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EFFECT OF FOLIAR-APPLIED BORON AND MANGANESE ON GROWTH AND BIOCHEMICAL ACTIVITIES IN SUNFLOWER UNDER SALINE CONDITIONS

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Abstract

Experiments were conducted to assess whether exogenous application of some essential micronutrients (B and Mn) through foliar spray could ameliorate the adverse effects of salt stress on growth and biochemical activities of sunflower plants. Sunflower (NuSun 636 cv) were grown under normal and different saline conditions, created through 0.4% (ECiw 6.1 dS/m) and 0.8% (ECiw 10.8dS/m) of sea salt irrigation. Salinity was found to cause a significant reduction at all the vegetative and reproductive growth parameters. Salt stress showed inhibition in biochemical activities such as amount of protein, nucleoprotein and Hill reaction in sunflower leaves. Foliar applications of B through H_3BO_3 and Mn through $MnCl_2$ and their mixture were found to improve all the studied growth parameters and biochemical activities of sunflower plant irrespective to their growth under non-saline or saline conditions. The growth and yield component as a result of the mixture of foliar spray was higher than spray of single element. Their spray appeared to ameliorate the toxic effects of excessive sodium present in growing medium which is reflected by producing economically feasible yield even beyond the threshold value of salinity (ECe 5.5ds/m).

Introduction

The greatest challenge of mankind in the 21st century might not be war, hunger, disease or even the collapse of civic order, it may be the lack of fresh water. Though water is the commonest stuff on earth, but only 2.53% is of good quality, while the rest is saline. Water for irrigation usually contains varying amount of different salts and salinity either of soil or of irrigation water cause disturbances in plant growth and nutrient balance (Parvaiz & Satyawati, 2008). According to Dubey (1997) and Yeo (1998) salts in irrigation water cause both ionic and osmotic effects on plants and most of the known responses of plants to salinity are linked to these effects. Ionic imbalance occurs in the cells due to excessive accumulation of Na^+ and Cl^- and reduces the uptake of other essential minerals, such as B, Mn, Fe and Cu (Lutts *et al*., 1999). Boron is involved in many processes including sugar transport, cell wall synthesis and maintenance, membrane integrity, and RNA, indole acetic acid (IAA) and phenol metabolism (Loomis and Durst, 1992; Dordas and Brown, 2001). The need of boron for seed and grain production is higher than vegetative growth (Vaughan, 1977). External application of boron was found to increase the vegetative and reproductive growth of the sunflower plant (Asad *et al*., 2003).The function of Mn at the cellular level of plant is to bind firmly to lamellae of chloroplast, possibly to the outer surface of thylakoid membranes, affecting the chloroplast structure and photosynthesis (Lidon and Teixeira, 2000). Mn is required in both lower and high plants for the Hill reaction – the water splitting and oxygen evolving system in photosynthesis. Photosystem II contains a Manganoprotein which catalyses the early stages of O_2 evolution. Exogenous application of manganese in adequate amount may result an increase in photosynthetic activity and growth rate of cells in barley under salinity (Cramer & Nowak, 1992).

The general effect of plants to salinity is reduction in growth (Romero-Aranda *et al*., 2001; Ghoulam *et al*., 2002). Salt stress limits plant growth by adversely affecting various physiological and biochemical processes including photosynthesis (Ashraf, 2004). Reduction in reproductive yield under salinity may be due to decrease in number of flowers (Sharma, 1992), resulting in faulty development of pollen grain and ovules which cause improper fertilization and denaturing of embryo, reduction in size of capitulum, number of seeds per capitulum and production of shriveled seeds (Kumar *et al*., 1980). All these problems created by the presence of extra sodium ions in rhizophere can be avoided by providing some sodium antagonistic minerals through foliar spray that function to minimize osmotic stress or ion disequilibrium or alleviate the consequent secondary effects caused by the salt stress (El-Fouly & Abou El- Nour, 1998). Foliar application of minerals like iron, boron, manganese and copper may be more practical than application to soil where they adsorbed to the soil particles and less available to the rooting medium (Sarkar *et al*., 2007; Wissuwa *et al*., 2008). Their two to three foliar applications may be all that are needed to meet crop demands.

