Progress in Nutrition 2016; Vol. 18, N. 3: 288-298

Original Article

Effect of foliar-applied iron and zinc on growth rate and essential oil in sweet basil (*Ocimum basilicum* L.) under saline conditions

Khalid Elhindi[1,2], *Nasser A. Al-Suhaibani*[1], *Ahmed F. Sharaf El-Din*[1,3], *Sobhy M. Yakout*[4], *Salem M. Al-Amri*[5]

[1]Plant production Department, College of Food and Agriculture Sciences, King Saud University, P.O. Box 2460, Riyadh 11451, Saudi Arabia - E-mail: elhindikhalid2010@yahoo.com; [2]Vegetable and Floriculture Department, Faculty of Agriculture, Mansoura University, Egypt; [3]Ornamental Plants and Landscape Gardening Research Department, Horticulture Research Institute, Giza, Egypt; [4]Biochemistry Department, College of Science, King Saud University, PO Box, 2455, Riyadh, 11451, Kingdom of Saudi Arabia; [5]Department of Biology, College of Science and Art, Shaqra University, Saudi Arabia

Summary. Foliar application of some micronutrients (Fe and/or Zn) were conducted to determine whether exogenous could mitigate the adverse influences of salt stress on growth rate and essential oil content of sweet basil (*Ocimum basilicum* L.) plants. Sweet basil were grown under normal and various saline stress conditions, with 0.4% (ECiw 6.1 dS/m) and 0.8% (ECiw 10.8 dS/m) of sea salt irrigation. Salinity was recorded to cause a significant decrease at all the vegetative and reproductive growth characters. However, essential oil percentage, proline as well as reducing and non-reducing sugars content were increased under saline conditions. Applications of Fe through $FeSO_4$ and Zn through $ZnSO_4$ and their mixture by foliar were establish to increase all the growth characters and biochemical activities of sweet basil plant regardless to their growth rate under non-saline or saline stress. The growth rate and yield component by reason of the mixture of foliar spray was greater than spray of single nutrient. Regarding essential oil constituents, linalool and eugenol were the main components. Salinity treatments at 0.4% and 0.8% levels enhanced the content of linalool and, however, there was a reduction in eugenol content. Addition of micronutrients by foliar spray reduced linalool in normal conditions; on the contrary, there was an increase in linalool content by using salinity treatment. In conclusion, our results support the hypothesis that foliar application of Fe and Zn could mitigate the seawater stress of sweet basil plants.

Keywords: sweet basil, foliar spray, salinity, essential oil, chemical constituents.

Introduction

Salt stress, a main a biological stress in most of plant horticulture, negatively affects plant yield in many areas of the region. (1) reported that the sense of many plants to salt conditions and the resultant difference in growth rate and production, but, with some plants capable to suffer salt stress conditions while others are particularly sensitive to few rate of salt stress. Salt resistance in some aromatic plants required to examine both plant growth rate and secondary metabolite productivity, as these constituents supply to yield and content of the essential oil.

Salinity decreases the capability of many plants to uptake water, lead to a decrease in growth rate and a lot of metabolic differences identical to those induced by water stress. If excessive amounts of salt enter the plant, salt will eventually rise to toxic levels in transpiring leaves (2) and then decreasing the photosynthetic leaf area of the plant to a rate that cannot sustain growth rate. (2) stated that the mot salt-tolerant plants vary from salt-sensitive ones in having a low level of Na^+ and Cl^- transport to leaves and the capability to compartmentalize these ions in vacuoles to inhibit their build-up in cytoplasm or cell walls and thus prevent salt toxicity. Ionic imbalance remain in the cells

as a consequence of excessive addition of Na^+ and Cl^- and decreases the uptake of other necessary micronutrients, like Fe, Zn, B, Mn and Cu (3), micronutrients are required in small quantities for sufficient plant growth rate and yield; but, their deficiencies cause a large disruption in the physiological and metabolic operation in the plant (4). Plants normally absorb minerals from soils through their roots although minerals can be given to plants as fertilizers by foliar sprays application. Therefore, foliar nutrient application grown under salinity stress may mitigate a water or nutrient deficiency under salt stress and controversial procedure of encourage plants by applying liquid fertilizer directly to their leaves (5). Several researches have also been attended on the extension of nutrients to the soil medium in reply to salt stress condition, while foliar fertilizer application was being most able to increase the hardiness of salinity for the plant.

Micronutrients, especially Fe and Zn, act either as metal components of various enzymes or as functional, structural, or regulatory cofactors. Thus, they are associated with saccharide metabolism, photosynthesis, and protein synthesis (6). Moreover, essential oil biosynthesis in sweet basil *(Ocimum sanctum* L.) is greatly determined by Fe and Zn (7). Iron plays essential roles in the metabolism of chlorophylls. External application of Fe increased photosynthesis, net assimilation and relative growth in seawater-stressed (8). Zinc may be required for biomass and chlorophyll production, pollen function and fertilisation (9). From a knowledge theoretical point of outlook, external zinc concentrations could mitigate the adverse influence of NaCl by prohibiting Na^+ and /or Cl^- uptake (10).

Sweet basil (*Ocimum basilicum* L.) is an economically great plant (11). Its essential oils are synthesized and stored in glandular hairs and are apply as flavorings in meals and beverages, as fragrances, as fungicides, or insecticides in pharmaceutical and industrialized productions (12). To the best of our knowledge, information regarding application method efficiency of iron and zinc on morphological development, physiological responses and essential-oil production of sweet basil under salt stress conditions is not available.

Therefore, the purpose of this study is to investigate the effect of salinity on the plant growth, essential oil content and components of sweet basil to verify whether the direct supply of sole and combined application of Fe and Zn via foliar fertilization may play a primary role in increasing sweet basil tolerance to salinity.

