DOI : 10.5958/0974-0112.2019.00043.4

Indian J. Hort. 76(2), June 2019: 279-288

Improving of pomegranate aril paleness disorder through application of Fe and Zn elements

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ABSTRACT

Aril paleness disorder changed to the critical damage in the pomegranate orchards of Iran recently. In order to evaluate the effects of Fe and Zn application on reduction of colorless arils of pomegranate, a factorial experiment in a randomized complete block design with three replications was conducted during 2014-2015. In this study, cultivar treatments were cv. *Khazar* and *Shisheh-Cap* and fertilizer treatments were in form of foliar application including the use of ferrous sulfate, zinc sulfate, liquid Fe chelate, each treatment separately in three levels, 0, 0.5 and 1% and combination of ferrous sulfate and Zn sulfate in three levels, 0, each with equal concentrations 0.5% in one compound, and 1% in another combination. Treatments of soil application included: Fe chelate, Zn chelate on each one separately and on three level, 0, 5 and 10 kg/ha. The results showed significantly, different treatments of Fe and Zn increased the Fe, Zn and anthocyanin content of juice and reduced colorless arils in cultivars during both consecutive years. According to the results of presented study, foliar application of Zn sulfate and ferrous sulfate 1% either separately or combined and liquid Fe chelate 1% as superior treatments were recommended to improve the physiological disorder of pomegranate colorless arils in cv. *Khazar* and *Shisheh-cap.*

Key words: *Punica granatum,* fertilizer, anthocyanin, colourless arils.

INTRODUCTION

Pomegranate (*Punica granatum*) is very important due to many desirable traits, taste, abundant medicinal properties, lack of spraying against pests and its being organic. Based on available evidence, it is native to Iran and neighboring countries that are located in semi-arid and arid regions. The incidence of any problem in pomegranate quality, especially the issue of whitening of its fruit arils can lead to the loss of export market of this product (Zabihi and Rezayan, 15; Faragi and Basaki, 5). In recent years, the colorless arils of pomegranate changed to the critical damage in the pomegranate orchards of Iran. This issue has concerned the growers and exporters of this economic production. Thus, the several researchers had studied the physiological disorder of pomegranate colorless arils.

The phenomenon of ripening is a complex combination of many cellular processes such as cell composition changes, loss of cell walls, the accumulation of hydrocarbons, and secondary metabolites. The produced secondary metabolites during fruit ripening are an essential phenomenon that affects fruit quality (Faragi and Basaki, 5; Khaksar *et al*., 9). Among secondary metabolic compounds, flavonoids are a large and essential group. Anthocyanins are responsible for the color of pomegranate and a subgroup of flavonoids (Khaksar *et al*., 9). Lack of essential nutrients can be the cause of disturbances in plant metabolic processes. In the meantime, Fe is a low mobility nutrient which has an effective role in the formation of cytochrome, photosynthesis, respiration and nitrogen fixation (Taiz and Zeiger, 13). Supplying Fe needed in the pomegranate trees dramatically enhances the quality of its fruit. Fe is directly involved in the production of anthocyanin through intervention in chlorophyll structure and increasing photosynthesis (Davarpanah *et al*., 4).

Zn is another micronutrients that play a leading role in increasing the quality and quantity of pomegranate fruit. Zn regulates the metabolism of proteins and carbohydrates and plays a role in enzymatic reactions (Hasani *et al*., 8). Zn reduces the level of active oxygen production in stress condition and damage caused by it. This element is required to build auxin from tryptophan amino acid and can induce rooting (Amiriy Nejad *et al*., 2). Some researchers have suggested that there is a positive significant correlation between the anthocyanin content and the concentrations of Fe, Zn, Mg, and Ca in the *Morusnigra* L. and *Cornus mas*