The aim of the present study was to assess as to whether foliar applications of micronutrients was effective in inducing salt stress tolerance in sunflower plants.

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Materials and Methods

The seeds of single head Sunflower (NuSun 636) were sown in second week of November, 2008 in 60 plastic pots. These pots were of 0.28m in diameter, and 0.30m deep, having basal holes for leaching irrigation water, filled with 20 kg of sandy loam soil and cow dung manure (9:1) having pH 7.4. NPK ratio in fertilizer was given 4:3:2 through urea, DAP and sulphates of potash (SOP) as recommended by Nawaz *et al*., (2003), which amounts to 0.744g Nitrogen (N), 0.558g Phosphorus (P) and 0.372g Potassium (K) per pot, given at the time of sowing and at the time of flowering. A certain amount of micronutrients were given in soil vide Hoagland solution (Hoagland & Arnon, 1938) twice along with irrigation water.

A randomized complete block design with five replications was used. Sixty pots were divided in four sets comprising of 15 pots each.

Sets	Treatments
1.	Non-spray control
2.	Foliar spray H_3BO_3 for B
3.	Foliar spray $MnCl_2$ for Mn
4.	Foliar spray H_3BO_3+ $MnCl_2$ for B+Mn

Out of 15 pots of above mentioned each sets, 5 pots of each were subjected to following different levels of saline water irrigation.

a) Non saline water (control) (EC[1] 0.5 dS/m)
b) 0.4 % sea salt solution[2] (EC 6.1 dS/m)
c) 0.8 % sea salt solution (EC 10.8 dS/m)

[1]*Electrical conductivity for irrigation water.*
[2]*Sea salt solutions for irrigation were prepared by adding required amount of sea salt in tap water per liter.*

The seeds were sown in pots under non-saline condition and saline water irrigation was started after two and half week of germination to get seedlings of equal size. After thinning only one seedling was kept in each pot for further work. They were irrigated with gradual increasing sea salt concentration weekly up to reaching the desired salinity levels of the experiment mentioned above. To maintain the required soil medium salt levels the EC of the soil medium was measured periodically by portable EC meter.

Spray of foliar application of mineral solution (H_3BO_3, $MnCl_2$, and mixture of H_3BO_3, $MnCl_2$) was given thrice, 45, 75 and 95 days after planting (during seedlings establishment, incipient of floral heads and start of seed formation, respectively) at the rate of 5ppm. The calculated amount of B and Mn in their respective solution, applied to the plants through foliar application was 0.85ppm and 2.2ppm respectively. Tween-20 (0.1%) was used as a wetting agent for each treatment. A volume 350mL/plant, of the solution was sprayed on all pots with a manual sprayer. Spray was carried out between 09:00 and 11:00AM. The plants were sprayed with solutions with uniform coverage until the leaves were completely wet and the solution ran off the leaves. At the time of spray other plants were covered with plastic sheet to prevent the contamination of sprayed nutrients. Control (non-saline) plants were irrigated with 3.5L of tap water and plants under saline treatments were irrigated with 3.5L of their respective sea salt solution ensuring about 40% leaching. Pest management was carried out during the growth season according to local practice.

Vegetative and Reproductive growth was recorded at harvest in terms of plant height, stem diameter, number of leaves, average leaf area, fresh biomass, dry biomass, floral head diameter, number of seeds per floral head, along with their weight, pollen viability and their germination. For pollen viability test and its germination, the procedure was followed as described by Gul & Ahmad (2006). It was calculated on percentage basis by observing 100 randomly selected pollen grains in three replicate. Total proteins were estimated by using the method of Bradford (1976), and expressed in mg/g fresh weight. The nucleoprotein (RNA and DNA) contents were estimated by the method of Nieman & Poulson (1963), and expressed in mg/g fresh weight. Hill reaction activity was determined in sunflower leaves by using the method of Giebel (2006) and the rate of activity was expressed in mM DCPIP (2, 6-dichlorophenolindophenol) reduced/hr/mg ch.

