at Org.1, Org.2 and Org.3, respectively, as compared to the 40% stressed plants with no fertilization treatments.

The increase in chlorophyll content under low levels of salinity recorded in this study is in agreement with the finding of Hussein *et al.* (2012) on pepper plants and Liu *et al.* (2007) on *Aeluropus littoralis* plants who found that salt stress increased Chl a and Chl b contents. This increase may be attributed to the thickness of the leaves under salt stress rather than to the stimulation of pigment formation. With increasing salinity levels, the photosynthetic pigment concentrations significantly decreased, this reduction may be related to enhanced activity of the chlorophyll-degrading enzyme, chlorophyllase, as suggested by Yasar *et al.* (2008), who indicated that increasing saline increased oxidation of chlorophyll leading to its decreased concentration. The decrease in chlorophyll content under salinity conditions is reported by Kusvuran *et al.* (2010), and Nazarbeygi *et al.* (2011) and might have been due to salt-induced increase in the activity of the chlorophyll degrading enzyme, chlorophylase (Noreen and Ashraf, 2009).

Earlier studies reported that the reduction in leaf chlorophyll content of the plants grown in NaCl stress has been attributed to the destruction of chlorophyll pigments and instability of the pigment protein complex. Furthermore, increased salt content also interferes with protein synthesis and influences the structural component of chlorophyll (Jaleel *et al.*, 2008). The decrease in chlorophyll content under stress conditions is a commonly observed phenomenon (Kumar *et al.*, 2011), and might be attributed to reduced synthesis of the main chlorophyll pigment complexes encoded by the chl. gene family (Nikolaeva *et al.*, 2010), or to destruction of the pigment protein complexes which protect the photosynthetic apparatus, or to oxidative damage of chloroplast lipids and proteins, therefore formation of chlorophyll a, b and carotenoids decreases. In this regard Akça and Samsunlu (2012) reported that the negative effects of abiotic stress on photosynthetic pigments could be due to the inhibition of chlorophyll biosynthesis or increase of its degradation by chlorophyllase enzyme,

which is more active under stresses. An oxidative stress could happen due to salt and water stress leading to deterioration in chloroplast structure, and consequently decrease in chlorophyll content (Kumar *et al.*, 2011).

As recorded in the figure, fertilization treatments increased chlorophyll and carotenoid contents in wheat during the growing season. Several functions are proposed for the accumulation of these compounds in plant tissues submitted to fertilization including osmotic adjustment, stabilization of proteins and membranes, being a scavenger of free radicals, improvement of the stability of some cytoplasmic and mitochondrial enzymes, and increased protection of proteins and enzymes or membranes (Simaei *et al.* 2011). The greatest part of the yield of cultivated plants is known to result from work of the photosynthetic apparatus, in which the chlorophyll molecule occupies a key place. In the present study, photosynthetic pigments content was investigated in plant leaves. The N fertilization was the most favorable variant for leaf chlorophyll content. This is in keeping with published data indicating that nitrogen exerts the greatest influence on chlorophyll content. Nitrogen is a structural element of chlorophyll and protein molecules, and it thereby affects formation of chloroplasts and accumulation of chlorophyll in them (Ray Tucker, 2004). The influence of nitrogen on formation of green pigments in the leaf depends primarily on its concentration. It affects the stability of chlorophyll in plants. Even though nitrogen is the most important mineral element in the process of chlorophyll biosynthesis, adding nitrogen to the soil can have negative as well as positive effects, since an excess of nitrogen shortens the life of leaves, increases their sensitivity, and lowers their resistance to plant diseases, which leads to decrease of leaf chlorophyll content (Bojović and Stojanović, 2005), therefore, in the present study the pigment concentration was decreased at the highest level of chemical fertilizer (Chem.3).