Materials and Methods

A pot experiment was conducted under greenhouse conditions (225 $\mu mol \cdot m^{-2} \cdot s^{-1}$ light intensity, 25/20°C day/night temperatures, 75-80% relative humidity and 14-h photoperiod). Seeds of sweet basil (*Ocimum basilicum* L.) were sterilized for 15 min in 1% sodium hypochlorite (NaClO) and were cleaned with deionized water and were grown in beds on 1st February. Uniform 45 days old seedlings were grown per earthenware pot (30 cm in diameter) of 10 kg of sandy loam soil. Properties of physical and chemical of the soil used in the study were estimate according to (13): The soil form was sandy loam in texture with pH of 8.7, 23% clay, 48% silt and 29% sand, organic matter 1.66%, specific electrical resistance 0.47 $mmhos \cdot cm^{-1}$. The soil analysis, 2.95% including $CaCO_3$, available 4.45, 23.47, 169 and 32.2 mg 100^{-1} g soil of P, K, Mg and Na, respectively and also available 7.3, 9.5, 2.83 and 4.85 ppm soil of Fe, Mn, Cu and Zn, respectively. Fertilization was conducted for each pot at proportion of 1.5 g ammonium nitrate (33.5% N), 2.5 g calcium superphosphate (15.5% P_2O_5) and 1 g potassium sulphate (48% K_2O). These fertilizers were applied in two doses at 60 and 75 days after planting.

Sixty pots were split in four sets including of 15 pots each. Five replications was used with a randomized complete block design.

Treatments
Non-spray control
Foliar spray $FeSO_4$ for Fe
Foliar spray $ZnSO_4$ for Zn
Foliar spray $FeSO_4$ + $ZnSO_4$ for Fe + Zn

Moreover of 15 pots of mention suggested each sets, 5 pots of each were arranged to successive varies rates of saline water irrigation.

a) Non saline water (control) (EC 0.5 dS/m, ECe: 1.8 ds/m)
b) 0.4% sea salt solution* (EC 6.1 dS/m, ECe: 7.4 ds/m)
c) 0.8 % sea salt solution (EC 10.8 dS/m, ECe: 12.2 ds/m)
* For irrigation sea salt solutions were determined by calculate needed supply of sea salt in tap water per liter.

Seeds of sweet basil were grow in pots under non-saline status and saline water irrigation was initiated after three weeks of transplanting. To preserve the needed soil medium salt rates the EC of the soil medium was calculate through portable EC meter. Foliar application of nutrient solution ($FeSO_4$, $ZnSO_4$, and mixture of $FeSO_4$ and $ZnSO_4$) by spray was added thrice, 45, 75 and 95 days after tranplant (through seedlings formation, beginning of floral heads, respectively) at the level of 7 ppm. The estimated quantity of Fe and Zn in their various solution, tested to the plants during foliar application was 0.85ppm and 2.2ppm respectively. For each treatment, Tween-20 (0.1%) was applied as a wetting assistant. Spray was conducted between 10:00 and 12:00 AM. The tap water was analyzed (Table 1) as reported by (14).

Table 1. Chemical characteristics of irrigation water

pH	7.4
EC (dS/m)	0.4
Total dissolved solids (mgL^{-1})	512
Nitrite (NO_2) as nitrogen (mgL^{-1})	1.2
Chloride (mgL^{-1})	273
Sulphate (mgL^{-1})	250
Ca^{2+} (mgL^{-1})	132
Mg^{2+} (mgL^{-1})	43.2
K (mgL^{-1})	8
Na (mgL^{-1})	30
Fe^{2+} (mgL^{-1})	0.1
Zn^{2+} (mgL^{-1})	0.3
Mn^{2+} (mgL^{-1})	0.01
Cu^{2+} (mgL^{-1})	0.7

The plant vegetation was cut 10 cm above the soil surface at full bloom and data including plant height, number of branches, leaves and inflorescence length per plant and fresh and dry weights of herb for plant growth rate character was registered. Leaf area samples were determined using a Li-3100 area meter (LI, Lincoln, Nebraska, USA), samples of the fresh weight the leaves were oven-dried at 70°C for 48 hours and dry weight was listed. For the determination of N, K and Na, the dried, ground material (0.1 g) was digested with sulphuric acid and Hydrogen peroxide (Merck) and Na, and K in the digests were determined with a flame photometer (Jenway, PFP-7). Nitrogen was estimated by micro-Kjeldhal's method (15).

Concerning reducing and non-reducing sugar content was determined according to (16). Chloride was determined using an ion chromatography analyser (Model LC20-1, Dionex, Sunnyvale, CA 94086, USA). Nitrate in leaf tissue was measured as according to (17). Leaf contents of Chl *a* and Chl *b* were determined according to (18). Free proline was measured according to the method illustrate by (19). Soluble protein concentrations were measured using Coomassie brilliant blue (20).

For determinations of chemical constituents were conducted 180 days from growing before flowering stage. The essential oil percentage was measured in the air dried plant according to the method described by (21).

The data of this study were statistically analysed using the COSTAT computer package (CoHort Software Inc., Berkeley, USA) except for the compositions of the essential oils. The least significance difference among the mean values was computed following (22). Test the difference among mean values was also used by Duncan's New Multiple Range test (DMRT) at 5% level of probability.