Statistical analyses were carried out using SPSS version 13. Data sets were subjected to two-way analysis of variance (ANOVA). Duncan's multiple range test (DMRT) was used to measure least significant differences (LSD) between treatment methods and controls.

Results

Vegetative growth

Effect of foliar application of H_3BO_3, $MnCl_2$, and their mixture on vegetative growth of sunflower plant under different salinity levels (i.e., 0.4% and 0.8% conc. of sea salt) is presented in Fig. 1.

Plant height: The increasing levels of salinity significantly decreased plant height. The reduction percentages at ECiw: 6.1dS/m was 13.5 %, and at ECiw: 10.8 dS/m 36.5% in comparison with non saline control. A significant difference was observed in height of plant with foliar spray of micronutrients (i.e., B and Mn) but their combined effects were more significant, irrespective to plants growth under non-saline (control) or saline water.

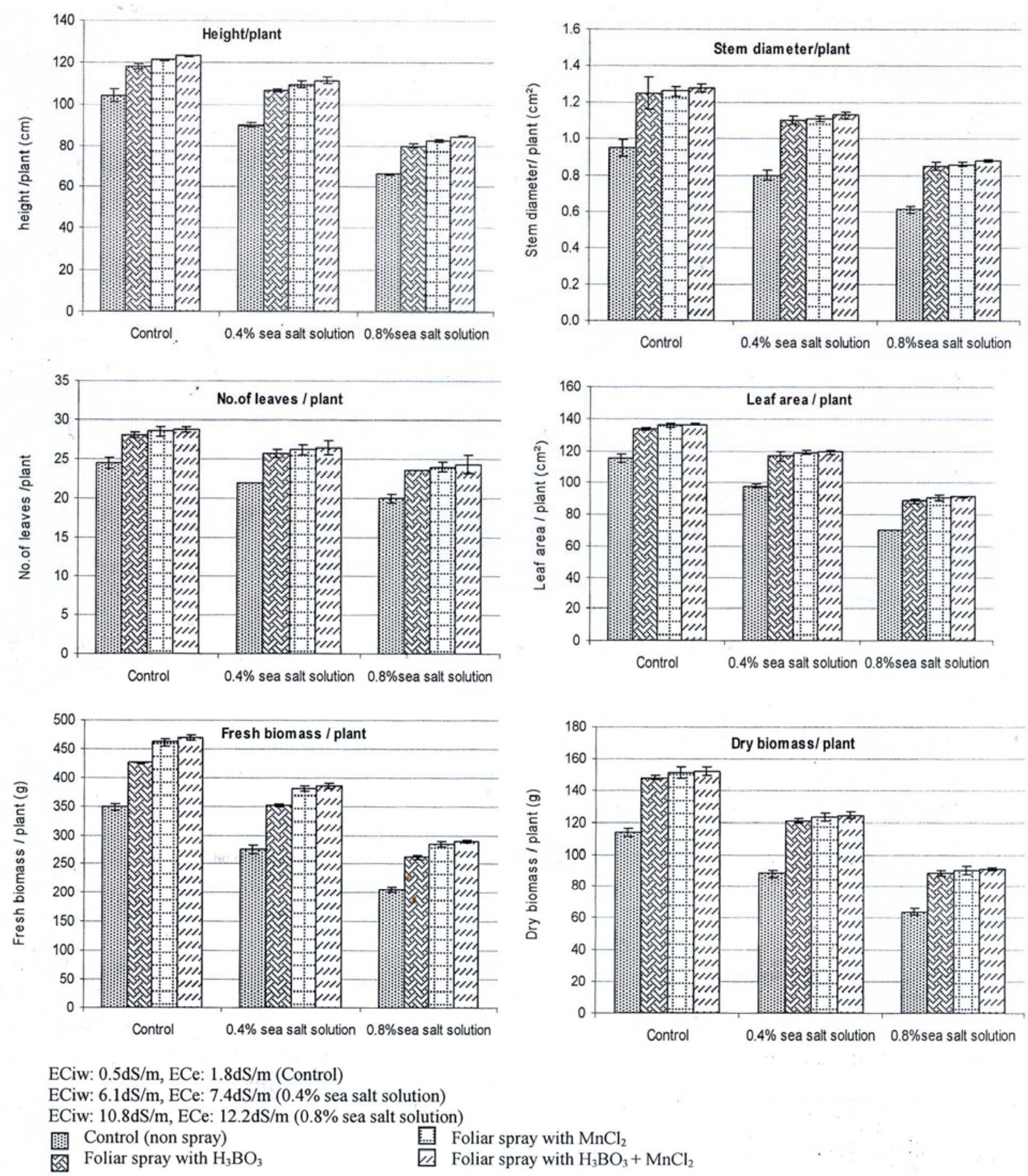

Fig. 1. Effect of foliar application of H_3BO_3, $MnCl_2$, and their mixture on vegetative growth of sunflower plant under irrigation of different salinity levels.

Stem diameter/plant: Different levels of saline irrigation significantly reduced stem diameter. At ECiw: 6.1 and 10.8dS/m, the reduction was 15.8% and 35.8% respectively in comparison with non saline control. The foliar spray with minerals at all the salinity regime showed a significant improvement in stem diameter.

Number of leaves/plant: A significant reduction was observed in number of leaves at different saline irrigation. The reduction percentages were 10.2, and 18.4 at ECiw: 6.1 and 10.8dS/m respectively in comparison with non-saline control. Leave number is significantly increased in plants sprayed with foliar minerals irrespective to their growth under non-saline or saline irrigation.

Average leaf area/plant: Average leaf area was significantly reduced with increasing levels of salinity. At the highest salinity levels i.e. 0.8% ECiw: 10.8dS/m the reduction was 38.9% and it was improved by 22.5% in those plants sprayed with the mixture of Mn and B. The spray solution of above mentioned micronutrients offset the toxic effects of salinity and has improved leaf area of plant irrespective to their growth in non-saline or saline irrigation.

