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FOLIAR APPLICATION OF SOME ESSENTIAL MINERALS ON TOMATO (*LYCOPERSICON ESCULENTUM*) PLANT GROWN UNDER TWO DIFFERENT SALINITY REGIMES

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Abstract

Tomato (*Lycopersicon esculetum* var. Avinash, F1 hybrid) was grown under two different regimes of irrigation water (0.2% Sea salt EC_{iw} = 2.9 dS m^{-1} and 0.4% Sea salt EC_{iw} = 5.8 dS m^{-1}) and subjected to foliar application of Potassium, Iron and Boron. Their growth was monitored on some vegetative, reproductive and biochemical parameters. Ripe fruits were collected and total yield was determined in terms of their number, circumference and weight. Reduction in growth was found proportional with increasing salinity of irrigation at all the parameters but foliar application of above mentioned essential minerals minimized the deleterious effects of salinity up to various extents. The significant improvement was observed in the number of fruits, circumference and weight of fruits, chlorophyll and protein contents of leaves, due to the foliar application of Potassium, Iron and the combined treatment of (K+Fe+B) under both non saline and saline conditions. Foliar spray of Boron was not found much effective to improve all the growth parameters. Reason of the growth promotion was assigned to the availability of these essential minerals for growth through foliar spray, as presence of excessive sodium in rooting medium would have reduced their uptake due to ion antagonism.

Introduction

Soil salinity is considered as one of the major environmental stress which adversely affects plant growth and metabolism resulting in considerable losses in crop productivity. Salinity has affected more than 800 million hectares of land throughout the world which is almost 6% of the world's total land area (Anon., 2008). Out of 20.36 million hectare cultivable lands, 6.3 million is affected with salinity of various degrees in Pakistan (Qureshi & Barret Lennard., 1998). In arid and semiarid regions, use of low quality water for irrigation, limited rainfall, high evapotranspiration, high temperature and faulty soil management have further contributed to the salinity problem. Apart from naturally existing saline lands, a significant proportion of recently cultivated agricultural land has become saline due to secondary salinity as a result of the human activity. Soil salinity affects plants in two ways viz., high concentrations of salts in the soil make it harder for roots to extract water while high concentrations of salts within the plant become toxic for growth (Munns & Tester, 2008).

To cope with toxic effect of salts, plants develop biochemical and molecular mechanisms which include compartmentalization of ions at cellular and whole-plant level, synthesis of compatible solutes, change in photosynthetic pathway, alteration in membrane structure, induction of antioxidative enzymes and plant hormones (Ashraf & Harris, 2004; Parida, 2005; Türkan & Demiral, 2009; Flowers *et al*., 2010).

Ion uptake and compartmentalization are crucial not only for normal growth but also for growth under saline conditions (Adams *et al*., 1992). Plants, whether glycophyte or halophyte, cannot tolerate large amounts of salt in the cytoplasm and therefore they either restrict the excess salts in the vacuole or compartmentalize the ions in different tissues under saline conditions to facilitate their metabolic functions (Zhu, 2003). Excessive sodium present in the rhizosphere, apart from its own toxic behavior in plant metabolism, causes physiological droughts. Being antagonistic to other cations, sodium inhibits their entry in root system; hence plants suffer deficiency of other mineral elements, which are essential for growth (Ahmad & Jabeen, 2005). The above mentioned problem created by the presence of excessive sodium in root zone could be avoided if potassium and other essential mineral elements are provided through foliar application to the plants. Potassium, Iron and Boron are essential minerals required for normal physiological processes of plants. Increasing evidence suggests that mineral nutrient status of plants plays a critical role in increasing plant resistance to environmental stresses (Marschner, 1995). Among the mineral nutrients, potassium plays an important role in contributing to the survival of crop plants under environmental stress conditions. Potassium is essential for many physiological processes, such as photosynthesis, translocation of photosynthates into sink organs, maintenance of turgor, activation of enzymes, and reducing excess uptake of ions such as sodium and iron in saline soils (Marschner, 1995; Mengel & Kirkby, 2001). Iron deficiency could induce different biochemical changes in the photosynthetic apparatus. It decreases the content of light-harvesting pigments (Abadía & Abadía, 1993; Morales *et al*., 2000) and the antenna disconnection in photosystem II (PSII) (Morales *et al*., 2001; Moseley *et al*., 2002). Iron import from the metabolic pathways that lead to the assembly of Fe into apoproteins, is crucial for proper photosynthetic activity, and thus represents an essential process for optimal plant productivity (Briat *et al.,* 2007).

