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IMPROVING SAFFLOWER (*Carthamus tinctorius* L.) GROWTH AND BIOLOGICAL ACTIVITIES UNDER SALINE WATER IRRIGATION BY USING IRON AND ZINC FOLIAR APPLICATIONS

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ABSTRACT

The present study aimed to evaluate growth and biological activities of Safflower *Carthamus tinctorius* L. *treated with* Iron and Zinc foliar application under Irrigation with saline water at Wadi El-Natrown, El-Behera Governate Egypt during two winter seasons; 2011/ 2012 and 2012/ 2013. It aimed to find out the individual and combination effects of foliar application of Iron (Fe) and Zinc (Zn) on vegetative growth, reproductive and yield characters, in addition to some chemical constituents of Safflower plants. All the vegetative growth characters; plant height, number of primary and secondary branches were significantly affected by the foliar application. Foliar application by Zn at 0.6% significantly promoted the plant height followed by application by the combination between Fe: Zn at 0.3:0.6%. The later treatment gave the greatest number of primary and secondary branches followed that of Zn at 0.6% where there was no significant difference between the two treatments. The highest number of inflorescences and seed yield per plant or plot or fadd was obtained by using the combination between Fe : Zn at a ratio of 0.3:0.6% or by Zn only at 0.6%. The percentage of Zinc, Iron, lipid, protein and pigments in addition to biological activity in different plant treatment were determined. All of chemical parameters were significantly affected by Zn, Fe foliar application at different concentration.

Keywords: *Carthamus tinctorius*, growth, yield , chemical composition, biological activities

INTRODUCTION

Safflower (***Carthamus tinctorius*** L.) is an annual herbaceous plant is adapted to hot and dry environments (Li and Mundel, 1996). Safflower is native of Asia. It is a multipurpose oilseed plant grown mainly as cut flower, vegetables and for its high quality oil. The plant is 30 to 150 cm tall and most genotypes have many sharp spines on the leaves and bracts. It is considered as a drought tolerant plant, which is capable of obtaining moisture from levels not available to the majority of crops (Weiss, 2000). It is classified also as a moderately salt-tolerant plant (Siddiqi *et al.*, 2007). Safflower plant can also be grown successfully on soil with poor fertility and in areas with relatively low temperatures (Koutroubas and Papadoska, 2005).

Zinc is necessary for root cell membrane integrity. As suggested by (Parker *et al.*, 1992), root cell membrane permeability is increased under zinc deficiency which might be related to the functions of zinc in cell membranes. From this point of view, external zinc concentrations could mitigate the adverse effect of NaCl by inhibiting Na and /or Cl uptake or translocation. (Alpaslan *et al.*, 1999) concluded that in the salt affected areas, zinc

application could alleviate possible Na and Cl injury in plants. Foliar spraying under these conditions could be much more efficient than any application of nutrients to the soil.

Safflower has been grown from a long of time for its colorful petals, which was used in food coloring and flavoring agent, as a source of vegetable oils and also for preparing textile dye in the Far East, Central and Northern Asia and European Caucasian (Esendal, 2001). The most important characteristics of safflower that determine its ornamental value are a combination of flower color (orange and yellow) and few to no spines on the flowers and bracts. (Pahlavanim, 2003). Safflower is also being used as a source of alternative fuel (biodiesel) (Nosheen *et al.*, 2011). Regarding the human health and nutritional physiology, vegetable oil is one of the fundamental components in foods that have important functions. Consumers have demanded healthier oils, naturally low in saturated fats. From this perspective, safflower has received a lot of importance as a source of vegetable oil. The seeds of safflower contain 35 to 50% oil, 15 to 20% protein and 35 to 45% hull fraction (Rahamatalla *et al.*, 2001). The tender leaves and shoots of safflower are used as pot herb and salad. The plants are very rich in vitamin A, iron, phosphorus and calcium. Bundles of young plants are commonly sold as a green vegetable in markets in India and some neighboring countries (Nimbkar, 2002).

In saline conditions, the solubility of micronutrients is particularly low, and plants grown on such soil often suffer from deficiencies in these elements. Soil salinity may reduce micronutrients uptake due to stronger competition by salt cations at the root surface (Marschner and Romheld, 1994). Soluble ferrous (Fe) tended to become oxidized to ferric oxide which was insoluble as well as the limitation of iron uptake by root cell cytosol (Nikolic and Kastori, 2000) and inhibit iron transport to shoots and its transfer from apoplasm to cytoplasm in shoot tissues (Nikolic and Romheld. 2002).