Fresh and dry biomass/plant: A corresponding significant decrease was observed in fresh biomass of plant under different concentration of sea salt irrigation. The reduction in fresh biomass of plants treated with 0.4% and 0.8% sea salt solution was 20.9%, and 41.1% respectively in comparison with non saline control. The reduction in Dry biomass of the plant treated with 0.4% and 0.8% sea salt solution was 22.6% and 43.7% respectively in comparison with non saline control. A significant difference was observed in fresh and dry biomass of plant with foliar spray of micronutrients (i.e., B and Mn) irrespective to their growth under non-saline or saline conditions.

Reproductive growth

Effect of foliar application of H_3BO_3, $MnCl_2$, and their mixture on reproductive growth of sunflower plant under different salinity levels is presented in Fig. 2.

Floral head diameter: The floral head diameter was reduced by 37% in those plants irrigated with highest concentration of sea salt solution i.e., 0.8%ECiw: 10.8dS/m. The largest head diameter was recorded in plants sprayed by the mixture of micronutrients (i.e., B + Mn).

No. of seeds per head: Increasing levels of salinity significantly reduced seed number/head. Reduction percentages were 13.5% and 37 % under irrigation of 0.4% and 0.8% sea salt solution respectively in comparison with non-saline control. Number of seeds per head significantly increased with the application of foliar spray of minerals and the highest number was recorded in those plants sprayed with the mixture of Mn and B as compared to non-sprayed plants irrespective to their growth under non-saline or saline water.

Weight of seeds per head: Weight of seeds per head also showed reduction with the increase in salinity levels. 60.6% reduction was recorded at highest salinity levels i.e. ECiw: 10.8dS/m, in comparison with non-saline control. A significant increase in seed weight was found in those plant sprayed with the nutrient solution of Mn+B

Pollen viability and their germination: The data in Fig. 2 also showed that both the pollen viability and germination was inhibited with increasing concentration of salts. It may be mentioned here that since a certain percentage of tetrazolium salt viable pollen fail to germinate, a separate account has been given for those which have shown germination. Data showed that viability as well as germination of pollen increased significantly with the application of foliar minerals. The highest percentage was recorded in plants sprayed by the mixture of micronutrients (i.e., B + Mn). It was further observed that effect of boron on pollen germination is more significant than manganese. The overall comparative pattern of promotion in pollen viability and its germination with the interaction of irrigation of sea salt solution and spray of micronutrients under both the non saline and saline conditions is as follows: *Non spray (control) < Mn < B < Mn+B.*