Importance of Boron is also well documented for great variety of physiological processes in plants and it seems to exert different effects such as root elongation, indole-3-acetic acid oxidase, sugar translocation, carbohydrate metabolism, nucleic acid synthesis and pollen tube growth (Blevins & Lukaszewski, 1998; Goldbach & Wimmer, 2007; Marschner, 1995). Moreover, more than 90% of the boron is found in cell walls. Its functions are also related to cell wall synthesis, lignifications and maintenance of cell wall structure (Hänsch & Mendel, 2009). Its deficiency predominantly damages actively growing organs such as shoot and root tips and effects flower retention, pollen formation, pollen tube growth or germination, nitrogen fixation and nitrate assimilation (Camacho-cristobal *et al*., 2008; Agustin, 2008).

The diverse physiological functions of K, B and Fe in plant metabolism suggest that their exogenous application through foliar spray could avoid their deficiency in plant created in presence of excessive sodium in rooting medium.

Tomato is one of the most important and widespread crop in the world. The total production of tomato in Pakistan is reported 468,146 tons in 2006 (Anon., 2008). Salinity is a well known factor affecting negatively growth and production of tomato (Tantawy, 2007). It is classified as moderately salt sensitive crop and the threshold value of saline rooting medium is given as 2.5 dS m^{-1}(Mass, 1986). The aim of present research work is to increase the level of salinity tolerance in tomato above their threshold value by the help of foliar supply of essential minerals, so that they could be grown under a bit higher level of salinity up to profitable extent.

Material and Methods

Seed germination: Seeds of tomato *(Lycopersicon esculetum)* cv. Avinash (F1 hybrid) were obtained from local market. The seeds were surface sterilized prior to germination, with 0.5% sodium hypochlorite solution for 2 minutes and washed 3 times with tap water, then allowed to imbibe in distilled water for one hour. The sea salt solutions of different concentrations viz., 0, 0.1, 0.2, 0.3, 0.4, 0.5 and 0.6% having EC_{iw} = 0.5, 1.4, 2.8, 4.2, 5.7, 7.1 and 8.5 dS m^{-1}, respectively, were prepared. The filter paper in each Petri plate was soaked with respective solutions and 10 seeds were placed in each Petri plate under sterilized conditions. The Petri plates having non saline tap water was considered as controls. Each treatment consisted of four replicates. The Petri plates were placed in temperature controlled incubator at 28±1 °C in dark. The germination percentage was recorded upto 10th day on daily basis.

Experimental setup and foliar application: Five seeds of tomato were sown in large size plastic pots having upper diameter of 12 inches, containing about 20 Kg of sandy loam soil. A hole was made in base for leaching purpose. Thinning of seedlings was done at three leaf stage by leaving a single well developed seedling of approximately same size in each pot. One hundred and eight pots were divided in six sets containing 18 pots in each set. These sets were subjected to three different treatments (six plants in each treatment) for creating saline substrate through irrigation with following sea salt solutions i.e.:

i. Non-saline control irrigated with tap water (EC_{iw} = 0.6 dS m^{-1}, EC_e =1.9 dS m^{-1})
ii. Irrigated with 0.2% sea salt solution (EC_{iw} = 2.9 dS m^{-1}, EC_e = 4.31 dS m^{-1})
iii. Irrigated with 0.4% sea salt solution (EC_{iw} = 5.8 dS m^{-1}, EC_e = 6.5 dS m^{-1})

The salinity was increased gradually by starting from 0.1% sea salt solution till the required salinity level was reached. The Electrical conductivity of soil (EC_e) was monitored throughout the experiment. These six sets were subjected to following foliar sprays:

Set#1. Non-Spray Control
Set#2. Water Spray (with non saline tap water)
Set#3. KNO_3 Spray (500 ppm) containing 193 ppm of K
Set#4. Fe-EDTA Spray (10 ppm) containing 1.5 ppm of Fe
Set#5. H_3BO_3Spray (10 ppm) containing 1.7 ppm of Boron
Set#6. KNO_3 + Fe EDTA + H_3BO_3 Spray containing 500 ppm + 10 ppm + 10 ppm respectively)

Foliar spray was started at 5 leaves stage, and then repeated at incipient flowering, and mid fruiting stage. Spray medium was containing 0.1% Tween 20 (polyoxyethylene sorbitan monolaurate) as a surfactant and plants were completely sprayed with 200 ml/plant of respective spray on both the upper and lower surface of leaves.

Vegetative and reproductive growth: Three plants of each treatment were randomly harvested at grand period of growth when flower initiation take placed and their height, number of branches, fresh and dry biomass of shoot was recorded. Ripe fruits were collected throughout the growth period; their circumference, number and weight per plant was recorded.