High salinity in water (or soil solution) causes a high osmotic potential. In simple terms, the salts in solution and in the soil "compete" with the plant for available water. Some salts can have a toxic effect on the plant or can "burn" plant roots and/or foliage. Excessive levels of some minerals may interfere with relative availability and plant uptake of other micronutrients. Soil pH, cation exchange capacity (CEC) and other properties also influence these interactions. High concentration of sodium in soil can lead to the dispersion of soil aggregates, thereby damaging soil structure and interfering with soil permeability. Hence special consideration of the sodium level or "*sodicity*" in soils is warranted (Fipps, 2003).

Iron (Fe) and Zinc (Zn) are considered two of the most important essential micronutrients for plants and humans (Hao *et al.*, 2007). A deficiency of one of these micronutrients can greatly reduce plant yield and even cause plant death. Zinc plays an essential role in plant physiology where it activates some of enzymes and related to metabolism of carbohydrates, auxins, RNA and ribosome functions. The beneficial effect of Zinc on several ornamental plants were studied by Farahat *et al.*,(2007) on *Cupressus sempervirens* L., (Halder *et al.*, 2007a and Halder *et al.*, 2007b)

on gladiolus. Iron is necessary for the biosynthesis of chlorophyll and cytochrome, besides the function of iron in the metabolism of chloroplast RNA, leading to increase in the biosynthesis materials (produced and accumulated), consequently, the growth was enhanced. . Iron is required at several steps in the biosynthetic pathways. However, iron deficiency inhibited leaf growth, cell number, size and cell division, as well as chlorophyll, protein, starch and sugar content, then the fresh and dry weights of herb could be decreased (Marschner1995). Iron (Fe) is a cofactor for approximately 140 enzymes that catalyze unique biochemical reactions (Brittenham, 1994). The stimulatory effect of zinc and/or iron was recorded by Pande *et al.,* (2007) and Said *et al.*, (2009).*Carthamus tinctorius* is a traditional Chinese medicine widely used to improve blood circulation (Li and Mundel 1996), extending the coagulation time in mice and exhibiting a significant antithrombotic effect (Zhu *et al.*, 2005). However, *Carthamus tinctorius* is used not only for its traditional medicinal purposes but is also effective for treating breast cancer (Koutroubas and Papadoska, 2005). The oil extracted from the seed of *Carthamus tinctorius* is reported to contain alkane-6,8-diols, which have the activity to inhibit 12- tetradecanoylphorbol- 13-acetate-induced tumor promotion in two-stage carcinogenesis in mouse skin. In addition, Nferuloylserotonin and N-(p-coumaroyl) serotonin strongly inhibit the melanin production of Streptomyces bikiniensis and B16 melanoma cells (Parker *et al.*, 1992). These compounds are suggested to have potential antitumor effects.

The aim of the present work is to evaluate the influences of foliar application with Iron (Fe) and Zinc (Zn) either individually or in combination on vegetative growth parameters, yield characters, chemical constituents and its biological activities as antioxidant and anticancer of *Carthamus tinctorius* L. plants irrigated with saline water

MATERIALS AND METHODS

Field experiment

The present investigation was carried out at farm of Agricultural Researches Station, Wadi El-Natrown, Faculty of Agriculture, Cairo University, during 2011/ 2012 and 2012/ 2013 seasons. It intended to find out the individual and combined effects of Fe and Zn foliar application on growth, yield , chemical constituents and biological activities of safflower ***Carthamus tinctorius*** L. plant. Seeds of safflower were obtained from a documented nursery in Crop Research Institute, Giza governorate. Planting dates were Nov.25 and Nov.29 for the two seasons respectively. Three seeds were sown in hills spaced 30 cm and in 50 cm in between. After one month from planting, the seedlings were thinned to one /hill. After 40 days from planting, safflower plants were sprayed twice of 15 days intervals by using freshly prepared solutions of two micronutrients in a chelated form. These micronutrients were

Iron in the form of $FeSO_4$ and Zinc in form of $ZnSO_4$. The micronutrient concentrations, either individually or in combination were; Fe (0.3%), Fe (0.6%), Zn (0.3%), Zn (0.6%), Fe: Zn (0.3: 0.6 %), Fe: Zn (0.3: 0.3 %), Fe: Zn (0.6: 0.6 %) and Fe: Zn (0.6: 0.3 %), in addition to the control treatment (only saline water). The traditional recommended doses of soil fertilizers were added before planting and the agricultural procedures for safflower plants were applied.