The alleviation effect of fertilizers under saline conditions on the growth and Chlorophyll content of some plants is reported in the literature (Al-Aghabary *et al.*, 2004). This positive effect of fertilizers on the photosynthetic pigments may be due to the improvement of chlorophyll formation, and photochemical efficiency of leaf. Fertilizers alleviate salt stress with maintenance of cell form through improving permeability of plasma membranes due to the increase of anti-oxidative enzymes (Al-Aghabary *et al.*, 2004) and improvement of plant water status (Parveen and Ashraf, 2010). Among the positive effects of fertilizers in the counteraction of the adverse effects of salt and water stress are the stabilization and protection of the photosynthetic pigments and the photosynthetic apparatus from oxidization (Khan *et al.* 2010). Different fertilizers can mitigate the adverse effects of drought through increasing the content of IAA and GA3 and decreasing ABA level, which may be involved in protecting the photosynthetic apparatus and consequently increasing the photosynthetic pigments (Saeidi-Sar *et al.*, 2013). The ones most recently used are based on the evaluation of performance of the photosynthetic system. A positive correlation between leaf N fertilization and rate and the chlorophyll content is well documented for a number of plant species and has been investigated for rapid N determination for most major crops including corn, rice, wheat (Houles *et al.*, 2007). Regulation of metabolic and developmental processes by photosynthetic pigments often depends on nitrogen supply, therefore, the assay of wheat photosynthetic pigment contents may serve to optimize wheat fertilization technologies (Tranavičienė *et al.*, 2008). Results of the present study are in agreement with that reported by Ramakrishnan and Selvakumar (2012) who found that *Azotobacter* treated plants had the highest chlorophyll and protein contents. Similarity biofertilizer significantly improved chlorophyll concentration in chilli (Selvakumar and Thamizhiniyan, 2011) and in black gram (Selvakumar *et al.*, 2012). This is because, N is the chief constituent of protein, essential for the formation of protoplasm, which leads to cell enlargement, cell division and ultimately resulting in increased plant growth. *Azotobacter* augment the plant growth mainly due to the biosynthesis of growth promoting substances like vitamins and auxins.

Carbohydrate contents

Effects of fertilizer treatments and salinity stress on soluble and non-soluble sugars (Fig. 2) and on total carbohydrates (Fig. 3) of wheat plants were well illustrated. It was clear that the content of total carbohydrates decreased with increasing levels of salinity to give the lowest value of total carbohydrates at the highest level of salinity stress compared with those of non-salt stressed plants. In this regard the 20% salinity stress resulted in a decrease of about 12.2% in total carbohydrates as compared with control plants. At 40% seawater salinity level the effect on total carbohydrates was even worse, at this level of salinity total carbohydrates decreased by about 19.7% as compared with salt unstressed control plants.