Biochemical analysis: Following biochemical analysis were undertaken at grand period of growth in leaves of the plants undergoing various treatments.

Chlorophyll contents: The chlorophyll contents of leaves were determined at grand period of growth. Leaf material was crushed with 6 ml 80% cold acetone and carefully transferred into centrifuge tubes. The extracts were centrifuged at 2000 rpm for 5 minutes. The supernatant was separated and resulting residue was again mixed with 2 ml of 80% cold acetone for two times to remove maximum chlorophylls. All the supernatant were combined and the volume of each was then adjusted to 10 ml with acetone. The absorbance was recorded at 645 and 663nm on spectrophotometer (Jenway 6305 UV/Vis Spectrophotometer). The chlorophyll contents were determined according to the method of Arnon (1949).

Carbohydrate contents: Carbohydrate contents of leaves were determined in hot water extract by the method of Yemm & Willis (1954). Dry leaf sample (0.2 g) was taken in 10 ml distilled water and kept on boiling water bath for 30 min. For the determination of carbohydrates, 1 ml diluted sample was mixed with 5 ml of Anthron's reagent and then kept on boiling water bath for 30 minutes. The absorbance was measured at 620 nm after cooling against glucose as standard.

Soluble protein contents: For soluble protein contents in leaves, 0.2 g fresh leaf material was homogenized in 5 ml of potassium phosphate buffer (pH 7), then centrifuged at 17,000 x g for 20 minutes and carefully transferred the supernatant into test tubes. The amount of soluble proteins in this extract was measured according to the method of Bradford (1976) against Bovine serum albumin (BSA) as standards.

Results and Discussion

Seed germination: Germination percentage showed a decrease as well as delay proportional to the increase of salinity levels of irrigation (Fig. 1). Seeds did not germinate beyond 0.6% sea salt solution (EC_w = 8.5 dS m^{-1}). Cuartero & Fernandez-Muñoz (1999) also observed similar results for seed germination of tomato. Seeds are not capable of imbibing enough water to germinate under saline conditions due to osmotic stress (Ghoulam & Fores, 2001). The higher concentration of salt reduces the water potential in medium which hinders water absorption in germinating seeds thus reducing germination (Jamil *et al*., 2006). There are many reports about delay and decrease in germination of seeds of various plants under salt stress (Puppala *et al*., 1999. Volkmar & Miller, 2001). The effect of salinity on the seed germination is attributed to osmotic or ionic stress which can alter physiological processes including enzyme activation (Begum *et al*., 1992; Croser *et al*., 2001; Essa & Al-Ani, 2001). The most important enzyme involved in seed germination is α-amylase which is influenced by salt stress. Sodium ions are reported to block the active surface of this enzyme which destroys structure of this enzyme (Saboury & Karbassi, 2000).