Analysis of irrigation water and Soil.

Chemical analysis of irrigation water and physic-chemical parameters of the experimental soil used as reported by APHA,(1998) are presented in Table (1 and 2).

Table 1. Physical and chemical characteristics of soil

Parameters	Value
A)- Physical parameters	
Dry Sieve	Zero
Gypsum%	1.28
Gravel%	8.60
C.S %	12.72
F.S%	78.94
Silt%	5.48
Clay%	2.86
Ca CO3%	6.24
O.M%	0.28
Soil texture	Sand
B)- Chemical parameters	
PH 1:25	7.6
Sp%	20
EC (ds/m)	5.12
T.S.S%	0.82
Soluble cations (meq/l)	
Na+	23.48
K+	4.62
Ca++	8.12
Mg++	6.23
Soluble anions (meq / 1)	
HCO- 3	1.38
Cl-	32.64
SO - 4	17.52
CEC meg / 100gs	4.92
Available amounts (mg/kg) of macro nutrients	
N	9.6
P	2.42
K	320
Total amounts (mg/kg) of macro nutrients	
N	320
P	240
K	760

Table 2. Chemical analysis of irrigation water sample

SAR	Soluble cations (meq/l)				Soluble anions (meq/l)			ppm	EC dS/m	pH
	Mg^{++}	Ca^{++}	K^{+}	Na^{+}	SO^{4-}	Cl^{-}	HCO^{3-}			
15.32	3.94	3.86	0.36	30.24	12.98	21.20	4.22	2470	3.84	7.55

Six plants from each foliar treatment application were chosen randomly and the following characters were recorded:

1- Growth and yield characters.

Plant height , number of primary branches/plant, number of secondary branches/plant, number of inflorescences, inflorescences diameter and dry weights of the whole plant , inflorescences, seed yield/plant, seed yield/plot and seed yield/fadd. was recorded.

2- Chemical compositions

Samples were digested by wet digestion with concentrated sulfuric acid in the presence of digestion catalysts (perchloric acid). In resulted solution: Fe and Zn were measured using Atomic Absorption Spectrometer (SOLAAR – UNICAM 989) according to 30 (AOAC,1995).Lipids were extracted by a modified method described by Xu *et al.* .Determination of total nitrogen was carried out according Micro-Kjeldahel method. The crude protein was calculated by multiplying total nitrogen percent by the factor of 6.25 32 (AOAC. 1990).The amount of carotenoides was determinate according to Hartmut and. Wellburn,(**1983**)..

3-Biological activities

a- Extraction of active ingredients

Fifty grams of the plant flowers samples were subjected to extraction three times with ethanol solvent (1: 10 V/V) according to Rosenthaler, (1930). Solvent extract was evaporated to dryness using rotary evaporator (40-50 °C) and weighed.

b-Antioxidant activity using DPPH radical assay

The 2, 2 diphenyl-1-picrylhydrazyl (DPPH) tests were carried out as described by Burits and Bucar (2000). One ml of plant extract (50 and 100 µg/ml) was mixed with 1ml DPPH reagent (0.002% (w/v) /methanol solution). After an incubation in the dark at room temperature for 30 min., the absorbance was measured at 517nm (using jenway 6130 spectrophotometer). BHA (50 and 100 µg/ml) was used as positive control this test was carried out in triplicate and the antioxidant activity was calculated as the following:

Activity % =Ac-At / Ac x 100

Where: At was the absorbance of samples and Ac the absorbance of methanolic DPPH solution.

c-Anticancer activity (Viability test)

Induction of tumor cell lin: Female Swiss Albino Mice, (kept under environment and nutritional condition for 2 weeks) was injected intrapertoneal (i.p) by Ehrlich Ascites Carcinoma Cells (EACC), in order to preparation of tumor cell line.

Tumor cells (cell line): A line of Ehrlich Ascites Carcinoma resistant to endoxan has been used. The parent line was first supplied through the coursty of Dr. G. Klein, Amestrdam, Holland. The tumor line is maintained in the National Cancer Institute, Egypt in Female Swiss Albino Mice by weekly transplantation of $2.5x10^6$ cells were centrifuged at 1000 xg for 5 min at 4 °C. The pellet was washed with saline (0.9% NaCl) then the needed number of cells was prepared by suspending the cells in the propitiate volume of saline.