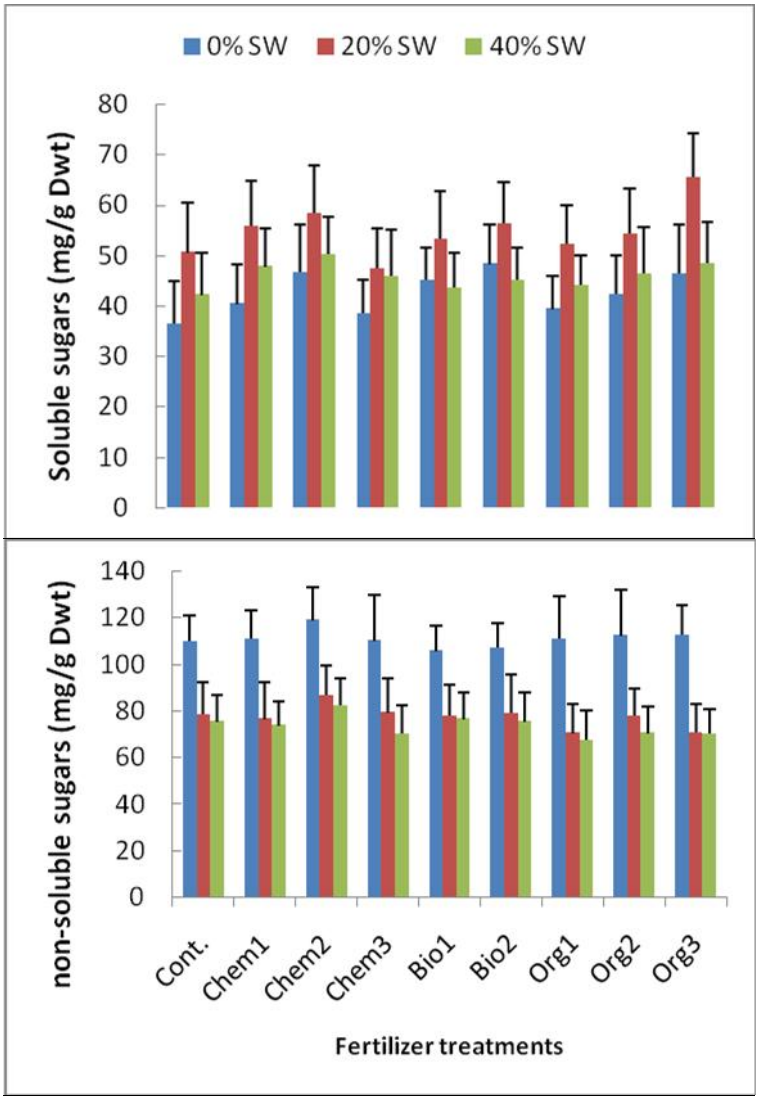

Figure 2: Effect of chemical fertilizer, biofertilizer and organic fertilizer on soluble and non-soluble sugars of wheat plants grown under different concentrations of seawater (SW). (Cont = Control; vertical lines indicate SD values).

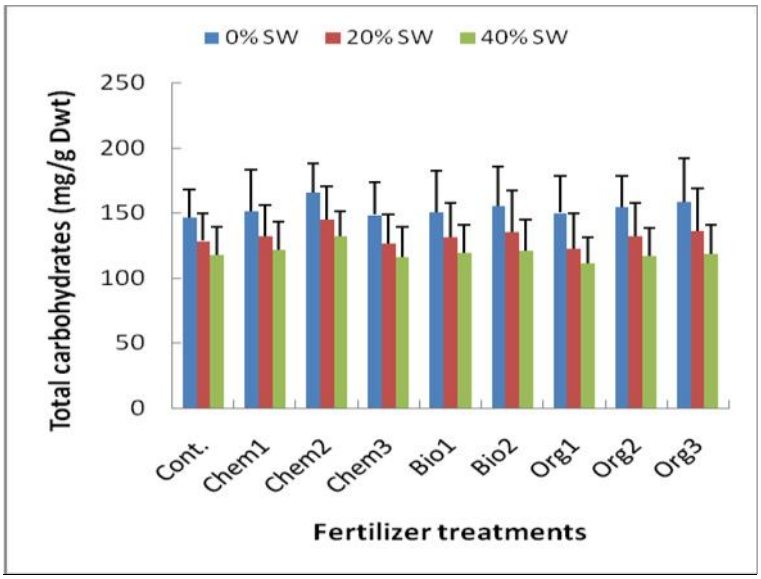

Figure 3: Effect of chemical fertilizer, biofertilizer and organic fertilizer on total carbohydrates of wheat plants grown under different concentrations of seawater (SW). (Cont = Control; vertical lines indicate SD values).

Soluble sugars, on the other side, increased significantly at salinity level of 20% seawater then started to decrease at higher salt stress (40% concentration of seawater) however it was still higher than that of control plants by about 15.6%. As for non-soluble carbohydrates, data recorded in the figure showed that seawater caused an observed decrease in sugar contents. At 20% level of seawater the decrease in non-soluble carbohydrates was about 28.8%, while at 40% saline sea water the concentration was decreased by about 31.5% as compared with salt unstressed control plants. Soluble sugars showed slight negative correlation with sea water concentration (R^2 = 26%), while insoluble sugars and total carbohydrates showed slight positive correlation (R^2 =84% and 82%, respectively).

Fertilizer treatments resulted, generally, in significant increases in the contents of total carbohydrates and soluble sugars in salt stressed and unstressed wheat plants. In salt unstressed plants, total carbohydrate, soluble sugars and non-soluble carbohydrates increased by chemical fertilizer treatments. The maximum increase in carbohydrate fractions was recorded at Chem.2 treatment, at which total carbohydrate, soluble sugars and non-soluble carbohydrates increased by about 13%, 28.5% and 8.1%, respectively, as compared with unfertilized salt untreated control plants.

The biofertilizers showed also an improvement in carbohydrate fractions as compared to unfertilized plants. In this regard, Bio.1 and Bio.2 treatments resulted in an increase of about 3% and 6%, respectively, in total carbohydrate and about 24% and 32%, respectively, in soluble sugar content of salt untreated plants as compared to salt untreated control plants. No significant changes were recorded in non soluble carbohydrates of biofertilizer treated plants grown under salt unstressed condition. Organic fertilizer treatments, particularly Org.3 treatment, showed also an improvement of carbohydrate fractions in salt unstressed plants. The Org.3 treatment caused an increase of about 8.3% in total carbohydrate and about 27% in soluble sugars of salt untreated plants compared with unfertilized control plants. Again, non-soluble carbohydrates were not affected significantly by organic fertilizer treatments.