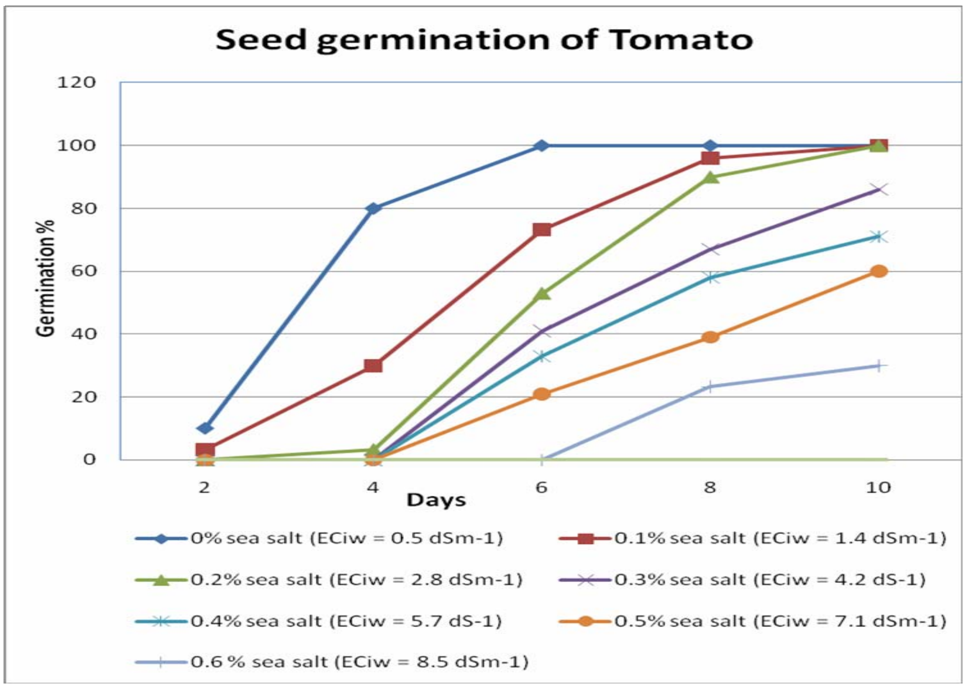
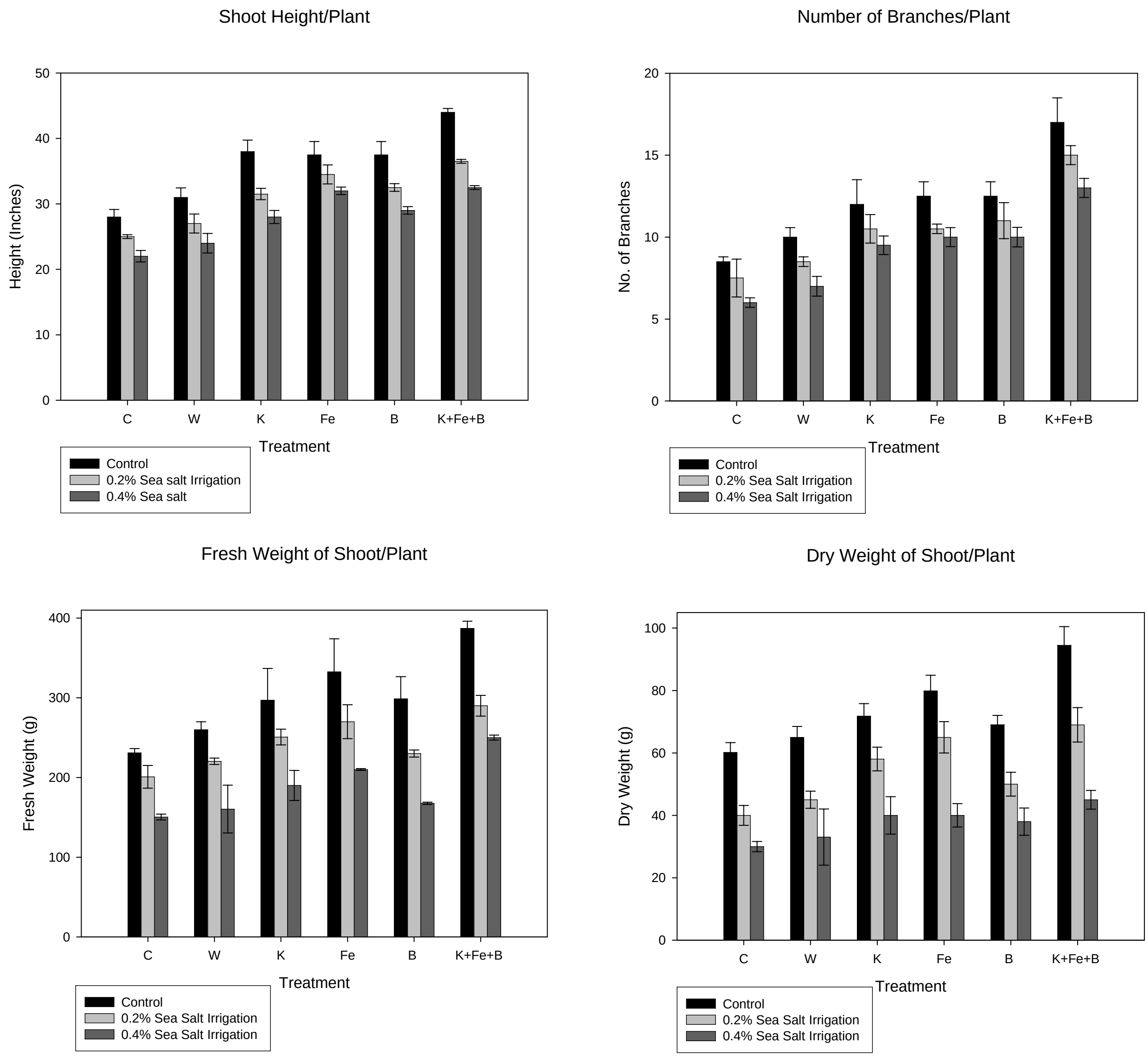


Fig. 1. Germination percentage of tomato seeds (*Lycopersicon esculentum*) under different salinity regimes.