Methods: (Viability of tumor: The viability percentage of tumor cells was measured by the modified cytotoxic trypan blue exclusion technique (Bennett *et al.*, 1976).

Medium and reagents

The culture medium used was prepared using RPMI media, 10% fetal bovine serum and 10% l-glutamine. Trypan blue (0.4%) then kept in brown closed glass bottle.

Procedure

The viability percentage (V %) of tumor cells was measured after incubation with the tested plant extracts as well as DMSO as control. Two ml of cells ($4x10^6$ cells) were transferred into a set of tubes, then different algal extracts (200 µg/ml) were added into the propitiate tubes as well as DMSO. The tubes were incubated at 37 ^{0}C for 2h. Then, in a test tube containing 80 µl saline and 10 µl trypan blue, 10 µl of cell suspension were added and mixed then the number of living cells was calculated using a hemocytometer.

Statistical Analysis: The experiment layout was a randomized complete block design, with three replicates of each treatment. Data were subjected to convenient statistical analysis methods for calculations of means, variance and standard error using MSTATC software. Mean separations were estimated by calculating LSD values according to Snedecor and Cochran (1980).

RESULTS AND DISCUSSION

The vegetative growth

Generally, results in Table (3) show that, all Fe or Zn and their combination at different concentrations cause an increase in the values of all the studied characters, to some extends, over the control treatment.

The tallest plants (129.0 cm) were observed when the plants of safflower were sprayed by Zn at 0.6% followed by the combination between Fe: Zn at 0.3:0.6% where there was no significant difference between the two treatments. The shortest plants (119.5 cm) were those sprayed with Fe at 0.6%. The tallest plants exceeded those of control treatment by 9.60%. The current data may be due to the role of Zn and/or Fe as cofactor for different defense enzyme e.g: superoxide dismutase and catalase, these enzymes play significant role against various a biotic stress especially high salinity and drought stress. Various investigators demonstrated the beneficial effect of Zinc and or Iron on several plants. Misra and Sharma, (1991) who found that Zn improved plant height of *Mentha arvensis*.

Data show also that, the greatest number of primary and secondary branches (10.78 and 34.40) of safflower plant were obtained by using the

foliar application of the combination between Fe: Zn in 0.3:0.6 % ratio, followed by using Zn at 0.6% where there was no significant difference between the two treatments. On the other hand, the lowest values of these characters (7.73 and 24.07) were noticed with the treatment Fe at 0.3%. The highest values of these characters exceeded the control values by 42.40 and 61.35%, respectively. These results were in agreement with that obtained by Hussein *et al.*, (2006). They mentioned that the Iron is among the essential micronutrients needed for better plant growth and high quality of branches. In addition, El-Sherbeny *et al.*, (2007) reported that such enhancement effect of Zinc might be attributed to the favorable influence of zinc on metabolism and biological activity and its stimulating effect on the enzyme activity which in turn encourage vegetative growth of faba bean plants. Kobraee *et al.* (2013) reported that the adequate supplies of the micronutrients will have favorable effects on soybean quality and growth. Foliar spray by Fe:Zn at 0.3:0.6% had the same superior effect on the dry weight, followed by Zn at 0.6% application. This result is confirmed by Aziz *et al,\`1.* (2010) .

Table 3. Measurements of vegetative growth characters of *Carthamus tinctorius* L as affected by Iron and Zinc Foliar Applications.

Treatments	Plant height (cm)	Mean over control %	No. primary branches	Mean over control %	No. secondary branches	Mean over control %	D.W shoots (gm/plant)	Mean over control %
Control	117.7	--------	7.57	-------	21.32	---------	38.2	------
0.3% Fe	120.6	2.46	7.73	2.11	24.07	12.90	49.9	30.63
0.6% Fe	119.5	1.53	8.15	7.66	26.52	24.39	55.2	44.50
0.3% Zn	123.7	5.10	8.55	12.95	28.73	34.76	59.4	55.50
0.6% Zn	129.0	9.60	10.32	33.82	32.33	51.64	67.9	77.75
0.3:0.6%Fe:Zn	126.4	7.39	10.78	42.40	34.40	61.35	71.6	87.43
0.3:0.3%Fe:Zn	123.5	4.93	10.13	36.33	30.55	43.29	60.1	57.33
0.6:0.6%Fe:Zn	122.3	3.91	9.93	31.18	29.43	38.04	61.8	61.78
0.6:0.3%Fe: n	124.5	5.78	8.88	17.31	26.97	26.50	56.6	48.17
$LSD_{0.05}$	4.42		0.62		2.21		4.84	