Similarly, several studies had been used saline irrigation water as a water management practices for irrigation of some moderate and high salinity tolerant crops. A greenhouse experiment was carried-out by Hajiboland *et al* (2009) in clay soil. They cultivated sugar beets (*Beta vulgarris* L) as a salt resistance crop under 4 saline irrigation water treatments namely: 0.98 (fresh water), 4, 8 and12 dS/m as well as 2 water regimes 100% (WI) and 75 % (WII) of the plant water requirement. They found that the sugar % increased with increasing salinity levels of irrigation water under both irrigation regimes and the sugar % was higher in WI compared to WII under different salinity levels. The results indicated that under salt treatment the sugar production showed values nearly 52.5 higher than that for fresh water. In another study on wheat plants grown under salinity stress conditions, Russo *et al*. (2009) found that fructose and glucose percentage was enhanced by salinity levels. They reported that, in saline treatments the contrasting trend of enzyme activity, corresponding to an increase in glucose and fructose is a strategy to bring about osmotic adjustment, required to overcome salt stress depending on the large transport of Na+ to leaves which the plant achieves by increasing the production osmo-compatible components at foliar level. Sugars and total carbohydrates of leaves were markedly decreased in salt-stressed wheat plants. Such inhibition in sugar accumulation was recorded by other authors (Kafi *et al.*, 2010). The decrease in sugars and photosynthetic pigment contents were directly proportional to the applied concentration of NaCl. These results led to the conclusion that NaCl may inhibit photosynthetic activity or increase partial utilization of carbohydrates in other metabolic pathways. Application of fertilizers generally stimulated the accumulation of sugars in salt-treated plants and the inhibitory effects of salt stress were partially alleviated.

The enhancement effect of fertilizers on carbohydrate biosynthesis, especially soluble sugars, is considered to be the principle organic osmotica in a number of glycophytes subjected to saline conditions (Hassanein, 2004). This effect highlights another possible mechanism by which fertilization plays a positive role in alleviation of the harmful effects of salt stress. Subjecting salinity stressed wheat plants to fertilization synergistically increased the amounts of soluble sugars than in untreated stressed ones which indicated that accumulation of these compounds by fertilization plays a key role in retaining the water capacity of stressed cells which thereby can tolerate severe salinity stress (Abdalla, 2011). Concerning biofertilizer treatments, results of the present study are in agreement with that reported by Ramakrishnan and Selvakumar (2012) who found that *Azotobacter* treated plants had the highest carbohydrate contents. Similarity biofertilizer significantly improved sugar concentration in chilli plants (Selvakumar and

Thamizhiniyan, 2011) and in black gram plants (Selvakumar *et al.*, 2012).

Data recorded in the present study showed clearly that the magnitude of carbohydrate reduction was increased with increasing salinity stress level. The reduction in soluble sugars and total carbohydrates in wheat plants under high salt stress could be attributed to the nutritional imbalance and reduced photosynthesis as recorded by Ramezani *et al.* (2011). Moreover, the reduction of sugar content under salinity stress may be attributed to the negative effect of the stress on photosynthetic pigments and consequently on photosynthesis as indicated by the data of photosynthetic pigments. In this regard, Yazdanpanah *et al.* (2011) found that net photosynthesis, transpiration rate and stomatal conductance were significantly affected by salt stress due to changes in chlorophyll content and chloroplast structure.

In a previous study by Jalal *et al.* (2012) they found that stress conditions decreased chl a, chl b, carotenoids and caused stomatal closure in *P. tenuiflorus* plants. Stomatal closure, in turne, restricts CO_2 entry into leaves thereby decreasing CO_2 assimilation and carbohydrate formation (Chaves, 2002). On the other side, the present study showed that fertilizer treatments improved plant tolerance against salinity stress and sugars approached near its normal condition. Increasing amount of sugars and thus the osmosis gradient in plant tissues treated with fertilizers would lead to the resistance against loosing water, protect chloroplasts and accelerate plant growth and carbohydrate formation under stress conditions (Amin *et al.*, 2009).

Nutrient elements

Collected data showed clearly that, N, P, Ca and Mg concentrations in shoots and roots of stressed plants decreased with increasing salinity stress to reach their lowest values at 40% salinity level, at which, N, P, Ca and Mg were decreased by nearly 12%, 8%, 6% and 14%, respectively in salt stressed shoots and by about 33%, 14%, 15% and 20%, respectively in salt stressed roots as compared to salt untreated control plants (Tables 1 and 2). All microelement nutrients (Fe, Mn and Zn) either in shoots (Table 3) or in roots (Table 4) of wheat plants decreased with increasing salinity stress.