Vegetative and reproductive growth: Data presented in Fig. 2 showed that vegetative growth is inversely proportional to the salinity up to significant level ($p<0.001$). Threshold value of tomato for salinity is 2.5 dS^{-m} and the % reduction in growth by increase of one dS/m of salinity is reported by 9.9% and tomato plant is considered as moderately salt sensitive crop (Mass, 1986). Adverse affects of salinity on growth has been observed in plants by many scientists such as Satti & Al-Yahyi (1995), in tomato. Leidi & Saiz (1997), in cotton. Chartzoulakis & Klapaki (1998) and Li & Stanghellin (2001), in tomato. Kaya *et al*., 2001, in cucumber and pepper, Tantawy (2007), in tomato and sweet pepper. Exogenous application through foliar spray of essential elements like K, Fe, and B was found promising to enhance the growth at different parameters in present investigation both under non saline as well as saline conditions. Height of tomato plant is significantly increased by the application of K, Fe and B individually as well as by their mixture. Maximum shoot height was observed in plants sprayed with mixture (K+Fe+B). This trend persisted even under saline water irrigation. Increase in number of branches were less under spray of individual mineral element in comparison with their mixture but still more than non spray control. Combined treatment of these minerals (K+Fe+B) significantly enhanced the number of branches under both saline and non saline conditions. Kaya et al., (2001) also observed the ameliorating affect of KH_2PO_4 spray even at higher level of salinity in cucumber, spinach and pepper. Similar improvement on vegetative growth through foliar application of KNO3 has been observed in cotton plant under saline as well as non saline condition (Ahmad & Jabeen, 2009). Iron deficiency which impairs adverse affects on the vegetative growth due to Iron chlorosis (Larbi *et al*., 2006) could be mitigated by foliar spray of Fe under non saline and saline conditions. Application of micronutrients is reported to enhance photosynthetic activities which lead to an increase in cell division and elongation, and increasing vegetative biomass. Iron plays an important role in promoting growth, as it is a component of ferrodoxin, an electron transport protein associated with chloroplast and promote photosynthesis (Hazra *et al*., 1987).

There appeared significant increase in vegetative biomass of shoot raised under non saline as well as saline conditions sprayed with only K and Fe but the increase due to spray of their mixture (K+Fe+B) was comparatively greater. This relative difference persisted in plants growing under 0.2% and 0.4% sea salt irrigation. Additionally reduction in vegetative growth under saline environment in cotton was found by Qadir & Shams (1997); Ye *et al*., (1997) and Kaya *et al*., (2001) reported significant reduction in fresh biomass of Spinach at 60 mM salinity, but foliar sprays of 5 mM KH_2PO_4 mitigated the detrimental effect of high salt. Jabeen and Ahmad (2009) observed the decrease in total leaf area and biomass at EC = 6.2 and 10.8 dS m^{-1} in Cotton (*Gossypium hirsutum)*. The dry biomass of shoot followed same trend as shown by fresh shoot biomass. Huang & Redmann (1995) found that osmotic stress induced by salinity was responsible for the reduction in fresh and dry biomass in canola and wild mustard. In the present investigation Boron spray did not show any significant improvement in non saline as well as saline condition in both fresh and dry biomass of shoot, it may be probably due to Boron approaching to toxic level in tomato. Various plants are sensitive to higher Boron concentration up to different extent. Excessive boron can cause deleterious effects on plant growth. In present experiment spray of 10 ppm H_3BO_3 appears not suitable concentration for tomato plant. According to Grieve & Poss (2000) excess exogenous B concentration (10 and 15 ppm) and salinity (8 and 12 dSm^{-1}) interact to limit growth, decrease all the vegetative growth parameters resulting decrease in yield components of wheat. Boron tolerance mechanism of plants has a similarity to salt tolerance mechanism. Plant species or genotypes are susceptible to B toxicity generally have higher concentrations of B in shoots than do tolerant species and genotypes (Nable *et al*., 1997). According to Alpaslan & Gunes (2001) Boron toxicity symptom appeared at 5 mg kg^{-1} B treatments and was more severe in the high boron, 10 and 20 mg kg^{-1}, treatments in tomato plants. Cucumber plants did not survive at the highest B, 20 mg kg^{-1} treatment. Hence it appears that critical value of the toxicity of these micronutrients vary in different crops depending upon the physiology of their growth.