The reproductive and yield characters

Table (4 and 5) show that spraying the safflower plants by the combination between Fe:Zn at a ratio of 0.3:0.6% lead to obtain the highest values (53.07, 35.05 gm, 344.1gm and 963.4gm) of number of inflorescences and seed yield per plant or plot or fadd., respectively. These values were exceeded the control values by 45.80, 123.96, 147.73 and 147.72%, respectively. The only exception was with the inflorescence diameter where the greatest value was noticed with Zn at 0.6% application followed by using Fe:Zn (0.3:0.6%) the greatest values exceeded the control by 60.07%. There was no significant difference in the values of these characters between spraying the plant by the combination of Fe:Zn or Zn alone.

Although, the lowest values of these characters were obtained when using either Fe at 0.3 or at 0.6% but still exceeded the control treatment. These results were in accordance with Fathy *et al.*, (2000) and Mady, (2009). They reported that foliar application with Zinc improved seed yield of faba bean plants due to increasing flower formation and the reduction of flowers and pod shedding as well as increasing their ability to accumulate more bio constituents. These positive effects of Zinc upon seed yield and its characteristics could be considered as a reversion of the effect upon the early vigorous growth of faba bean plants. Zeidan *et al.* (2010) using Fe and Zn as foliar application on wheat. They stated that the grain yield and number grain/spike as well as protein content were significantly increased by application of these elements compared with control. Aziz and El- Sherbeny (2004) stated that the micro-nutrients (Fe+Mn+Zn) at 150 ppm were the most suitable treatment for increasing the productivity and quality of *Sideritis Montana*.

Generally, Zinc and Iron applied as single or combined have been proven effective in increasing the productivity, yield and constituents of *Cymbopogon citratus* grown in newly reclaimed land. These increases may be due to the beneficial vital role of Zn in plant growth and development. Zinc which is closely involved in the metabolism of RNA and ribosomal content in plant cells which lead to stimulation of carbohydrates, proteins and the DNA formation. Zinc is an essential factor in tryptophan metabolism and consequently affected the auxin content of the plant. Several enzymes are often activated by Zn, which therefore has a considerable effect on protein synthesis. Zinc has three functional: catalytic, co catalytic and structural. In the last decade the role of Zinc in protein molecules involved in DNA replication and in the regulation of gene expression has attracted much interest. Changes in metabolism brought about by Zinc deficiency are quite complex.

Table 4. Measurements of reproductive characters of Carthamus tinctorius L as affected by Iron and Zinc Foliar Applications.

Treatments	No. Inflo.	Mean over control %	Inflor. Diameter (mm)	Mean over control %	D.W inflorescence (g/ plant)	Mean over control %
Control	36.40	———	24.97	———	73.6	———
0.3% Fe	41.10	12.91	31.13	24.67	94.3	28.12
0.6% Fe	42.33	16.29	30.57	22.43	101.5	37.91
0.3% Zn	44.08	21.10	34.72	39.05	104.5	41.98
0.6% Zn	49.45	35.85	39.97	60.07	115.4	56.79
0.3:0.6% Fe: Zn	53.07	45.80	39.00	56.19	129.4	75.81
0.3:0.3% Fe: Zn	49.30	35.44	38.34	53.54	107.8	46.47
0.6:0.6% Fe: Zn	48.90	34.34	36.42	45.86	112.3	52.58
0.6:0.3% Fe: Zn	43.88	20.55	35.28	41.29	98.1	33.29
LSD0.05	2.95	———	1.63		7.64	

Table 5. Measurements of yield characters of Carthamus tinctorius L as affected by Iron and Zinc Foliar Applications.