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L. fruit (Pliszka *et al.*, 12). Supplying micro-elements nutrition for pomegranate trees including Fe, Zn, and Mn increases phenolic compounds in pomegranate arils and peel (Mirdehghan and Rahimi, 10). The researchers reported that foliar application of 3 mg/L of Zn-EDTA and Fe-EDTA significantly increased soluble solids in the pomegranate fruit (Zare *et al.*, 16). Some researchers also announced that application of 0.4% Zn sulfate and 0.4% Ferrous sulfate with boric acid increased the size of canopy, chlorophyll content and the high percentage of pomegranate fruit set (Yadav *et al.*, 14). Other researchers also reported that by the soil application of Fe-EDTA plus ZnSO4.7H2O, near the roots, pomegranate fruit weight and diameter, one hundred arils weight, and Brix index increased significantly, especially in the second year (Mirzapoura and KhoshGoftarmanesh, 11). In another study, Fe-EDDHA at a concentration of 2000 mg/L increased the number of fruits, total soluble solids and Fruit dry weight in each tree (Davarpanah *et al.*, 4).The utilizing of Zn sulfate increased leaf photosynthesis and also the content of Total Soluble Solid, tannins, total phenols, flavonols, flavonoids, and anthocyanin in berries and berry skin of red grapes (Chang-Zheng *et al.*, 3). Moreover, in conducting several experiments, it was announced that using Fe and Zn fertilizer increased the amount of total solids and anthocyanin in red grapes (Abd El-Razek *et al.*, 1).

Given the above, this study was conducted to study the role of Fe and Zn fertilizers to reduce the physiological disorder of pomegranate colorless arils.

MATERIALS AND METHODS

This study was conducted during 2015 in Bajestan, Khorasan-Razavi province, Iran, which is located in semi arid and arid area, longitude 58°11'9.73", latitude 34°31'25.09" and altitude of 1250 meters above the sea level. The average rainfall is 120 mm per year. (Zabihi and Rezayan, 15). Soil physical and chemical parameters are given in Table 1.

Table 1. Result of physical and chemical soil analysis.

depth (cm)	0-50	50-100
pH	8	7.9
Electrical conductivity (ds/m)	4.1	4.7
Clay (%)	10	10
Silt (%)	21	10
Sand (%)	69	80
Saturation percent (%)	31	28.5
Organic carbon (%)	0.32	0.25
Total nutrient value (%)	15.4	14
N (%)	0.03	0.26
P (ppm)	0.5	39.8
K (ppm)	29.9	70.9
Zn (ppm)	0.5	0.3
Fe (ppm)	4.5	4

This experiment was conducted using factorial experiment in a randomized complete block design with three replications where cultivar treatment was at two levels cv. *Khazar* (V_1) and *Shisheh-Cap* (V_2) and fertilizer utilized treatments were in form of foliar application including the use of 1) Ferrous sulfate 21% at three levels of 0 (control), 5 (1.05 kg of pure Fe) and 10 kg (2.1 kg of pure Fe) per thousand liter water ($F_{Fs\ 0,\ 0.5\ and\ 1\%}$), 2) Zn sulfate 34% at three levels of 0, 5 (1.7 kg of pure Zn) and 10 kg (3.4 kg of pure Zn) per thousand liter water ($F_{Zs\ 0,\ 0.5\ and\ 1\%}$), 3) Ferrous sulfate and Zn sulfate combination at three levels of 0 (control), 5 kg Fe sulfate and 5 kg Zn sulfate per thousand liter water and 10 kg Fe sulfate and 10 kg Zn sulfate per thousand liter water ($F_{Fs+Zs\ 0,\ 0.5\ and\ 1\%}$), 4) Liquid Fe chelate 6% (Fe-EDTA 6%) at three levels of 0, 5 and 10 liter per thousand liter water ($F_{LFc,\ 0,\ 0.5\ and\ 1\%}$). Fertilizer treatments in form of soil application include the use of 1) Three levels of Fe chelate as 0, 5 and 10 kg/ha ($S_{Fc\ 0,\ 5\ and\ 10kg/ha}$), 2) Zn chelate on three levels: 0, 5 and 10 kg/ha ($S_{Zc\ 0,\ 5\ and\ 10kg/ha}$). Zn and Fe chelates were used in from of Zn-EDTA 15% and Fe- EDDHA 6%. Each plot consisted of three 9-years-old pomegranate trees at a distance of 3 meters between rows and 2.5 meter on the row. Qualitative traits of colourless arils (aril paleness) percentage in fruits was measured by counting the number of colourless arils per three fruit and calculating the mean values based on percentage. Biochemical trait including total anthocyanin of fruit juices (mg/L) was calculated based on cyanidin 3-glucoside as the dominant anthocyanins of pomegranate and via using pH difference between the two buffer systems (Hamouda *et al.*, 7). To measure the colour of the samples Hunter lab device model Chroma Meter CR-410, Japan. Also the microscopic images were captured by microscope model Motic SFC-28 series. MSTAT-C software was used for data analysis and Excel software was used for drawing the diagrams. Mean comparison of traits was done using Duncan's multiple range tests at the level of 5%.