Results and Discussions

Effect of foliar application of $FeSO_4$, $ZnSO_4$, and their mixture on vegetative characteristics (Leaves number, branches number, plant height, and herbs weight (fresh and dry) of sweet basil plant treated with salinity (Sea salt mixed water with concentrations of

0.4% and 0.8%) the data is showed in Table (2). The enhancing rates of salinity significantly reduced vegetative growth. The growth decrease was positively related to salt concentration in the irrigation liquid. Despite leaching 40% of irrigation water, the EC of the soil has increased as the salinity level increased. Raising the salinity level decreases the plants ability to uptake water and, hence a fast growth rate reduction occurs; this may be explained by the low osmotic potential in the soil, which as a final result negatively affected the water uptake ability, reduced Photosynthesis, and stomatal conductance control, all of this resulted in growth rate reduction (23). Reference (24) has published similar results on *Chrysanthemum morifolium*, where the growth rate reduced by the raising of salinity level in the soil. This was also the case with what (25) reported, as salinity irrigation treatments (sea salt solutions 0.4% (ECiw 6.1 dS/m) and 0.8% (ECiw 10.8 dS/m)) significantly reduced the growth rate of treated plants. The treatment of micronutrients by foliar spray (*i.e.*, Fe and Zn) has resulted in significantly positive effects on plants growth characteristics, the combined effect of those micronutrients was more pronounced than that of individual micronutrients, this positive effect manner was found on plants under non-saline (control) and saline water. The foliar treatment with micronutrients has the ability to raise plants growth under salinity conditions through providing the plants with balanced nutrient, and enhancing metabolic operations, enzyme activity and support the positive effects of photosynthetic pigments (26). Treatment with the mixture of Fe and Zn has resulted in a better growth parameter when compared to the results of foliar spraying with other individual micronutrients.

Analysis of variance indicated that the saline growth medium significantly affected the leaf area of the sweet basil plants (Table 2). As (27) reported there might be some possible reasons explaining the leaves growth reduction shown in plants planted in

Table 2. Effect of foliar application of $FeSO_4$, $ZnSO_4$ and their mixture on growth characteristics of sweet basil plant under irrigation of different salinity levels

Sea salt concentration (%)	Foliar spray treatments	Plant height (cm)	FM of herb (g plant^{-1})	DM of herb (g plant^{-1})	Branch number (plant^{-1})	Leaves number (plant^{-1})	Leaf area (plant^{-1})	Inflorescence length (cm)
Control (ECiw:0.5dS/m)	Control-1 (non-spray)	53.34±0.80c	68.48±1.18c	23.18±0.51c	12.13±1.07b	336.32±1.10c	146.6±1.36d	4.98±0.32d
	$FeSO_4$	60.35±1.15b	86.49±1.19b	26.59±0.85b	13.94±0.29a	345.36±1.18b	157.34±1.03b	6.16±0.49b
	$ZnSO_4$	61.55±0.66b	87.03±1.3b	25.83±0.73b	14.5±1.03a	345.65±1.00b	154.62±0.70c	5.52±0.53c
	$FeSO_4$+ $ZnSO_4$	71.27±1.12a	103.49±1.2a	30.61±0.64a	15.03±0.74a	488.04±3.27a	194.15±1.37a	9.06±0.15a
0.4 (ECe:6.1dS/m)	Control-2 (non-spray)	45.25±0.96f	60.61±0.84f	19.05±0.37e	8.97±0.86de	304.52±0.85f	134.73±1.02g	3.64±0.02f
	$FeSO_4$	49.31±1.02e	63.27±1.02e	21.62±1.23d	9.84±0.34cd	313.74±1.03e	139.53±1.0f	4.24±0.18e
	$ZnSO_4$	41.24±0.56e	61.07±0.86f	21.11±0.86d	10.09±0.13c	312.56±1.40e	138.85±0.72f	4.31±0.31e
	$FeSO_4$+ $ZnSO_4$	51.37±0.80d	66.61±1.36d	23.64±0.70c	10.74±0.76c	326.84±1.17d	142.19±0.76e	5.39±0.41cd
0.8 (ECe:10.8dS/m)	Control-3 (non-spray)	38.82±0.37i	50.48±0.67i	15.94±0.90g	6.23±0.63g	283.83±1.31i	114.49±0.81j	2.54±0.11h
	$FeSO_4$	41.24±0.28h	54.61±0.66h	17.81±0.37f	7.07±0.30fg	291.38±0.80h	122.51±1.19i	2.76±0.19gh
	$ZnSO_4$	40.74±0.95h	53.93±1.63h	17.28±0.35f	6.87±0.40g	293.51±0.82h	123.17±1.74i	2.89±0.05gh
	$FeSO_4$+ $ZnSO_4$	43.37±0.83g	57.02±0.21g	19.86±0.68e	8.09±0.18ef	301.33±0.75g	131.37±1.21h	3.07±0.11g
LSD at 0.05		0.69	1.13	0.53	0.41	1.91	1.24	0.49

saline medium, those reasons are the turgor pressure reduction in expanding tissues, lower rates of photosynthetic activities in leaf cells (27). The spray with solution of micronutrients (as mentioned previously) offset the toxic growth retracting effects of salinity for example it improves the leaf area parameter of treated plant wither in non-saline or saline irrigation.

The inflorescence length was badly affected by salinity in salinity treated plants of salt 0.4% (ECiw 6.1 dS/m) and 0.8% (ECiw 10.8 dS/m). The data present in Table 2 shows a clear negative relation between the salinity level and the length of floral heads. Salinity stress disturbed the photosynthetic, enzymatic, metabolic activities and thus reducing the metabolites transfer to the flowers, hence reducing the yield this comes in accordance with (28) reports. Plants treated with the mixture of Fe and Zn yielded the highest inflorescence length, this may be due to the enhanced mineral status in treated plants compared to none-treated ones.

There was a significant positive relationship between the foliar treatment with minerals and the leaves content of chlorophyll a and b under different concentration of sea salt irrigation (Figs. 1, 2). Higher salinity stress means higher osmotic which leads to cell enlargement reduction, this may be a reason for raising the content of a and b chlorophyll pigments in salinity treated plants as reported by (29). Results showed that plant grow under salt stress had higher chlorophyll a and b which associated with higher proline content. The higher levels of chlorophyll a and b may be due the breaking of excess of proline and starting the biosynthesis of glutamic acid, which is a very important compound in the biosynthesis of chlorophyll (30).The foliar spray with Fe and Zn has caused a significant chlorophyll a and b content increase in plants (whether under control or saline conditions). The positive effect of Fe and Zn on chlorophyll content may be a result of their stimulating effect on the enzymes and pigments related to the photosynthetic activity, adding that Fe is of extreme importance in the operation of chlorophyll biosynthesis, this was the case in the present study as the foliar application of Fe And Zn has increased the chlorophyll content in treated plants, these results agree with those of (24).