Treatments	Seed yield/ plant (gm)	Mean over control %	Seed yield/ plot (gm)	Mean over control %	Seed yield/ fadd. (kg)	Mean over control %
Control	15.65	-------	138.9	-------	388.9	-------
0.3% Fe	22.30	42.49	206.2	48.45	577.5	48.50
0.6% Fe	22.92	46.45	230.0	65.59	643.9	65.57
0.3% Zn	26.76	70.99	285.2	105.33	798.7	105.37
0.6% Zn	33. 50	114.06	329.5	137.22	922.6	137.23
0.3:0.6 % Fe: Zn	35.05	123.96	344.1	147.73	963.4	147.72
0.3: 0.3 % Fe: Zn	30.73	96.36	286.2	106.05	801.4	106.07
0.6: 0.6% Fe: Zn	28.25	80.51	298.9	115.19	836.9	115.20
0.6: 0.3 % Fe: Zn	27.43	75.27	269.6	94.10	754.9	94.11
LSD 0.05	1.80		23.6		65.9	

Concentration of Zn and Fe in shoot of safflower

Application of Zn and Fe significantly increased the nutrient plant uptake. The plant receiving Fe: Zn at 0.3:0.6% foliar spray recorded the highest uptake (12.77 and 8.18 mg/100 g D.W) followed by the treatment by Zn alone at 0.6%. These values exceeded the control treatment (Table **6**) by 101.26 and 166.81 % for Fe and Zn, respectively. These finding were in agreement with those obtained by Pasricha and Aulakh, (1991). They stated that the combination of sulphur and micronutrients had marked influence on Zn and Fe uptake. The lowest Zn and Fe uptake was recorded in control treatment.

Table 6. Influence of spraying zinc and Fe on concentrations of zn, Fe in shoots of safflower.

Treatments	Fe (mg/100g D.W)	Zn (mg/100g D.W)
Control	6.35	3.07
0.3% Fe	7.69	5.58
0.6% Fe	6.73	5.15
0.3% Zn	7.14	5.28
0.6% Zn	5.15	7.85
0.3:0.6 % Fe: Zn	6.45	8.19
0.3: 0.3 % Fe: Zn	5.61	6.66
0.6: 0.6% Fe: Zn	5.48	5.69
0.6: 0.3 % Fe: Zn	4.99	5.86
LSD at 0.05	0.26	0.28

Carotenoids pigments content

Effect of micronutrients on the concentrations of pigments in petals of safflower, could be observed when spraying Zn and Fe on the plant especially the Carotenoids. Spraying safflower plants with Fe: Zn (0.3: 0.6%) recorded the highest concentration of carotenoids (6.45 mg/g). This increase significantly exceeded the other treatments (Figure 1). The present data of

carotenoids pigments were in parallel with the data of vegetative parameters as shown in Table (3). These results were in agreement with those reported by El-Sherbeny *et al.*, (2007) on faba bean plants. They stated that such enhancement effect of Zinc might be attributed to the favorable influence of Zinc on metabolism and biological activity and its stimulating effect on the enzyme activity which in turn encourage vegetative growth.

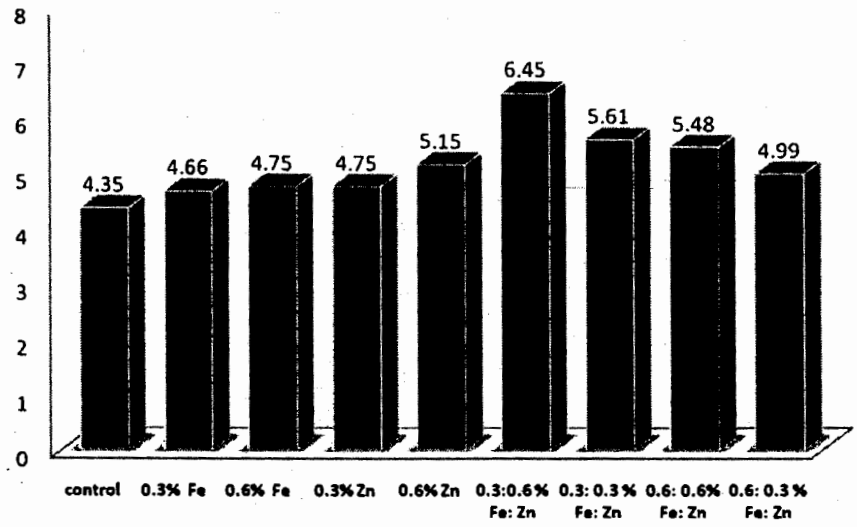

Figure 1. Influence of spraying zinc, Fe on concentrations of carotenoids of safflower petals.