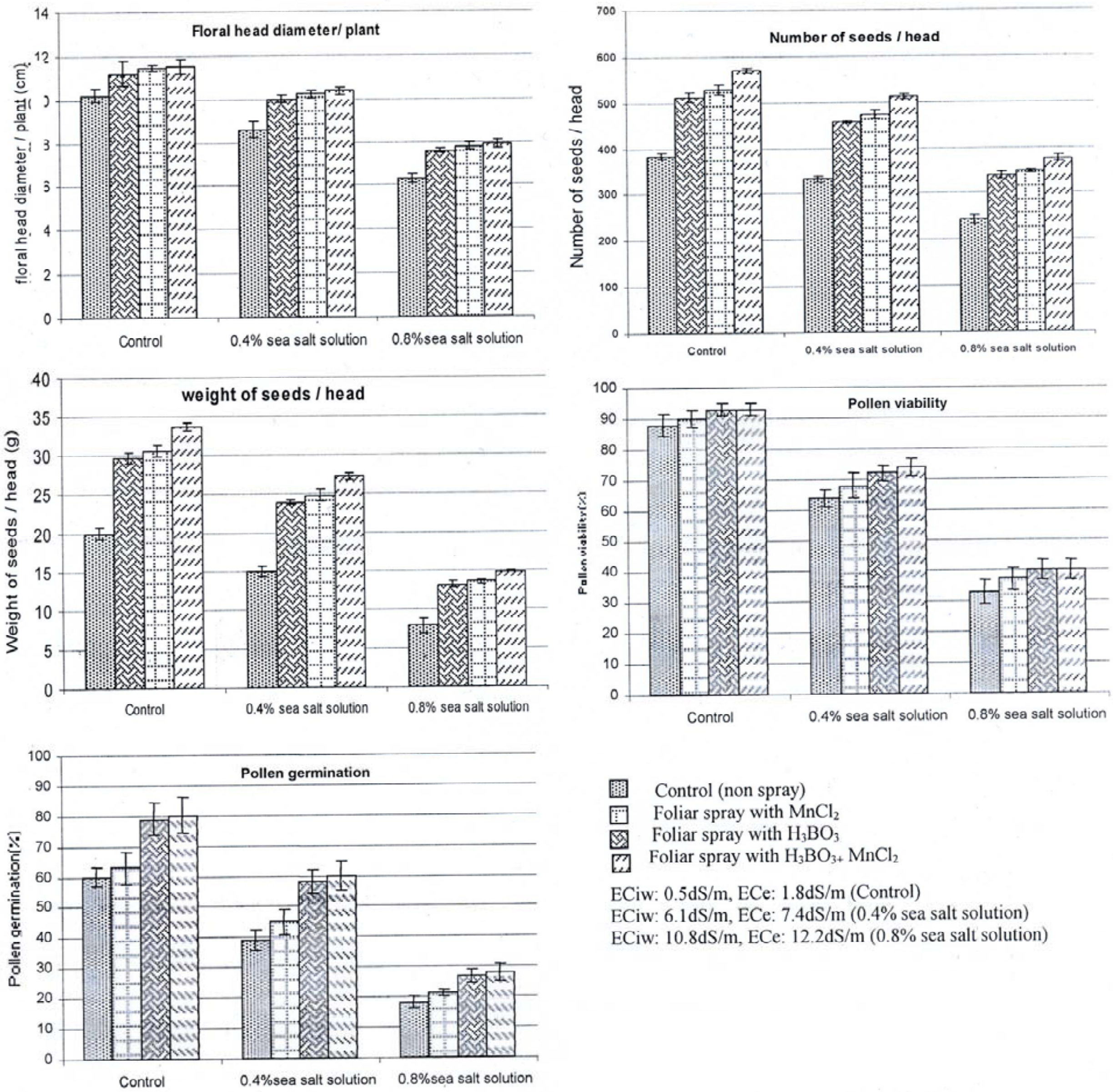

Fig. 2. Effect of foliar application of H_3BO_3, $MnCl_2$, and their mixture on reproductive growth of sunflower plant under irrigation of different salinity levels.