Table (3) showed that the data related to the leaves

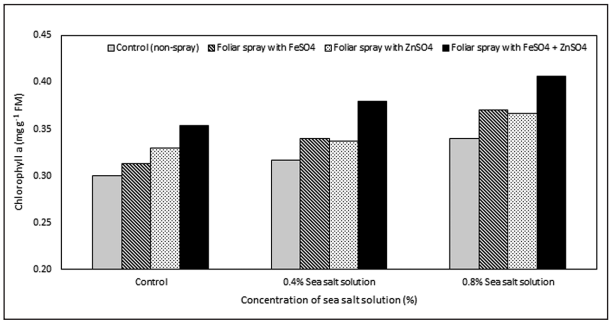

Figure 1. Effect of foliar application of $FeSO_4$, $ZnSO_4$ and their mixture on chlorophyll a of sweet basil plant under irrigation of different salinity level. Values in each bar followed by the same letter are not significantly different at P≤0.05 (Duncan's multiple range test). Values shown are means ± SE of three replicates. FM – fresh mass

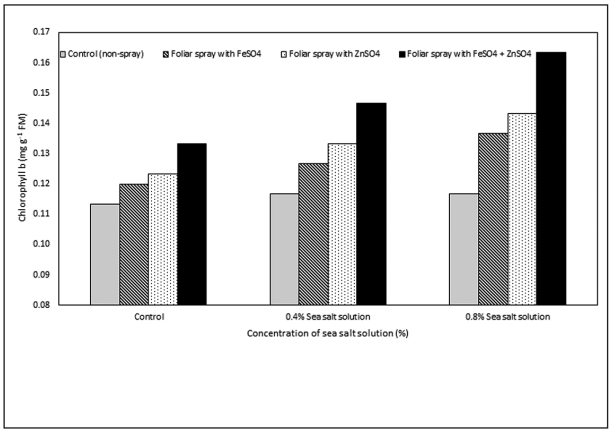

Figure 2. Effect of foliar application of $FeSO_4$, $ZnSO_4$ and their mixture on chlorophyll b of sweet basil plant under irrigation of different salinity level. Values in each bar followed by the same letter are not significantly different at P≤0.05 (Duncan's multiple range test). Values shown are means ± SE of three replicates. FM – fresh mass.

content of ions the sweet basil plants under salinity and (Fe and/or Zn). This ion toxicity as Na^+ displaces K^+ in the presence of high application of NaCl salt in root-zone, induce metabolic imbalances which cause decrease in growth rate and dry mass productivity. The greater K^+ compositions in plant tissue grown under salt stress could be as a result of capability of plant to do selective K^+ absorb and selective cellular K^+ and Na^+ mentation and their transport in the shoots (31). The ions concentration and balance in the root zone was significantly affected by salinity, as the concentration of K^+ was significantly decreased, while the concen-

tration of Na^+ and Cl^- was significantly increased. The K^+/Na^+ ratio under the (Fe + Zn) application showed the maximum K^+/Na^+ ratio (Table 3). The capability to remain high K^+: Na^+ ratio by roots shows the selectivity for plant for K^+ over Na^+ through absorb of K^+ ion inside xylem rather than Na^+ (31). The foliar spraying of Fe and Zn on plants treated with salinity has succeeded in alleviating the toxic effects of salinity, this may partially be a result of decreasing the accumulation of Na^+ ions and enhancing the nutrients balance in the aerial parts of the plants where spraying occurred, This came agreeing what has been reported by many authors on many plant species (32 and 8).

Increased Salinity resulted in increased Cl^- concentration in the leaves with the highest rate in the plants treated with the highest salinity level (ECe: 10.8 dS/m) (Table 3), increasing salinity also leads to decreasing NO_3^- concentration in the leaves of salinity stressed sweet basil plants(Table 3) . The decrease of NO_3^- may have resulted from the negative salinity effects on the roots membranes integrity (33), imbalanced decreased nitrate uptake (34), the negative competitive effect of Cl^- ions with nitrate may also decrease the uptake of nitrate, and raise the uptake of chloride, hence raise their level in the leaves (35) this toxic effect of salinity related ions has the ability to inactivate the nitrate transporters (36). (37) also reported that under salinity stress conditions the plant xylems loses their ability to exudate chloride, which leads to decreasing the plants ability to uptake nitrate, reducing the leaves content of nitrate and nitrate related enzymes like nitrate reductase. In the present study, the foliar spraying of (Fe and/or Zn) has significantly reduced the plants content of cl- in salinity stressed plants.

Salinity stress also negatively affects the leaves content of soluble protein (Data shown in Table 4).This may be explained by the increasing rate of proteolytic process because of salinity stress, which works on breaking down the protein because of salinity. The negative relationship between salinity and soluble protein content has been reported in many plants regardless of their tolerance to salinity stress (38). Whether under saline or none-saline conditions, the foliar spraying of (Fe and/or Zn) in the present study has raised the plants content of soluble protein. The application of Fe or Zn is associated to enhance NO_3^- uptake, its

Table 3. Effect of foliar application of $FeSO_4$, $ZnSO_4$ and their mixture on K^+, Na^+, Na^+/K^+ ratio, Cl^- and NO_3^- content of sweet basil under irrigation of different salinity levels