Antioxidant activity

Extracts of safflower by ethanol 70% solvents at 50 and 100 µg/ml concentrations and treated with the different micronutrients showed that, the highest concentration of ethanolic extract (100 µg/ml) exhibited higher antioxidant activity (Table 7) with those plants treated by Zinc at 0.6% and Fe: Zn at 0.3: 0.6% in comparison with control plant and synthetic standard antioxidant (BHA) at the same concentrations of ethanolic extract as shown in Table 7 (81.65, 81.09, 77.0 and 88.75 respectively).The obtained results revealed that the polar plant extract to which attributed the antioxidant activity mainly include carotenoide pigment as shown in Table 8. Moreover, phenolic compounds which are largely present in most species which exhibited pronounced antioxidant activity (Shalaby *et al.*, 2010).

Table 7. Influence of spraying zinc, Fe on antioxidant activity of ethanolic extract of Carthamus tinctorius L at 50 and 100 ug/ml against DPPH radical assay.

Treatments	Antioxidant activity (%)	
	50 ug/ml	50 ug/ml
control	51.85	77.00
0.3% Fe	49.65	76.85
0.6% Fe	52.63	80.62
0.3% Zn	52.60	79.62
0.6% Zn	55.45	81.65
0.3:0.6 % Fe: Zn	54.23	81.09
0.3: 0.3 % Fe: Zn	51.30	79.65
0.6: 0.6% Fe: Zn	54.62	80.23
0.6: 0.3 % Fe: Zn	49.65	78.65
BHA	58.95	88.75
L.S.D at 0.05	1.68	1.97

Anticancer activity

Viability assay was used to assess the cytotoxicity of ethanolic extract from safflower flowers against EACC tumor cell lines. The obtained results (Table 8) of the control extract showed acceptable potency against EACC cell lines ranged between 41 to 65% at 50 and 100 µg/ml. However, plant treated with Zn and Fe showed relatively higher activity ranged between 65.85 to 73 % at 100 µg/ml in plants treated with 0.3+0.6 Zn:Fe and 0.6+0.6 respectively. The recorded data were matching with carotenoids content as shown in Table 8. These results are in agreement with the results obtained by Loo *et al.* (2004) who found that, *Carthamus tinctoriu* is used not only for its traditional medicinal purposes but is also effective for treating breast cancer.

Table 8. Influence of spraying zinc, Fe on anticancer activity of ethanolic extract of Carthamus tinctorius L at 50 and 100 ug/ml against EACC cell line.

Treatments	Anticancer activity (%)	
	50 ug/ml	50 ug/ml
control	41.32	65.52
0.3% Fe	42.30	67.85
0.6% Fe	42.00	66.89
0.3% Zn	43.62	69.30
0.6% Zn	44.12	71.95
0.3:0.6 % Fe: Zn	43.12	72.09
0.3: 0.3 % Fe: Zn	41.65	69.85
0.6: 0.6% Fe: Zn	47.62	73.00
0.6: 0.3 % Fe: Zn	41.32	65.85
LSD	0.87	1.25

Total lipids and protein

The data presented in Figure 2 represented that, the safflower plants that received Fe: Zn at any ratio, especially at 0.3: 0.6% show the highest value of lipid content (26.84%) and was significantly superior over the control by 18.66%. This might be due to the role of Zn and Fe as a co-enzyme in synthesis of oil. This confirmed by the findings of Mishra and Agarwal, (1994) in soybean.

Also, Application of Zn and Fe significantly increased the protein content of seeds. The treatment Fe: Zn at 0.6: 0.6% recorded the highest protein content (14.06%) and exceeded significantly the control value by 21.6 %. This result agreed with that finding by Babhulkar *et al.* (2000) on safflower treated by sulphur combined with micronutrients, and exhibited the marked influence on the protein content.