Biochemical estimation

Protein content: The effect of foliar application and different levels of salinity on protein content of leaves is presented in Table 1. There was found a reduction in total protein with the increasing levels of salinity. At 0.4% ECiw: 6.1dS/m the reduction was 14.7% and at 0.8% ECiw: 10.8dS/m the reduction was 38.8%. Foliar micronutrients enhanced the total protein content of leaf and offset the toxic effect of saline irrigation to some extent.

Nucleoproteins (RNA and DNA): The RNA and DNA content of sunflower leaf showed a significant decrease with increasing levels of salinity (Table1). The reduction in RNA at ECiw: 10.8dS/m was 39.3% and in DNA it was 30.3%. The foliar minerals partially alleviated the toxic effects of saline irrigation and enhanced the amount of nucleoproteins irrespective to the plants growth in non-saline (control) or saline conditions.

Table 1. Effect of foliar application of H_3BO_3, $MnCl_2$, and their mixture on total protein, nucleoproteins and Hill reaction activity of sunflower plant under irrigation of different salinity levels.

Sea salt concentration (%)	Foliar spray treaments	Total protein mg/g F.W	RNA mg/g F.W	DNA mg/g F.W	Hill activity (DCPIP photoreduction) mM DCPIP* reduced/ hr/ mg ch.
0 (ECiw:0.5dS/m, ECe:1.8dS/m)	Control -1 (non spray)	25.8 ± 0.23	0.488 ± 0.05	0.234 ± 0.03	0.070 ± 0.037
	H_3BO_3	27.8 ± 0.29	0.529 ± 0.02	0.253 ± 0.02	0.161 ± 0.042
	$MnCl_2$	26.7 ± 0.29	0.499 ± 0.03	0.239 ± 0.03	0.268 ± 0.120
	H_3BO_3+$MnCl_2$	28.0 ± 0.29	0.575 ± 0.03	0.269 ± 0.02	0.300 ± 0.110
0.4 (ECiw:6.1dS/m, ECe:7.4 dS/m)	Control -2 (non spray)	22.0 ± 0.29	0.400 ± 0.04	0.200 ± 0.03	0.060 ± 0.015
	H_3BO_3	24.7 ± 0.23	0.481± 0.03	0.235 ± 0.03	0.144 ± 0.051
	$MnCl_2$	23.6 ± 0.29	0.450 ± 0.02	0.220 ± 0.02	0.242 ± 0.060
	H_3BO_3+$MnCl_2$	24.8 ± 0.18	0.522 ± 0.04	0.250 ± 0.03	0.272 ± 0.080
0.8 (ECiw:10.8dS/m,ECe:12.2 dS/m)	Control -3 (non spray)	15.8 ± 0.29	0.296 ± 0.03	0.163 ± 0.02	0.044 ± 0.017
	H_3BO_3	18.2 ± 0.35	0.369 ± 0.02	0.192 ± 0.02	0.109 ± 0.038
	$MnCl_2$	17.4 ± 0.29	0.341 ± 0.04	0.180 ± 0.04	0.184 ± 0.040
	H_3BO_3+$MnCl_2$	18.3 ± 0.35	0.396 ± 0.05	0.204 ± 0.05	0.206 ± 0.120

(*2, 6-dichlorophenolindophenol)

Hill reaction activity: Table1 showed inhibition of Hill reaction activity (with DCPIP as electron acceptor) in thylakoids isolated from salt stressed plants, with increasing level of salt concentrations. Highest reduction percentage in Hill activity i.e. 37.1%, was recorded in those plant irrigated with 0.8% sea salt solution (ECiw:10.8dS/m) in comparison with non-saline control. Hill reaction activity was enhanced, reducing the toxicity created by saline irrigation water, in those plants which were sprayed by micronutrients (i.e., B + Mn). Effect of manganese was more significant than boron irrespective to the plants growth under non-saline (control) or saline conditions.