Sea salt concentration (%)	Foliar spray treatments	K^+	Na^+	$K+/Na^+$	Cl^-	NO_3^-
Control (ECiw:0.5dS/m)	Control-1(non-spray)	18.34±1.16c	4.13±0.02h	14.15±0.17c	9.0±0.62h	25.27±0.35c
	$FeSO_4$	25.33±1.21b	3.1±0.14i	16.44±1.12b	7.86±0.35i	27.8±0.66b
	$ZnSO_4$	24.67±0.49b	2.95±0.03i	14.38±0.80c	6.86±0.35ij	27.67±0.71b
	$FeSO_4$+ $ZnSO_4$	35.3±0.75a	1.95±0.10j	20.27±1.02a	5.76±0.15j	29.9±0.46a
0.4 (ECe:6.1dS/m)	Control-2(non-spray)	15.35±1.20ef	17.44±1.21b	3.44±0.02ef	46.93±0.51c	23.47±0.31d
	$FeSO_4$	18.12±0.61c	15.43±0.20de	4.12±0.03e	40.9±30e	25.43±0.15c
	$ZnSO_4$	17.48±0.85cd	15.03±0.73ef	4.12±0.01e	39.47±1.22f	25.3±0.70c
	$FeSO_4$+ $ZnSO_4$	18.71±0.52c	13.41±0.20g	5.64±0.45d	32.4±0.92g	27.1±0.26b
0.8 (ECe:10.8dS/m)	Control-3(non-spray)	13.94±0.29g	20.86±0.70a	2.23±0.10g	61.73±0.54a	20.73±0.49f
	$FeSO_4$	15.27±034ef	16.81±0.37bc	2.98±0.14fg	51.71±0.50b	22.07±0.25e
	$ZnSO_4$	14.95±0.31fg	15.98±0.34cd	3.0±0.14fg	50.62±0.61b	22.0±0.56e
	$FeSO_4$+ $ZnSO_4$	16.24±0.30de	14.4±0.22df	3.99±0.15e	44.4±0.98d	23.76±0.40d
LSD at 0.05		1.27	0.83	0.87	1.11	0.81

Table 4. Effect of foliar application of $FeSO_4$, $ZnSO_4$ and their mixture on essential oil percentage, reducing and non-reducing sugars, protein percentage and proline content of sweet basil plant under irrigation of different salinity levels

Sea salt concentration (%)	Foliar spray treatments	Essential oil content (%)	Reducing sugar	Non-reducing sugar	Protein Content (mg/g FM)	Proline Content (µg/g DM)
Control (ECiw:0.5dS/m)	Control-1(non-spray)	0.58±0.01h	0.96±0.01g	7.27±0.56f	25.46±0.49cd	5.14±0.41h
	$FeSO_4$	0.67±0.01f	1.14±0.02ef	8.09±0.13e	27.4±0.53b	7.94±0.29f
	$ZnSO_4$	0.63±0.01g	1.17±0.03de	8.07±0.10e	26.1±0.20c	6.36±0.16g
	$FeSO_4$+ $ZnSO_4$	0.74±0.02e	1.36±0.21ab	9.0±0.30d	28.93±0.67a	8.33±0.18f
0.4 (ECe:6.1dS/m)	Control-2(non-spray)	0.62±0.01g	1.05±0.01fg	8.01±0.10e	22.2±0.26g	6.84±0.32g
	$FeSO_4$	081±0.01cd	1.21±0.01cde	8.09±0.40d	24.43±0.55e	10.82±0.72d
	$ZnSO_4$	0.75±0.01e	1.2±0.01de	9.07±0.10d	23.37±0.15f	9.57±0.11e
	$FeSO_4$+ $ZnSO_4$	0.85±0.01b	1.26±0.02bcd	10.14±0.14c	25.13±0.29d	12.83±0.73c
0.8 (ECe:10.8dS/m)	Control-3(non-spray)	0.67±0.01f	1.13±0.01ef	8.91±0.24d	15.5±0.26c	5.04±0.51h
	$FeSO_4$	0.83±0.04bc	1.14±0.01b	10.95±0.20b	18.73±0.15h	14.73±0.84b
	$ZnSO_4$	0.79±0.01d	1.17±0.01bc	11.41±0.8b	17.67±0.25i	11.72±0.50d
	$FeSO_4$+ $ZnSO_4$	0.93±0.01a	1.44±0.01a	12.72±0.13a	19.17±0.25h	19.38±1.01a
LSD at 0.05		0.03	0.004	0.53	0.64	0.31

decrease and assimilation (39). The foliar spaying if micronutrients has succeeded in enhancing the plant protein content, and in partially facing the negative effects of salinity. Zinc shows important function in protein and carbohydrate synthesis, nucleic acid and lipid metabolism. One of the first indications of Zn lack is an inhibition of cell growth rate and proliferation. Zinc affects growth rate of shoots and roots and growth marks of Zn toxicity in crops, commonly, are identical to those of Zn lack (32). (40) stated that protein composition was improved in canola plants being sprayed by elements *i.e.*, Fe + Zn.

Plants content of proline has increased as the salinity level increased (Table 4). In control non-saline treated plants (whether sprayed with Fe and or Zn or not) the difference between them in the proline content was not significant. On the other hand, in salinity treated plants, the foliar spray with Fe and or Zn decreased the amount of accumulated proline in those plants under the same level of salinity (Table 3). Control plants (EC 0.5 dS/m) has recorded the lowest amount of proline (5.14 µg/g), where on the contrary the highest amount (19.38 µg/g) were recorded by salinity treated plants with the salinity level of (EC 10.8 dS/m) those plants were treated by Fe+Zn foliar spray.

The data shown in Table 4 presented a clear positive relationship between the level of salinity and amount of accumulated sugars (reducing and non-reducing sugars) with the highest amount in the plants treated with the salinity level of (ECiw 10.8 dS/m) and treated with Fe + Zn, as the amount of reducing and non-reducing sugar in those plants was (1.44 and 12.72 µg/g DM) respectively. The part of results of the present study concerning the proline and the reducing and non-reducing sugars comes with the same pattern like those of other ornamental plants (41). The higher reducing and non-reducing sugar percentages could be associated to the increase in the amount of metabolites synthesized by the plants, which in turn, accelerated the various plant growth characters and dry weight of

herbs as a result of foliar applications of Fe, Zn and their mixture, and finally reflected on the reducing and non-reducing sugar percentages. Moreover, under saline stress, the plants consume a lot of energy to absorb water, this might be a way of facing the excess accumulation of monovalent ions in the vacuole. Plants faces the osmotic pressure through accumulating osmolytes, such as carbohydrates and proline in the vacuoles, this helps balancing the osmotic potential of the vacuole, and unlike monovalent ions not harming the enzymatic system.