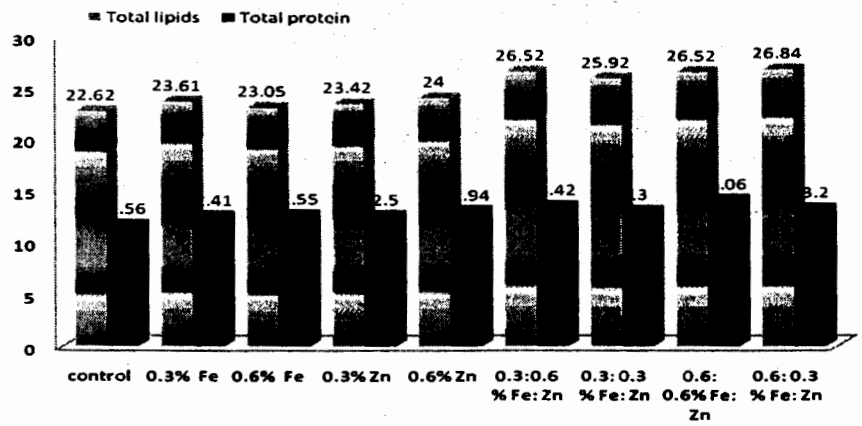

Figure 2 : Influence of spraying Zn, Fe on concentrations of Total lipids and protein (%) of Carthamus tinctorius L seeds.

Conclusion

From the previous results it could be concluded that foliar spray by Fe: Zn at a ratio 0.3:0.6% is necessary and important for safflower plants grown on the reclaim soils in addition to it's positively effect on growth parameters when irrigated with saline water using. This ratio represented the highest values on the majority of morphological, yield and chemical characters under study, except the average of inflorescence diameter and the antioxidant activity, hence application of Zinc at 0.6% exhibited the greatest values for both characters, followed by Fe: Zn at 0.3:0.6%

It could be noticed also that spraying the plants by the combination between Fe: Zn at 0.6:0.3% or 0.6:0.6% gave the highest values for anticancer activity and total protein percentage.

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تحسين النمو والانشطة الحيوية لنبات القرطم (*Carthamus tinctoriu* L.) تحت ظروف ملوحة ماء الري باستخدام الرش الورقي بالحديد والزنك

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تهدف هذه الدراسة الي تقييم النمو والانشطة الحيوية لنبات القرطم *Carthamus tinctorius* L تحت ظروف الري بالماء المالح بمنطقة وادي النطرون – محافظة البحيرة برش النباتات بتركيزات من الحديد والزنك سواء منفردة او مجتمعة ، ولهذا الغرض اجريت تجربتان حقليتان في محطة بحوث البيئة الصحراوية بوادي النطرون – كلية الزراعة – جامعة القاهرة خلال موسمي الشتاء ٢٠١١/٢٠١٢ و ٢٠١٢/٢٠١٣ لدراسة تاثير الرش الورقي بالحديد والزنك المنفرد والخليط علي النمو الخضري والثمري وصفات المحصول بالإضافة بعض المركبات الكيمائية لنبات القرطم. وكانت معاملات الرش الورقي : الحديد في صورة FeSO4 والزنك في صورة ZnSO4. بتركيزات الحديد (٠.٣٪) و (٠.٦٪)، الزنك (٠.٣٪) و (٠.٦٪)، الحديد: الزنك (٠.٣: ٠.٦٪)، الحديد: الزنك (٠.٣ : ٠.٣٪)، الحديد: الزنك (٠.٦: ٠.٦٪) والحديد: الزنك (٠.٦: ٠.٣٪). اظهرت النتائج تأثر جميع الصفات الخضرية المدروسة (طول النبات – عدد الأفرع الرئيسية – عدد الأفرع الثانوية) معنويا بمعاملات الرش الورقي. حيث سجلت معاملة الرش الورقي بالزنك بتركيز ٠.٦٪ اعلي طول للنبات تلاها معاملة الرش الورقي ٠.٣:٠.٦% حديد:زنك والتي سجلت اعلي قيمة لعدد الأفرع الرئيسية والثانوية علي النبات بدون فرق معنوي عن معاملة الرش المنفرد بالزنك بتركيز ٠.٦%. أكبر عدد من النورات/نبات وكذلك محصول البذور/نبات عند الرش بمزيج من الحديد والزنك بتركيز ٠.٣:٠.٦% علي الترتيب بدون فرق معنوي عن معاملة الرش المنفرد بالزنك بتركيز ٠.٦٪ . تم تقدير تركيز الحديد والزنك والدهون والبروتين الكلية بالإضافة الي تقدير مضدات الأكسدة ومضدات السرطان و اظهرت النتائج تاثر جميع الصفات الكيمائية والأنشطة البيولوجية معنويا نتيجة الرش الورقي بالحديد والزنك بجميع التركيزات.

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