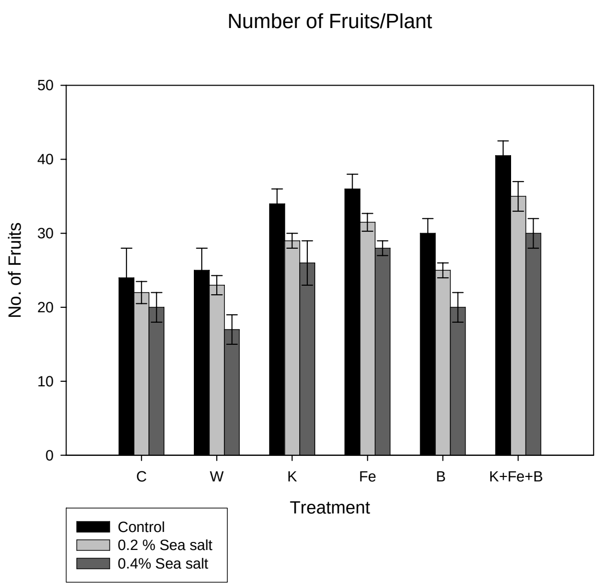
Control (EC_{iw}= 0.6 dS^{-m}, EC_e = 1.9 dS^{-m}), 0.2% Sea salt Irrigation (EC_{iw} = 2.9 dS^{-m}, EC_e = 4.31 dS^{-m}), 0.4% Sea salt Irrigation (EC_{iw} = 5.8 dS^{-m}, EC_e = 6.5 dS^{-m})
C = Non spray, W = water spray, K = Potassium spray, Fe = Iron spray, B = Boron spray, K+Fe+B = Potassium+Iron+Boron spray

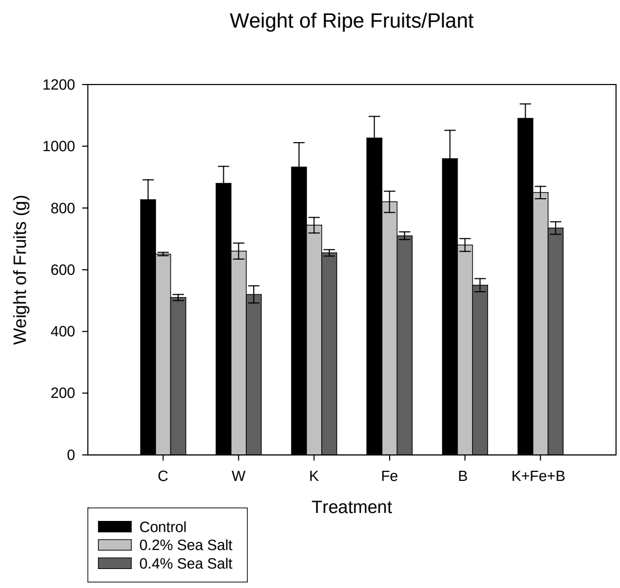
Fig. 2. Vegetative growth parameters of Tomato (*Lycopersicon esculentum*) plant grown through irrigation with water of two different salinity regimes and exposed to foliar spray of Potassium, Iron, Boron and their mixture.

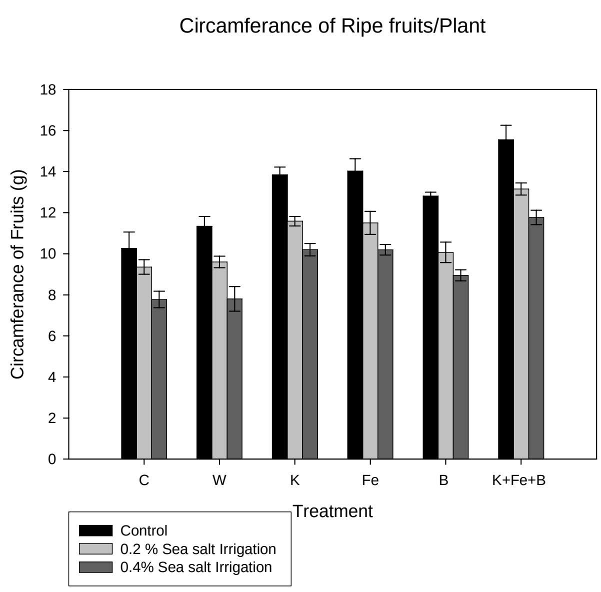
Data presented in Fig. 3 showed significant (p<0.001) decrease in fruit yield per plant under saline water irrigation in terms of weight of total fruits produced by plant. Mitchel *et al*., (1991) also observed the decrease in fruit yield under saline condition in tomato under saline soil, Awang *et al*., (1995) and Saied *et al*., (2005) in strawberry in saline soil. A significant increase was noticed in the weight of ripe fruits (p<0.001) by foliar spray of K, Fe and their mixture (K+Fe+B) on plants raised under non saline condition, in 0.2% sea salt irrigation water this effect was more pronounced under spray of Fe and their mixture of sprayed minerals. The yield of plants irrigated at 0.4% sea salt solution was also significantly increased by foliar spray of K, Fe and the combined treatment of all the three mineral elements in comparison with their non spray control. Increased yield due to above mentioned treatments could be attributed to enhanced photosynthetic activity, and increased production and accumulation of carbohydrates have favorable effect on vegetative growth and retention of flowers and fruits, which increased the number of fruits per plant besides increasing the size. Similarly, the Kumbhar & Deshmukh (1993) and Bose & Tripathi (1996) revealed that the increased dry matter production may be attributed to greater accumulation of photosynthates by vegetative parts and fruits in tomato. Significant improvement in number and circumference of fruit was observed in non saline condition under the foliar spray of K, Fe and combined treatment (K+Fe+B). Significant (p<0.001) decrease was observed for fruit number, fruit weight and circumference in 0.2% salinity but foliar spray treatment of K, Fe and the combine treatment (K+Fe+B) reduced the adverse effects of salinity both in 0.2% and 0.4% sea salt irrigation in comparison with non saline control. Uma & Patil (1996) reported that with increase in salinity levels all the growth and yield parameters were reduced. Abaye (1998) has shown that exogenous application of potassium increases yield compared with the untreated control. Fe spray has been shown to vary according to many plant related, environmental, and physico-chemical factors (Fernández & Ebert, 2005). There are various evidences that Fe-fertilisation increases fruit quality and yield in many crops (Álvarez-Fernández *et al*., 2006).