RESULTS AND DISCUSSION

The juice anthocyanin content was significantly different in pomegranate cultivars ($P<0.01$) and it was higher in V_2 compared to V_1 (35.26%) in the first year of experiment (Table 3).However statistically, there was no significant difference between the anthocyanin content of the cultivars in the second year (Table 3).

Regardless the effect of cultivar, applying fertilizer treatments significantly affected on fruit juice anthocyanin (P<0.01).As a superior treatment, utilizing $F_{LFc1\%}$ increased anthocyanin in fruit juice compared to the control in the first year (243.9mg/L) and second (219.1mg/L) year of experiment (Table 3).

Also, in consecutive both years, application of Fe and Zn fertilizers was significantly effective on fruit juice anthocyanin of V1 and V2 at 1% level (Table 2). In the first year of experiment, the highest amount of fruit juice anthocyanin was obtained through the $F_{LFc1\%}$ on V_2 (248.6 mg/L) and V_1 (239.3 mg/L) which is shown in Fig. 1. Furthermore, using $F_{LFc1\%}$ caused to maximum decreased anthocyanin fruit juice on V_1 (221.4 mg/L) and V_2 (216.8 mg/L) in second year (Fig. 2).

Table 2. Analysis of variance of the pomegranate traits effected by Fe, Zn fertilizer and cultivars.

Source of Variation	df	Mean square			
		Anthocyanin	PWA	Fe content of fruit juice	Zn content of fruit juice
Year	1	5.359 ns	3151.042 **	0.00 ns	0.010 ns
Replication	4	3158.779 ns	149.426 ns	0.007 ns	0.176 ns
Cultivar	1	23119.360 **	7455.375 **	0.003 ns	0.342 **
Y*C	1	14851.320**	1044.560**	0.004 ns	0.00 ns
Treatment	17	14371.677**	2802.642 **	0.005 *	0.101 **
Y*T	17	3725.346 **	575.855 **	0.004 ns	0.034 **
C*T	17	7187.780 **	859.895 **	0.004 ns	0.051**
Y*C*T	17	4548/240 **	577.688 **	0.005 *	0.012 ns
Error	140	665.581	48.764	0.003	0.014
CV (%)	….	20.72	19.95	13.99	16.01

(* significant at 5%; ** significant at 1%; ns non- significant).

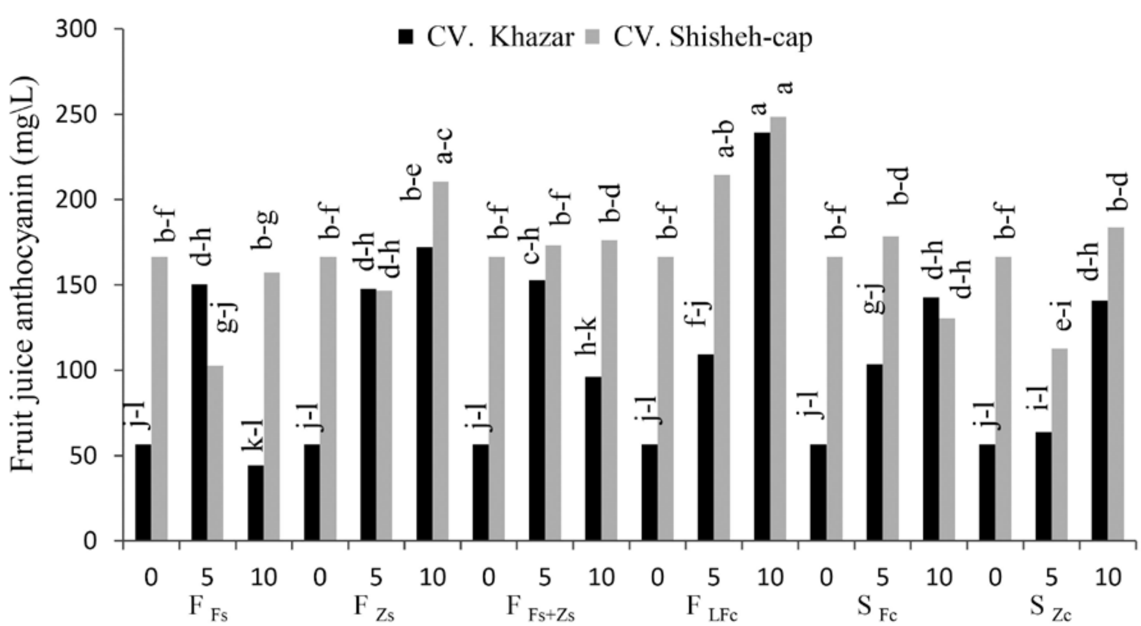

Fig. 1. Interaction effect of fertilizer treatment and cultivar on anthocyanin of fruit juice in the first year.