Table (4) showed that a significant increase in the essential oil % percentage under salinity conditions. The percentage of essential-oil content increased as the salt stress increased. Some suggestions are also possible for salinity influences on oil productivity, and favorable with our results for sweet basil, an enhanced percentage of oil in the plant tissues was establish to attend salinity-induced restriction of plant biomass productivity. Furthermore, the articles for enhanced accumulation of essential oil in the plant tissues under salinity are further promoted by two studies, which determined that under salinisation, conditions that improved tolerance to salinity are also conducteed by decreased percentage of essential oil in the crops (42). Reference (43) showed that the raise happening in the percentage of essential oil in salinity stressed plants might be a miss-leading parameter if the concern is on it alone, as this raise is not a sign of good growth as it is of the decreasing of the primary metabolites via degrading under salinity conditions, which produces the built block molecules needed to build secondary metabolites like essential oils. Under stress conditions such salinity there is a critical extent of stress level above it the decline in the growth parameter starts to occur, in the present study the foliar spray with Fe and / or Zn has considerably extended the critical extent of stress, as the spraying helps plants by providing it with minerals, and decreasing the negative effects of salinity allowing sweet basil plants to grow even under salinity conditions. According to our results, it can be summarized that foliar application of Fe and Zn can considerably increase the growth and essential oil content of sweet basil, especially if these elements were applied together regardless to their growth rate under non-saline or saline stress conditions. The produced amount of essential oil was the highest in Fe+Zn sprayed plants, then in Fe sprayed plants, then in Zn sprayed plants, and the lowest was in control unsprayed

Table 5. Effect of foliar application of $FeSO_4$, $ZnSO_4$ and their mixture on the chemical constituents of essential oil extracted from sweet basil herb under irrigation of different salinity levels

		Sea salt concentration (%)											
		Control (ECiw: 0.5dS/m)				0.4 (ECe: 6.1dS/m)				0.8 (ECe: 10.8dS/m)			
Compounds		Control-1 (non-spray)	$FeSO_4$	$ZnSO_4$	$FeSO_4$ + $ZnSO_4$	Control-2 (non-spray)	$FeSO_4$	$ZnSO_4$	$FeSO_4$ + $ZnSO_4$	Control-3 (non-spray)	$FeSO_4$	$ZnSO_4$	$FeSO_4$ + ZnSO4
1	α-pinene	0.29	0.35	0.29	0.33	0.28	0.33	0.26	0.29	0.21	0.29	0.24	0.27
2	β-pinene	0.41	0.43	0.47	0.45	0.39	0.42	0.46	0.44	0.39	0.41	0.43	0.42
3	Myrcene	0.29	0.38	0.36	0.41	0.38	0.43	0.39	0.42	0.34	0.39	0.36	0.37
4	1,8-cineol	4.43	5.15	5.86	5.42	3.98	4.46	5.23	4.95	3.72	4.53	4.76	4.61
5	Ocimene	0.35	0.38	0.36	0.39	0.36	0.31	0.34	0.36	0.33	0.3	0.35	0.38
6	Linalool	39.63	39.26	37.16	36.32	39.91	40.11	40.28	40.31	40.93	41.02	39.55	41.32
7	Linalyl acetate	0.49	0.51	0.41	0.56	0.58	0.47	0.46	0.52	0.39	0.44	0.41	0.46
8	Methylchavicol	3.13	4.15	3.16	3.21	3.59	4.65	3.76	4.12	4.37	5.12	3.94	4.36
9	Geraniol	0.28	0.25	0.24	0.26	0.37	0.36	0.3	0.31	0.36	0.39	0.38	0.37
10	Methyl eugenol	5.16	5.49	5.18	5.93	3.97	4.82	4.76	5.11	3.26	4.55	4.42	4.81
11	Eugenol	43.32	41.12	44.08	43.85	43.38	40.83	41.03	40.21	43.06	39.62	42.33	39.52
12	Farnesol	2.22	2.53	2.43	2.87	2.81	2.81	2.73	2.96	2.64	2.94	2.83	3.11

plants (Data shown in Table 4).

Chemical analyses showed that the data associated with qualitative and quantitative compositions of essential oils distilled from the sweet basil plants before flowering status (Tables 4 and 5). According to analysis of essential oils, twelve compounds were identified. The known compounds were grouped including three items *i.e.* major compounds (more than 10%), minor compounds (less than 10% and more than 1%) and trace ones (less than 1%). In this consideration, it is evident that linalool and eugenol exhibited as majors, 1,8-cineol, methylchavicol, methyl eugenol and farnesol were represented as minors, and α-pinene, β-pinene, myrcene, ocimene, linalyl acetate and geraniol were considered as traces.

It is clear from data tabulated in Table 5 that salinity level (0.4% or 0.8%) increased those components percentage compared to their percentage in the oil of the control. Salinity treatments induced enhance in the compositions of linalool, methylchavicol, farnesol, geraniol and myrcene compared to control. However, salinity treatments induced a reduced in the composions of eugenol and methyl eugenol as compared to control. Moreover, ocimene, 1, 8-cineol, α-pinene, β-pinene and linalyl acetate were reduced by application soil salinity rates at 0.4% and 0.8%. Also, the greatest composition of linalool, methylchavicol, farnesol, ocimene and geraniol were enhanced to note its greatest by application that of 0.8%.