It seems that nitrogen concentrations were reduced by increasing salinity stress and decreasing macronutrient level. Decreased N concentration may be associated with an increase in Na or Cl concentration (Taiz and Zeiger, 2010). The decrease in N concentration in shoots and roots may also be attributed to the accelerated reduction of NO_3 to NH_4 under salt stress (Bybordi, 2010), or to the decrease in nitrate reductase activity in salt stressed wheat plants which cause an inhibition of NO_3 uptake. The decrease in N content due to salt stress has been reported in various crops (Scagel *et al.*, 2011).

It is well known that, salinity affects every aspect of physiology and biochemistry of a plant (Parvaiz and Satyawati, 2008). Chemical analysis showed a significant elevation in the levels of sodium ion concentration while K decreased with application of higher concentrations of NaCl. The concentration of potassium (K) in wheat plants was decreased and sodium (Na) was increased in both shoots and roots with increasing salinity stress conditions. It is a fact that salinity stress is generally recognized as injurious to plants by disturbing the electrolyte balance, resulting in deficiency of some nutrients. It is well known that, salinity stress affects the availability of nutrients in the soil by its effects on the solubility and precipitation of salt, and alters physiological processes within the plant, including nutrient uptake and translocation (Netondo *et al.*, 2004). The rates of increase in Na content were higher in shoots than in roots. The distribution of Na varies among the organs of the plant. Due to this variation, accumulation of Na in different parts of plant differs (Loukehaich *et al.*, 2011).

Potassium content was found to be decreasing with increase in salt stress. These outcomes suggest that there was a competition between Na and K regarding their uptake. Similar findings were reported with soybean cultivars (Li *et al.*, 2006), green bean cultivars (Yasar *et al.*, 2008) and canola cultivars (Bandeh-Hagh *et al.*, 2008). The Na accumulation in plants causes many deleterious effects such as necrosis of leaves and reduced shoot and root growth (Munns, 2010). The accumulation of Na interferes with the K selective ion channels in the root plasma membrane and thus reduce the availability of many nutrients (Tester and Davenport, 2003) . It is generally accepted that Na disturbs the nutrient balance and causes specific toxicity. In this study, salinity caused a significant increase in sodium concentrations in plant shoots (Table 2) and roots (Table 4). This increase was accompanied by a decline in the K concentration, especially in shoots, indicating an apparent antagonism between K and Na. This antagonism may be due to the direct competition between K and Na at a site of ion uptake in the plasmalemma (Mukhomorov and Anikina, 2011). Sodium may also enhance the efflux of K into the growth medium, because of disturbed membrane integrity (Radi *et al.*, 2013).

Table (1): Effect of different fertilizer treatments on macro-elemental concentrations (%) of wheat shoots grown under different concentrations of seawater.

Fertilizer treatments	Seawater concentration														
	0%					20%					40%				
	N%	P%	K%	Ca%	Mg%	N%	P%	K%	Ca%	Mg%	N%	P%	K%	Ca%	Mg%
Control	2.13	0.72	1.85	0.90	0.35	2.05	0.76	1.28	0.96	0.42	1.87	0.66	1.03	0.85	0.32
Chem1	3.22	0.76	1.88	0.94	0.42	2.78	0.82	1.53	1.14	0.52	2.06	0.74	1.29	1.03	0.41
Chem2	4.64	0.82	1.92	0.98	0.46	3.06	0.86	1.67	1.08	0.58	2.56	0.67	1.32	1.11	0.34
Chem3	5.66	0.85	1.95	1.05	0.47	3.65	0.88	1.72	1.15	0.57	2.82	0.72	1.46	1.10	0.52
Bio1	2.76	0.84	2.05	1.11	0.38	2.55	0.86	1.45	1.31	0.48	2.17	0.73	1.18	1.21	0.38
Bio2	3.11	0.89	2.12	1.08	0.41	2.90	0.91	1.83	1.18	0.51	2.30	0.65	1.48	1.06	0.42
Org1	2.54	0.78	1.96	0.96	0.42	2.23	0.85	1.58	0.99	0.48	2.03	0.74	1.40	0.89	0.34
Org2	2.87	0.81	2.03	1.08	0.43	2.76	0.88	1.78	1.09	0.46	2.14	0.77	1.52	0.99	0.37
Org3	3.16	0.86	2.15	1.13	0.47	3.01	0.90	1.92	1.22	0.52	2.75	0.81	1.64	1.02	0.41
LSD 5%	0.56	0.08	0.22	0.07	0.06	0.34	0.04	0.65	0.05	0.07	0.22	0.08	0.12	0.05	0.06

a- Each value is the mean of 3 replicates ± standard errors.