F_{Fs} = Foliar application of Ferrous sulfate F_{Zs} = Foliar application of Zinc sulfate F_{Fs+Zs} = Foliar application of Ferrous sulfate + Zinc sulfate F_{LFc} = Foliar application of Liquid Fe chelate S_{Fc} = Soil application of Fe chelate S_{Zc} = Soil application of Zn chelate

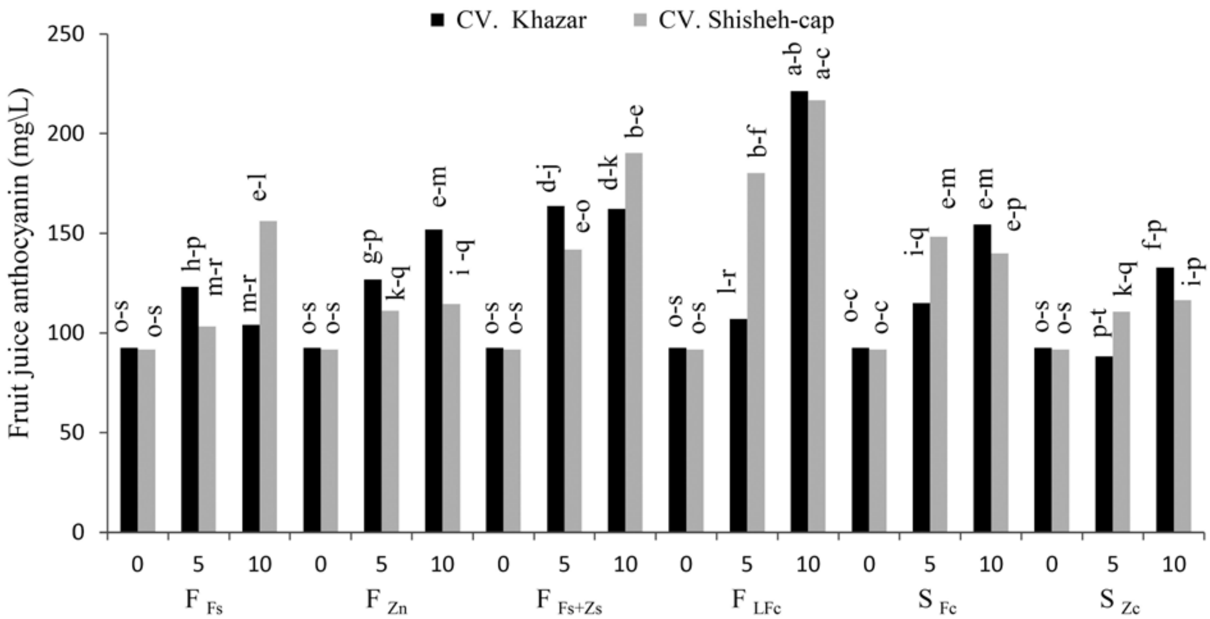

Fig. 2. Interaction effect of fertilizer treatment and cultivar on anthocyanin of fruit juice in the second year.

F_{Fs} = Foliar application of Ferrous sulfate F_{Zs} = Foliar application of Zinc sulfate F_{Fs+Zs} = Foliar application of Ferrous sulfate + Zinc sulfate F_{LFc} = Foliar application of Liquid Fe chelate S_{Fc} = Soil application of Fe chelate S_{Zc} = Soil application of Zn chelate

The results of data analysis showed the interaction effect of cultivar and year was significant at 1% level. The mean comparison showed that the amount of PWA in V_1 was 17.29% and 41.13% lower than the V_2 in first and second year respectively (Table 3).