The data in Table (5) showed that the highest amount of (α-pinene, myrcene and methylchavicol) was produced by the iron spray treatment, while the highest amount of (1,8-cineol and β-pinene) was produced by the zinc spray treatment, and the highest amount of (linalool, linalyl acetate, eugenol and methyl eugenol) was produced by the Fe+Zn spray treatment. However, control non-sprayed treatment gave the same amounts of (ocimene, geraniol and farnesol) under the saline conditions. The highest linalool percentage (41.32%) was produced under salinity with the foliar spraying of Fe^{+} Zn. The plants content of eugenol was positively affected by all spraying treatments (under salinity conditions) except for the Fe+Zn treatment .The highest percentage of eugenol (44.08%) was produced in Zn sprayed plants. The highest adversely lowering effect on linalool (36.32%) and eugenol (39.52%) was recorded under Fe+ Zn under control and saline conditions, respectively.

Under control (non-saline) conditions the content of linalool was higher in non-spray treatments compared to spray ones, on the contrary the content of eugenol has increased in spray treatments, as the highest content was recorded in the Fe+Zn mixture spray treatment, while the lowest was in the Fe spray treatment (the comparison in control non-saline condition). On the other face, under salinity conditions, the linalool content was increased in the spray treatments compared to non-spray ones, as the highest content of linalool under salinity conditions was found in plants sprayed with the mixture of Fe+zn followed by the plants sprayed by Zn Followed by those sprayed with Fe.

In this study the essential oil content was accelerated only under low stress, while it reduced at the great salinity rate. These results were in agreement with those obtained by (44) who found that the main components of the essential oil of *Ocimum basilicum* var. purpurascens were eugenol and linalool. Under the level of soil salinity of 1500 and 4500 mg kg^{-1} rates the composition of linalool increased and the content of eugenol decreased.

The stimulate of essential oil productivity under a low rate of salinity could be as a result of higher oil gland density and enhance in the number of glands formed prior to leaf appearance (45). Furthermore, salt stress conditions may also affect the essential oil addition indirectly during its impacts on either net assimilation or the partitioning of assimilate between growth rate and differentiation progress. It might be declare that the constraction and accumulation of essential oil was precisely dependent on ideal growth and development of the crops producing oils. The decrease in oil production might be due to the decrease in plant anabolism. Reference (43) reported that an enhance in oil composition in many of the salt stressed plants might be associated to failure the primary metabolites as a result of the impacts of salinity, inducing intermediary products to become possible for secondary metabolites synthesis.

Conclusions

Iron and zinc uptake are controlled by the two main factors, availability of these nutrients in the soil and the capability of crops to acquire them. Us-

ing methods of elements are very necessary to help the sweet basil plants to avoid Na^+ toxicity and nutrient uptake under salinity stress. Improvement of plant growth, water status of salt-stressed sweet basil plants makes it possible to recommend the treatment of plants grown under saline conditions.

In general, application of Fe and Zn by foliar can considerably increase essential oil yields of sweet basil, especially if these nutrients were used together.

Furthermore, we also recommend that Fe and Zn should be sprayed on plants to ability the perfect quality and quantity in sweet basil production. Moreover, to establish the present findings, further researches are needs in this direction with other varieties of sweet basil.

Acknowledgements

The authors would like to extend their sincere appreciation Deanship of Scientific Research, King Saud University, Saudi Arabia for its funding of this research through the Research Group No. RGP-1436-020.

References

1. Munns R. The impact of salinity stress. Plant Stress. Coping with Environ. Stress Found Sust Agric 2008; Accessed January, 12.
2. Munns R. Comparative physiology of salt and water stress. Plant Cell Environ 2002; 25: 239-250.
3. Lutts S, Majerus V, Kinet JM. NaCl effects on proline metabolism in rice (Oryza sativa) seedlings. Physiol Plantarum 1999; 105: 450-58.
4. Bacha MA, Sabbah AM, Hamady MA. Effect of foliar application of iron, zinc and manganese on yield, berry quality and leaf mineral composition of Thompson seedless and roomy red grape cultivars. Agric Sci 1997; 1: 127-40.
5. Bernal M, Cases R, Picorel R, Yruela I. Foliar and root Cu supply affect differently Fe and Zn-uptake and photosynthetic activity in soybean plants. Environ Exp Botany 2007; 60: 145-50.
6. Marschner H. Mineral nutrient of higher plants. Second Ed., Academic Press Limited. Harcourt Brace and Company, Publishers, London 1995; 97: 347-64.
7. Misra A, Dwivedi S, Srivastava AK, Tewari DK, Khan A, Kumar R. Low iron stress nutrition for evaluation of Fe-efficient genotype physiology, photosynthesis, and essential monoterpene oil(s) yield of Ocimum sanctum. Photosynth 2006; 44: 474-77.
8. Sultana N, Ikeda T, Kashem MA. Effect of foliar spray of nutrient solutions on photosynthesis, dry matter accumulation and yield in sea water-stressed rice. Environ Exp Bot 2001; 46: 129-40.
9. Pandey N, Pathak GC, Sharma CP. Zinc is critically required for pollen function and fertilisation in lentil. J Trace Elem Med Biol 2006; 20: 89-96.
10. Alpaslan M, Inal A, Gunes A, Cikili Y, Oscan H. Effect of zinc treatment on the alleviation of sodium and chloride injury in tomato (Lycopersicon esculentum L.) grown under salinity. Tr J Bot 1999; 23: 1-6.
11. Werker E, Putievsky E, Ravid U, Dudai N, Katzir I. Glandular hairs and essential oil in developing leaves of *Ocimum basilicum* L. (Lamiaceae). Ann Bot 1993; 71: 43-50.
12. Pascual-Villalobos MJ, Ballesta-Acosta MC. Chemical variation in an *Ocimum basilicum* germplasm collection and activity of the essential oils on Callosobruchus maculatus. Biochem Syst Ecol 2003; 31: 673-79.
13. Jackson ML. Soil Chemical Analysis Constable Co. London, Prentice Hall Inc., Englwood Cbifis, 1973; New Jersy.
14. Chapman HD, Pratt PF. Methods of Analysis for Soils, Plants and Water; Chapman Publisher: Riverside 1982; CA.
15. Bremner JM. Total nitrogen and inorganic form of nitrogen. In: Methods of Soil Analysis, (Ed.): C.A. Black Am Soc Agron Madison, Wisconsin 1975; 43: 1149-1237.
16. AOAC. Association of Official Analytical Chemists. Official Methods of Analysis. 18th Ed 2005; Arlington.
17. Cataldo DA, Haroon M, Schrader TE, Youngs VL. Rapid colorimetric determination of nitrate in plants tissue by nitration of salicylic acid. Soil Sci Plant Anal 1975; 6: 71-80.
18. Hiscox JD, Israelstam GF. A method for the extraction of chlorophyll from leaf tissue without maceration. Can J Bot 1979; 57: 1332-34.
19. Bates LS, Waldern RP, Teare ID. Rapid determination of for proline for water stress studies. Plant and soil 1973; 39: 205-7.
20. Bradford MM. A rapid and sensitive for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochem 1976; 72: 248-54.
21. Guenther G. The essential oils VIII. Robert E.D. Nastrand Comp. Inc. Toronto, New York, 1961; London.
22. Snedecor GW, Cochran WG. Statistical methods. 7th edition, Iowa State Univ. Press, Ames, Iowa, 1980; USA.
23. Mantri N, Patade V, Penna S, Ford R, Pang EK. Abiotic Stress Responses in Plants: Present and Future. In: Abiotic Stress Responses in Plants: Metabolism to Productivity, (Eds.): Ahmad, P. and Prasad M. N. V. Springer, New York 2012; 56: 1-19.
24. Elhindi KM. Alleviation of adverse effects of seawater on growth and chemical constituents of Chrysanthemum morifolium by foliar Fe and Mn applications. Amer J Plant Nutr Fertil Tech 2013; 3: 1-11.
25. Nusrat J, Rafiq A. Effect of foliar-applied boron and manganese on growth and biochemical activities in sunflower under saline conditions. Pak J Bot 2011; 43: 1271-82.
26. Michail T, Walter T, Astrid W, Walter G, Dieter G, Maria SJ, Domingo MA. Survey of foliar mineral nutrient concentrations of Pinus canariensis at field plots in Tenerife. Forest Ecol Manag 2004; 189: 49-55.