b- Cont = control, Chem1= 100 kg chemical fertilizer/ha, Chem2=150kg chemical fertilizer/ha, Chem3=250kg chemical fertilizer/ha, Bio1= *Rhizobium Spp.*, Bio2= *Azotobacter spp*., Org1= 5kg humic acid/ha and Org2= 10kg humic acid/ha, Org3= 20 kg humic acid/ha.

Table (2): Effect of different fertilizer treatments on micro-elemental concentrations (mg/g dwt)) of wheat shoots grown under different concentrations of seawater.

Fertilizer treatments	Seawater concentration														
	0%					20%					40%				
	Na	Fe	Mn	Zn	Cu	Na	Fe	Mn	Zn	Cu	Na	Fe	Mn	Zn	Cu
Control	0.52	0.17	0.13	0.11	ND	0.72	0.19	0.12	0.11	ND	0.90	0.12	0.11	0.18	ND
Chem1	0.59	0.18	0.15	0.12	ND	0.61	0.20	0.15	0.12	ND	0.92	0.13	0.13	0.19	ND
Chem2	0.62	0.17	0.16	0.13	ND	0.69	0.21	0.14	0.13	ND	0.95	0.13	0.14	0.20	ND
Chem3	0.68	0.16	0.17	0.14	ND	0.77	0.19	0.14	0.14	ND	0.98	0.12	0.15	0.19	ND
Bio1	0.49	0.17	0.14	0.12	ND	0.64	0.19	0.12	0.13	ND	0.85	0.12	0.13	0.19	ND
Bio2	0.46	0.18	0.14	0.12	ND	0.65	0.18	0.13	0.12	ND	0.86	0.13	0.12	0.18	ND
Org1	0.48	0.17	0.15	0.13	ND	0.57	0.17	0.12	0.12	ND	0.87	0.11	0.13	0.18	ND
Org2	0.52	0.18	0.15	0.12	ND	0.63	0.18	0.13	0.13	ND	0.86	0.13	0.13	0.19	ND
Org3	0.55	0.18	0.16	0.13	ND	0.69	0.17	0.13	0.13	ND	0.89	0.12	0.14	0.18	ND
LSD 5%	0.082	0.011	0.012	0.009	00	0.015	0.012	0.086	0.075	00	0.024	0.075	0.01	0.008	00

a- Each value is the mean of 3 replicates ± standard errors.

b- Cont = control, Chem1= 100 kg chemical fertilizer/ha, Chem2=150kg chemical fertilizer/ha, Chem3=250kg chemical fertilizer/ha, Bio1= *Rhizobium Spp.*, Bio2= *Azotobacter spp*., Org1= 5kg humic acid/ha and Org2= 10kg humic acid/ha, Org3= 20 kg humic acid/ha.

Table (3): Effect of different fertilizer treatments on elemental concentrations (%) of wheat roots grown under different concentrations of seawater.

Fertilizer treatments	Seawater concentration														
	0%					20%					40%				
	N%	P%	K%	Ca%	Mg%	N%	P%	K%	Ca%	Mg%	N%	P%	K%	Ca%	Mg%
Control	1.57	0.42	0.95	0.65	0.25	1.26	0.35	0.82	0.77	0.32	1.05	0.32	0.71	0.55	0.20
Chem1	1.89	0.46	0.98	0.67	0.35	1.23	0.42	0.88	0.78	0.34	1.15	0.36	0.79	0.67	0.24
Chem2	2.11	0.52	1.02	0.78	0.42	1.85	0.44	0.92	0.82	0.42	1.75	0.38	0.88	0.70	0.26
Chem3	2.78	0.55	1.15	0.79	0.45	2.05	0.46	0.96	0.86	0.46	1.87	0.40	0.84	0.74	0.30
Bio1	2.15	0.74	1.45	0.85	0.36	2.00	0.36	0.88	0.76	0.45	1.66	0.37	0.80	0.78	0.24
Bio2	2.65	0.79	1.52	0.88	0.38	2.13	0.38	0.85	0.87	0.46	1.82	0.39	0.78	0.80	0.28
Org1	1.95	0.68	1.24	0.76	0.38	1.82	0.40	0.87	0.80	0.38	1.32	0.35	0.74	0.76	0.25
Org2	2.03	0.71	1.63	0.88	0.40	1.76	0.42	0.92	0.84	0.42	1.52	0.46	0.82	0.78	0.28
Org3	2.67	0.76	1.65	0.93	0.42	2.05	0.45	0.97	0.88	0.46	1.65	0.48	0.89	0.82	0.31
LSD 5%	0.20	0.15	0.14	0.12	0.09	0.11	0.09	0.07	0.06	0.06	0.11	0.06	0.04	0.03	0.05