Regardless the effect of cultivar, application of Fe and Zn fertilizer affected on PWA significantly in both years at 1% level (Table 2). In the first year of experiment, the minimum amounts of PWA were respectively obtained from $F_{Zs\ 1\%}$ (10%) and $F_{LFc\ 1\%}$ (13.67%) which placed statistically in one group (Table 3). In addition, $F_{LFc0.5\%}$, $F_{Fs+Zs0.5\%}$, $F_{Fs+Zs\ 1\%}$, $F_{Fs\ 0.5\%}$ and $S_{Zc10kg.ha^{-1}}$ reduced significantly the PWA as 75.36%, 74.46%, 71.76%, 70.86% and 74.43% respectively compared to their control treatments which were statistically in one group with the best treatment to improve the disorder of PWA (Table 3). In the second year, application of $F_{LFc\ 1\%}$ (11.17%) and $F_{Fs+Zs1\%}$ (13.67%) caused to the best effects on improving PWA (Table 3).

The results of data analysis related to the effect of fertilizer treatments on V_1 and V_2 represent a positive role of Fe and Zn fertilizer for significantly reducing the PWA physiological disorder in both years at 1% level (Table 2). The best treatments to improve the PWA were related to $F_{Zs\ 0.5\%}$ and $F_{Zs\ 1\%}$ on V_1 (6.33%) which reduced PWA 88.34% compared to their control treatment in the first year (Fig. 3). The $F_{Fs\ 1\%}$ had the maximum effect on PWA in V_2 (9.667%) and reduced PWA 82.94% compared to its control treatment as well (Fig. 3). In the second year of the experiment, the superior treatments of the first year had a significant effect on the reduction of PWA. Nevertheless, the highest effects were observed via $F_{Fs+Zs1\%}$ and $F_{LFc1\%}$, respectively (7.67% and 8.66% PWA) on V_1 (Fig. 4). Moreover, $F_{LFc1\%}$ (13.68%) and $F_{Fs+Zs1\%}$ (19.67%) were respectively the best treatments to improve the PWA in V_2 (Fig. 4).

The data analysis showed that the interaction effect between year, cultivar and applying different fertilizer treatments of Fe and Zn was significant on fruit juices Fe content at 5% level, but the interaction effect between year and applying different fertilizer treatments and interaction effect between year and cultivar was not significant (Table 2).

Mean comparison of data revealed that, $F_{Fs\ 0.5\%}$ and $F_{Fs\ 1\%}$ had the maximum significant effect on increasing the Fe content of fruit juice of V_1 (0.1667 and 0.2667 mg/L respectively in the first year (Table 4). Nevertheless, using Fe fertilizers had no significant effects on increasing the juice Fe content of V_1 in the second year and V_2 in both years of experiment (Table 5).

Based on the results of data analysis, the interaction effect between year and applying different fertilizer treatments of Fe and Zn was significant on fruit juice Zn content at 1% level, but the interaction effect between year, applying different fertilizer treatments and cultivars was not significant (Table 2).

Table 3. Mean comparison of the pomegranate traits under fertilizer treatment in the first and second year of experiment.

Traits	Anthocyanin of fruit juice (mg/ L)		PWA (%)	
Treatment	First year	Second year	First year	Second year
V1	105.7 c	122.6 b	35.15 c	23.11 d
V2	143 a	126.7 b	42.5 a	39.26 b
$F_{Fs\ 0}$	111.5 h-m	92.11 k-n	55.50 a-b	44.67 c
$F_{Fs\ 0.5\%}$	126.5 f-k	113.3 g-m	16.17 i-j	30.33 d-g
$F_{Fs\ 1\%}$	100.7 i-m	130.2 e-j	30.50 d-g	22.50 f-i
F_{Zs0}	111.5 h-m	92.11 k-n	55.50 a-b	44.67 c
$F_{Zs\ 0.5\%}$	147.2 d-g	119.0 f-m	43.33 c	31.33 d-f
$F_{Zs1\%}$	191.4 b-c	133.3 e-j	10.00 j	21.33 g-i
F_{Fs+Zs0}	111.5 h-m	92.11 k-n	55.50 a-b	44.67 c
$F_{Fs+Zs\ 0.5\%}$	86.73 m-n	152.8 d-f	15.67 i-j	19.00 h-j
$F_{Fs+Zs\ 1\%}$	136.2 e-i	176.3 c-d	14.17 i-j	13.67 i-j
$F_{LFc\ 0}$	111.5 h-m	92.11 k-n	55.50 a-b	44.67 c
$F_{LFc\ 0.5\%}$	161.8 c-e	143.6 d-h	50.67 b	22.67 f-i
$F_{LFc1\%}$	243.9 a	219.1 a-b	13.67 i-j	11.17 j
$S_{Fc\ 0}$	111.5 h-m	92.11 k-n	55.50 a-b	44.67 c
$S_{Fc\ 0.5\%}$	58.94 n	131.7 e-j	54.00 b	34.33 d-e
$S_{Fc\ 1\%}$	136.7 e-h	147.3 d-g	25.67 e-h	21.00 h-i
S_{Zc0}	111.5 h-m	92.11 k-n	55.50 b	44.67 c
$S_{Zc\ 0.5\%}$	88.40 m-n	99.60 j-m	61.83 a	38.67 c-d
$S_{Zc\ 1\%}$	90.26 l-n	124.8 f-l	14.17 i-j	27.33 e-h