Discussion

Findings of experiments revealed that plants irrigated with different concentrations of sea salt showed significant reduction in all growth parameters of the plant. This reduction is directly proportional with increase of sea salt in irrigation medium. It might be seen that inspite of 40% leaching of irrigation water, the EC values of soil also increased proportionally with increase of salt in irrigation. El-Kader *et al*., (2006) found similar reduction in sunflower growth at various parameters in plants growing at the soils of different salinity levels. A considerable decrease in sunflower growth was also reported by Ahmad & Jabeen (2009) under irrigation of different sea salt solutions (i.e., ECiw: 4.8dS/m and 8.6dS/m). There may be three possible mechanisms for the reduction in growth under saline rooting medium i.e., turgor pressure reduction in expanding tissues, reduction in photosystem activity in leaf cells and direct effects of accumulated salts on metabolic steps in dividing and expanding cells (Neumann, 1997). Foliar applications of above mentioned mineral partially overcomes the negative effects of stress and provide plants balanced nutrients. Spray medium of mixture of H_3BO_3 and $MnCl_2$ was found better in all the vegetative growth characteristics as compared to those undergoing with spray of other individual minerals. Kassab (2005) also observed a significant effect of micronutrients in growth parameters including yield in mung bean plants by foliar application of zinc, manganese and iron under water stress. Such enhancement effect of foliar application might be attributed to the favorable influence of these nutrients on metabolism and biological activity and its stimulating effect on photosynthetic pigments and enzymes activity which in turn encourage vegetative growth of plants (Michail *et al*., 2004). Fresh and dry biomass (root and shoot) of sunflower plant in present investigation was significantly reduced with the increasing salinity levels. Reduction in total biomass under different salinity levels was also reported by Khan *et al*., (1989) and Bassil *et al*., (2002). Foliar mineral spray significantly affect biomass production of plants irrespective to their growth under non-saline or saline conditions. The obtained results are in agreement with the findings of Asad *et al*., (2003), Basole *et al.,* (2003), Kassab, (2005) and Thalooth *et al*., (2006).

In present investigation salinity stress significantly depressed size of floral head, number and weight of seed per plant which is proportional to increasing levels of salinity. Salt stress resulted in a disturbance in photosynthesis, enzyme activities, protein synthesis energy and lipid metabolism which effects the metabolites transportation to the grains and hence reduced yield (Parvaiz & Satyawati, 2008). The foliar application of above mentioned minerals appear to have minimized these toxic affects through mitigating the nutrient demands of stressed plants. These minerals increased photosynthetic and enzymatic activities and an effective translocation of assimilate to reproductive parts resulting in higher yield (Sarkar & Malik, 2001).

The present investigation also showed that increasing salinity levels not only decrease the viability of pollens but also reduced their germination percentage. The degree of inhibition of pollen germination was more than pollen viability which shows that even viable pollen often fails to germinate. Similar results were also reported by Gul & Ahmad (2006) in pollens of different cultivars of canola under 0.2% (EC2.5dS/m), 0.4% (EC4.5dS/m) and 0.6% (EC6.5 dS/m) sea salt salinity. The inhibitory effect of salinity on pollen viability and germination was partially alleviated by the foliar spray of manganese and boron. Spray of Boron as an individual mineral element and the spray of its mixture with manganese were found better in stimulating the pollen viability and germination as compared to manganese alone. Wang *et al.*, (2003) observed that boron plays an essential role in pollen germination and pollen tube growth in *Picea meyeri*. High boron levels in the stigma and style are required for physiological inactivation of callose which is an important polysaccharide component of the pollen tube wall by the formation of borate callose complexes (Kyu-Ock & Bradford, 1998). When boron levels are low, callose levels increase and induce the synthesis of phytoalexins including phenols which can cause an injury to membrane structure and cellular functions and bring some alteration in the morphology and structure of pollen tubes (Wang *et al.,* 2003).