a- Each value is the mean of 3 replicates ± standard errors.

b- Cont = control, Chem1= 100 kg chemical fertilizer/ha, Chem2=150kg chemical fertilizer/ha, Chem3=250kg chemical fertilizer/ha, Bio1= *Rhizobium Spp.*, Bio2= *Azotobacter spp*., Org1= 5kg humic acid/ha and Org2= 10kg humic acid/ha, Org3= 20 kg humic acid/ha.

Table (4): Effect of different fertilizer treatments on elemental concentrations (mg/g dwt) of wheat roots grown under different concentrations of seawater.

Fertilizer treatments	Seawater concentration														
	0%					20%					40%				
	Na	Fe	Mn	Zn	Cu	Na	Fe	Mn	Zn	Cu	Na	Fe	Mn	Zn	Cu
Control	0.76	0.18	0.13	0.15	ND	0.87	0.16	0.11	0.10	ND	0.91	0.11	0.10	0.14	ND
Chem1	0.78	0.19	0.17	0.17	ND	0.86	0.17	0.12	0.11	ND	0.95	0.12	0.11	0.15	ND
Chem2	0.81	0.19	0.17	0.18	ND	0.87	0.17	0.13	0.12	ND	0.97	0.12	0.11	0.15	ND
Chem3	0.88	0.18	0.18	0.19	ND	0.93	0.18	0.13	0.12	ND	0.98	0.13	0.12	0.16	ND
Bio1	0.80	0.18	0.15	0.17	ND	0.87	0.17	0.12	0.11	ND	0.92	0.14	0.12	0.15	ND
Bio2	0.81	0.17	0.16	0.17	ND	0.87	0.18	0.12	0.11	ND	0.90	0.13	0.12	0.15	ND
Org1	0.81	0.18	0.15	0.18	ND	0.86	0.16	0.11	0.12	ND	0.89	0.12	0.13	0.15	ND
Org2	0.82	0.19	0.16	0.18	ND	0.91	0.17	0.12	0.12	ND	0.96	0.13	0.12	0.16	ND
Org3	0.90	0.19	0.16	0.19	ND	0.95	0.17	0.13	0.13	ND	0.99	0.14	0.14	0.17	ND
LSD 5%	0.04	0.005	0.006	0.007	ND	0.035	0.007	0.006	0.008	ND	0.03	0.006	0.009	0.006	ND

a- Each value is the mean of 3 replicates ± standard errors.

b- Cont = control, Chem1= 100 kg chemical fertilizer/ha, Chem2=150kg chemical fertilizer/ha, Chem3=250kg chemical fertilizer/ha, Bio1= *Rhizobium Spp.*, Bio2= *Azotobacter spp*., Org1= 5kg humic acid/ha and Org2= 10kg humic acid/ha, Org3= 20 kg humic acid/ha.

The obtained results showed that calcium and magnesium concentrations in shoots and roots declined with increasing salinity stress. High salinity level in the external medium may have greatly reduced the activity of Ca in the soil solution and may have resulted in a decrease in the amount of Ca available for uptake by the plants. Root growth and function may be inhibited by the high Na/Ca ratio (Abdul Kader and Lindberg, 2010), and may process whereby Ca is transported from the root to the shoot may be impaired. The Ca disorder was eliminated when external Na/Ca was reduced by fertilizer addition. A recent study has shown that K concentration in plant tissues is reduced as the Na/Ca ratio in the root medium increases (Iqbal *et al.*, 2014). In the present study, salinity affected Mg accumulation in shoots and roots similar to Ca behavior. The decrease in Mg concentration seems mainly occur due to ion competition between Na and Mg. Calcium is strongly competitive with Mg. The binding sites on the root plasma membrane appear to have less affinity for the highly hydrated Mg than for Ca (Guimarães *et al.*, 2012). Competition between Ca and Mg may have occurred in this study too. Phosphorus is one of the most important nutrients in the growth and development of plants. It plays a key role in cellular energy transfer, respiration and photosynthesis. Phosphorus uptake decreases with decreasing soil moisture in various crops such as chickpea (Goldani and Rezvani, 2007) and pepper (Cimrin *et al.*, 2010).