Biochemical parameters: Data presented in Fig. 4 showed significant decrease in chlorophyll and protein content due to the deleterious effect of the increasing salinity. The growth inhibiting effects of salinity is reported mainly due to the induction of growth inhibitor (abscisic acid) which cause premature senescence of leaves and reduced leaf area ultimately cause decrease in photosynthesis (Kashem *et al*., 2000). Data presented here shows the concentration of total Chlorophyll kept on increasing by foliar spray in plants growing under non saline conditions in following order Control < water spray < K and B < Fe < K+Fe+B. The significant ($p<0.001$) increase of total chlorophyll in plants being irrigated by 0.2% sea salt solution was found only under spray of Fe and K+Fe+B mixture. According to Kaya *et al*., (2001) foliar sprays of KH_2PO_4 mitigated the detrimental effect of high salt in plants sprayed with KH_2PO_4 which show almost the same chlorophyll values as those for unstressed spinach, cucumber and pepper plants. Exogenously applied K plays a vital role in stomatal regulation under salt stress, so an increase in plant photosynthetic rate (Chow *et al*., 1990; Kaya *et al*., 2003). In the present study, foliar application of K along with Fe and B was the most effective in enhancing the chlorophyll content which may have resulted in increased photosynt hetic rate.

The amount of carbohydrate in leaves was significantly increased in plants irrigated with 0.2% and 0.4% sea salt solution. Increase of carbohydrates in plants raised under 0.4% sea salt irrigation water was maximum which has been attributed to reduced ability to utilize assimilates/photosynthates and supply of assimilates to the growing regions (Munns *et al*., 1995; Ahmad *et al*., 2009). Carbohyderate accumulation in combined treatment of K+Fe+B was decreased may be due to the utilization of photosynthate in vigorous vegetative growth and production of yield which was maximum in this treatment. Increase in protein content of leaves is more prominent in plants undergoing foliar spray of Fe and mixture of K+Fe+B over their respective control. This increase was maintained under Fe and K+Fe+B mixture spray under both the above mentioned salinity levels. Higher accumulations of Na+ and Cl− concentrations result in decreased leaf protein content (Sultana *et al*., 1999) and inhibit the growth of plants. Foliar spray of above mentioned treatments could ameliorate these adverse effects and ultimately increase the protein content.







Control (EC_{iw}= 0.6 dS^{-m}, EC_e = 1.9 dS^{-m}),
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0.4% Sea salt Irrigation (EC_{iw} = 5.8 dS^{-m}, EC_e = 6.5 dS^{-m})

C = Non spray, W = water spray, K = Potassium spray,
Fe = Iron spray, B = Boron spray,
K+Fe+B = Potassium+Iron+Boron spray

Fig. 3. Reproductive parameters of Tomato (*Lycopersicon esculentum*) plant grown through irrigation with water of two different salinity regimes undergoing foliar spray of Potassium, Iron, Boron and their mixture.