The total proteins of leaves of sunflower decreased in salinity. This may be due to increases in break down of protein by proteolytic process under high salinity. Decrease in protein content of leaves has been reported in many plants under salt stress irrespective of their salt tolerance (Ashraf & Waheed, 1993; Parida & Das, 2005; Ahmad & Jabeen, 2009). The amount of proteins increased with the foliar application of above mentioned minerals irrespective to the plant growth under non saline or saline conditions. Bybordi & Mamedov (2010) reported that protein content was increased in canola plants being sprayed by micronutrients i.e., Iron + Zn. Rizk & Abdo (2001) found increased crude protein contents in mung bean with the foliar application of boron.

Amount of nucleoprotein of sunflower decreased under different salinity levels. High salinity levels may have increased the break down of proteins RNA and DNA due to proteolytic and nucleolytic process. Kessler *et al*., (1964) reported that salinity strongly suppressed RNA and DNA content particularly due to intensified activity of cyoplasmic RNase and impaired synthesis of DNA. Abo-Kassem (2007) reported that high salinity decreased total nucleic acid content in four different species of Chenopodiaceae plants. In present investigation decreased amount of RNA and DNA under salinity were partially restored by foliar application of boron and manganese. Boron foliar application had the greatest stimulatory effect on the amount of the nucleoprotein as compare to manganese foliar spray. The highest amount was recorded in plant being sprayed by the mixture of boron and manganese. If boron is not supplied sufficiently to plant the RNA is strongly and rapidly affected and has a much higher turn over than DNA. It also results impaired synthesis of uracil which is the major constituent of RNA and also a precursor of energy rich phosphate (Moore & Hirsch, 1983). Dzondo-Gadet *et al.*, (2002) examined the action of boron at the molecular level, using cell-free systems of transcription (isolated placenta nuclei) and translation (wheat germ extract). They found that 10 m*M* boric acid greatly increased RNA synthesis. Full-length functional mRNA was produced because proteins of 14–80 kDa were translated. These results demonstrate that boron may contribute to biological cell activities at both the transcription and translation levels. However, the mechanism of action is still not known.

In present investigation photosynthetic oxygen evolving activity (PSII activity) in sunflower was suppressed under different salinity levels. Similar data of inhibitory effect of salt stress on PSII activity were also reported by others (Hasegawa *et al*., 2000; Munns, 2002; Ashraf & Shahbaz, 2003; Ashraf, 2004). Maslenkova *et al*., (1993) reported that under salinity treatment the thylakoids membranes of control plants lost a great part of their polypeptide bands along with the substantially decreasing Hill reaction activity and the ability of chloroplast membranes to evolve oxygen under flash illumination completely disappeared. Maslenkova *et al*., (1995) observed that high salinity (100mM NaCl) markedly reduced oxygen evolution in isolated thylakoids of barley seedlings. Foliar application of micronutrients i.e., Mn and B on sunflower plant partially offset the inhibitory effect of salinity on PSII oxygen-evolving activity and enhanced the Hill activity in plant irrespective to their growth under non saline or saline conditions. Mn and mixture of Mn and B was found more stimulating than B (alone) in activating the Hill reaction. Lidon & Teixeira (2000) reported similar result in rice plant (cv.safari) treated with varying concentration of Mn, between 0.125 and 32 mg L^{-1}. They found a significant increase in the photosynthetic electron transport rates coupled to PSII and PSI until the 8-mg.L^{-1} Mn treatment. The highest Mn treatment was also observed to be associated to the synthesis of a new thylakoid protein and a Mn protein. Amao & Ohashi (2008) showed that DCPIP photoreduction with grana of spinach was promoted by addition of manganese (III) or (II) ion. El-Shintinawy (1999) demonstrated that low supply of boron in sunflower plant result slower rate of Hill reaction activity and a suppressed flow of electron transfer of the whole chain in thylakoid membranes as compared to sufficient supply of boron. This reduction was accompanied with a decline in the activity of PSII shown by a diminished rate of oxygen evolution. He concluded from the result that B is an important element for membrane maintenance, protection and function by minimizing or limiting production of free oxygen radicals in thylakoid membranes of sunflower leaves.

In present investigation the critical values of salts in rooting medium beyond which retardation occurs at all growth parameter (threshold value) was considerably extended due to foliar spray of above mentioned essential minerals which could provide incentive to grow sunflower plants through foliar application of essential minerals at a bit higher levels of soil salinities or saline water irrigation.

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