Iron is involved in the production of chlorophyll, and iron chlorosis is easily recognized on iron-sensitive crops growing on calcareous soils. Iron also is a component of many enzymes associated with energy transfer, nitrogen reduction and fixation, and lignin formation. Iron deficiencies are mainly manifested by yellow leaves due to low levels of chlorophyll. Salinity stress was found to reduce iron uptake by wheat plants in the present study. Uptake of iron decreases with increased soil salinity, and is adversely affected by the imbalance of other ions. Manganese is necessary in photosynthesis, nitrogen metabolism and to form other compounds required for plant metabolism. Manganese deficiencies mainly occur on organic soils, high-pH soils, sandy soils low in organic matter, and on over-limed soils. Soil manganese may be less available in dry, well-aerated soils. Conversely, manganese toxicity can result in some acidic, high-manganese soils. Uptake of manganese decreases with increased soil pH and is adversely affected by high levels of available iron in soils. Zinc is an essential component of various enzyme systems for energy production, protein synthesis, and growth regulation. Zinc deficient plants also exhibit delayed maturity. Zinc is not mobile in plants so zinc-deficiency symptoms occur mainly in new growth. The most visible zinc deficiency symptoms are short internodes and a decrease in leaf size. Zinc deficiencies are mainly found on sandy soils low in organic matter and on organic soils. Zinc uptake by plants decreases with increased soil pH. Uptake of zinc also is adversely affected by high levels of available phosphorus and iron in soils. The present study revealed that all the micronutrient studied were decreased in plant tissues when treated with salinity. The concentration of these nutrients decreased considerably with increasing level of salinity stress. This increase in micronutrients may be due to the reduction in their uptake by the plants or to the inhibition of root growth under salinity stress conditions (Munns *et al.*, 2010).

The role of fertilizers in increasing ionic content may be due to its effects on stabilizing cellular membranes through increasing antioxidants substances, saving cell membranes from oxidative stress and hence improving plant cell permeability (Farouk, 2011). Data showed that fertilizers increased significantly N, P and K, Ca and Mg as well as micronutrients in shoots and roots of salinity stressed wheat plants. The magnitude of enhancement was increased with increasing fertilizer concentration. These results are in good harmony with those obtained by Akça and Samsunlu (2012) who reported that application of some growth substances enhance the uptake of N, P, K and other nutrient elements. In this regard, they suggested that one of the main roles of fertilizers is to maintain a cation-anion balance in plant tissues by stabilizing cell membranes at high external abiotic stress. In this concern, It has been found that, exogenous supply of mineral fertilizers enhanced elemental concentration in stressed plants. This increase was attributed to the positive effect of fertilizers on the root growth, which consequently increased the absorption of different nutrients and alleviated the harmful effects of water stress (Saeidi-Sar *et al.*, 2013).

Bio and organic fertilizer applications reduced the harmful effects of saline treatment through reduction of inorganic Na ions accumulation. Such an effect may help the plants to avoid ions toxicity. Also, fertilizers improved K uptake under salinity stress, which effectively increased the K/Na ratio in the tissues. This effect is considered to be important in salt tolerance where maintenance of high cytoplasmic level of K is essential for survival in saline habitats (Gadallah, 1999) and the characteristic of K and Na transport are

determinant of the NaCl tolerance in plants (Benlloch *et al*, 1994).

Conclusion

From collected data in the present study it can be concluded that high ratios of sea water significantly reduced photosynthetic pigments, sugars and carbohydrate contents and mineral elements in wheat plants. On the other side, plant fertilization enhanced all parameters as compared with fertilizer un-treated plants. Biofertilizera and organic fertilizers were most affective in promoting chemical constituents than chemical fertilizers, particularly at high concentration treatments of seawater. Therefore, the use of organic and bio-fertilizers may be better than the use of chemical fertilizer to avoid the negative effects of the latter on health and environment.

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