27. Neumann P. Salinity resistance and plant growth revisited. Plant Cell Environ 1997; 20: 1193-98.
28. Parvaiz A, Satyawati S. Salt stress and phyto-biochemical response of plants- a review. Plant Soil Environ 2008; 54: 89-99.
29. Tavakkoli E, Fatehi F, Coventry S, Rengasamy P, McDonald G. Additive effects of Na+ and Cl– ions on barley growth under salinity stress. J Exper Botany 2011; 62: 2189-2203.
30. Ashraf M. Breeding for salinity tolerance in plants. Critical Review of Plant Sci 1994; 13: 17-42.
31. Carden DE, Walker DJ, Flowers TJ, Miller AJ. Single-cell measurements of the contributions of cytosolic Na+ and K+ to salt tolerance. Plant Physiol 2003; 131: 676-83.
32. Kabata-Pendias A, Pendias H. Biogeochemistry of Trace Elements. PWN, WarSaw, Poland 1999; pp, 398.
33. Carvajal M, Martnez V, Alcaraz FC. Physiological function of water channels as affected by salinity in roots of paprika pepper. Plant Physiol 1999; 105: 95-101.
34. Parida AK, Das AB. Effects of NaCl stress on nitrogen and phosphorus metabolism in a true mangrove Bruguiera parviflora grown under hydroponic culture. J Plant Physiol 2004; 161: 921-28.
35. Dean-Drummond CE. A comparison of regulatory effects of chloride on nitrate uptake, and of nitrate on chloride uptake into Pisum sativum seedlings. Plant Physiol 1986; 66: 115-26.
36. Lin H, Sandra SS, Schumaker KS. Salt sensitivity and the activities of the H-ATPase in cotton seedlings. Crop Sci 1997; 37: 190-197.
37. Tabatabaei SJ, Gregory P, Hadley P. Uneven distribution of nutrients in the root zone affects the incidence of blossom end rot and concentration of calcium and potassium in fruits of tomato. Plant Soil 2004; 258: 169-178.
38. Parida AK, Das AB. Salt tolerance and salinity effect on plants: a review. Ecotoxicol Environ Saf 2005; 60: 324-49.
39. Ruiz JM, Romero L. Nitrogen efficiency and metabolism in grafted melon Plant. Sci Hort 1999; 81: 113-23.
40. Bybordi A, Mamedov G. Evaluation of Application Methods Efficiency of Zinc and Iron for Canola (Brassica napus L.). Not Sci Biol 2010; 2: 94-103.
41. Zapryanova N, Atanassova B. Effects of salt stress on growth and flowering of ornamental annual species. Biotechnology and Biotechnology Equipments 2009; 9: 177-79.
42. Hendawy SF, Khalid KA. Response of sage (Salvia officinalis L.) plants to zinc application under different salinity levels. J Appl Sci Res 2005; 1: 147-55.
43. Morales C., Cusido R.M., Palazon J., and Bonfill M., 1993. Tolerance of mint plants to soil salinity. J. Ind. Soci. Soil Sci., 44, 184-186.
44. Said-Al Ahl HH, Mahmoud A. Effect of zinc and / or iron foliar application on growth and essential oil of sweet basil (*Ocimum basilicum* L.) under salt stress. Ozean J Appl Sci 2010; 3: 97-111.
45. Charles DJ, Joly RJ, Simon JE. Effect of osmotic stress on the essential oil content and composition of peppermint. Phytochem 1990; 29: 2837-40.

Correspondence:
Khalid Elhindi
Plant production Department, College of Food and Agriculture Sciences, King Saud University,
P.O. Box 2460, Riyadh 11451, Saudi Arabia
E-mail: elhindikhalid2010@yahoo.com