Mean followed by the same letters in each column are not significant (Duncan's multiple range test 5%).
V_1= cv. Khazar, V_2= cv. Shisheh-cap F_{Fs}=Foliar application of Ferrous sulfate F_{Zs}= Foliar application of Zinc sulfate F_{Fs+Zs}= Foliar application of Ferrous sulfate + Zinc sulfate, F_{LFc}= Foliar application of Liquid Fe chelate, S_{Fc}= Soil application of Fe chelate, S_{Zc}=Soil application of Zn chelate.

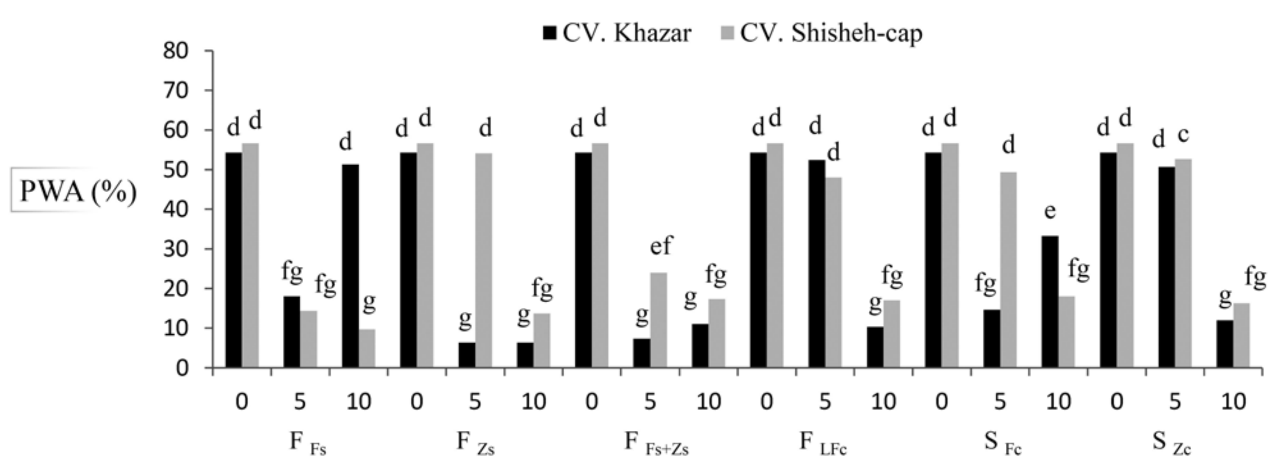

Fig. 3. Interaction effect of fertilizer treatment and cultivar on PWA in the first year of experiment.

F_{Fs} = Foliar application of Ferrous sulfate F_{Zs} = Foliar application of Zinc sulfate F_{Fs+Zs} = Foliar application of Ferrous sulfate + Zinc sulfate F_{LFc} = 3 Foliar application of Liquid Fe chelate S_{Fc} = Soil application of Fe chelate S_{Zc} = Soil application of Zn chelate.