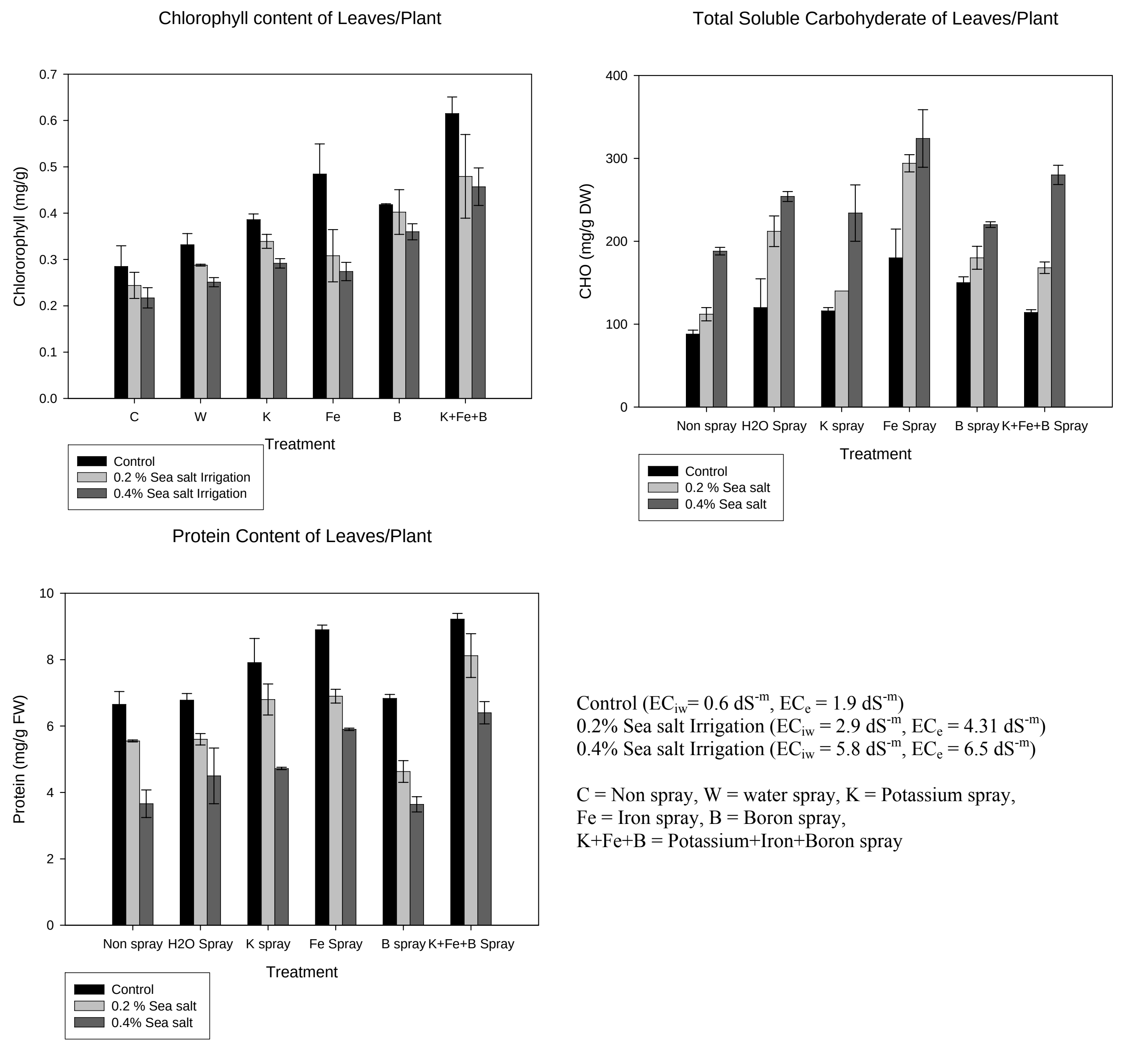

Fig. 4. Some Biochemical parameters of Tomato (*Lycopersicon esculentum*) plant grown through irrigation with water of two different salinity regimes undergoing foliar spray of Potassium, Iron, Boron and their mixture.

Conclusions

Tomato plant is considered as moderately salt sensitive crop. Threshold value of tomato for salinity is 2.5 dS m^{-1} and the % reduction in growth by increase of one dS m^{-1} of salinity is reported by 9.9% (Mass, 1986). The foliar application of potassium and iron alone contributed towards increase at all the studied growth parameters, whereas individual effect of 10 ppm Boric acid did not show any significant improvement. However in mixture its combined effect (K+Fe+B) contributed toward increase at all vegetative, reproductive and biochemical parameters of tomato plants irrespective of non saline and saline conditions. This combined treatment is more effective than individual application of above mentioned minerals. The deleterious effect of excessive sodium was significantly inhibited due to the foliar application of above mentioned minerals and if brought into practice could increase productivity of tomato under saline environment.

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