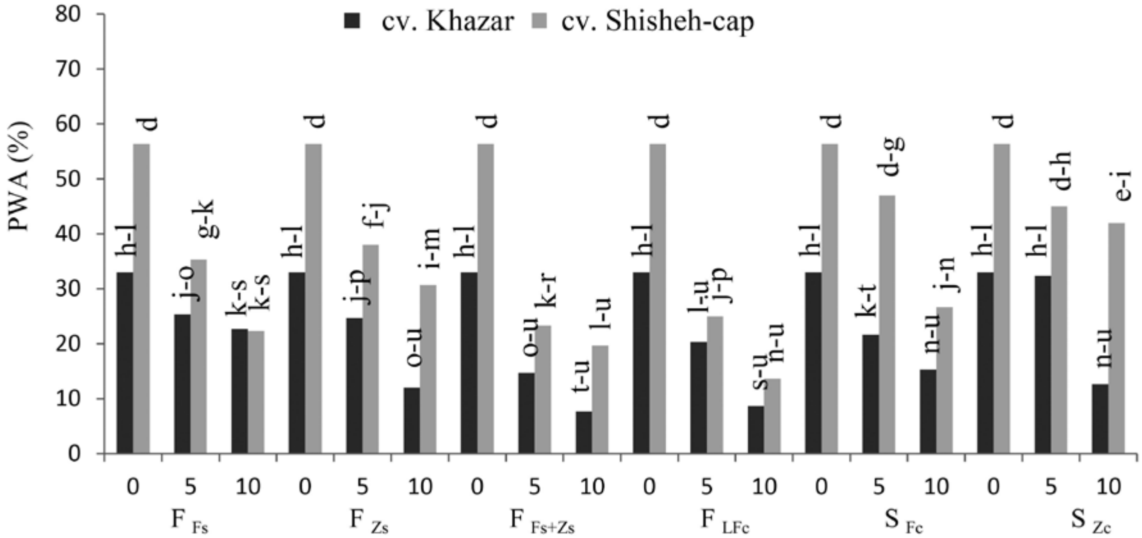

Fig. 4. Interaction effect of fertilizer treatment and cultivar on PWA in second year of experiment.

F_{Fs} = Foliar application of Ferrous sulfate F_{Zs} = Foliar application of Zinc sulfate F_{Fs+Zs} = Foliar application of Ferrous sulfate + Zinc sulfate F_{LFc} = 3 Foliar application of Liquid Fe chelate S_{Fc} = Soil application of Fe chelate S_{Zc} = Soil application of Zn chelate.

Table 4. Mean comparison of the pomegranate traits under fertilizer treatment in the first and second year of experiment.

Traits	Fe content of fruit juice (mg/L)		Zn content of fruit juice (mg/L)	
Treatment	First year	Second year	First year	Second year
V_1	0.06204 a	0.05184 a	0.7004 b	0.715 b
V_2	0.04624 a	0.05235 a	0.7811 a	0.7933 a
$F_{Fs\ 0}$	0.03500 b	0.03200 b	0.8083 b-g	0.7839 b-h
$F_{Fs\ 0.5\%}$	0.03117 b	0.06683a-b	0.8167 b-f	0.8177 b-f
$F_{Fs\ 1\%}$	0.08333a-b	0.1055 a-b	0.6617 e-i	0.7426 b-i
F_{Zs0}	0.03500 b	0.03200 b	0.8083 b-g	0.7894 b-h
$F_{Zs\ 0.5\%}$	0.1383 a	0.03933 b	0.6433 g-i	0.6395 h-j
$F_{Zs1\%}$	0.1333 a	0.05135 b	0.7867 b-h	1.019 a
F_{Fs+Zs0}	0.03500 b	0.03200 b	0.8083 b-g	0.7856 b-h
$F_{Fs+Zs\ 0.5\%}$	0.03167 b	0.04370 b	0.6267 h-j	0.8167 b-f
$F_{Fs+Zs\ 1\%}$	0.03667 b	0.06433a-b	0.8283 b-e	0.8544 b-d
$F_{LFc\ 0}$	0.03500 b	0.08150a-b	0.8083 b-g	0.7839 b-h
$F_{LFc\ 0.5\%}$	0.05500 b	0.05467 b	0.7150 c-i	0.6917 d-i
$F_{LFc1\%}$	0.05500 b	0.08167a-b	0.7300 b-i	0.7387 b-i
$S_{Fc\ 0}$	0.03667 b	0.03200 b	0.8083 b-g	0.7839 b-h
$S_{Fc\ 0.5\%}$	0.05500 b	0.04583 b	0.6333 h-j	0.4843 j
$S_{Fc\ 1\%}$	0.04667 b	0.07000a-b	0.6070 i-j	0.5940 i-j
S_{Zc0}	0.03833 b	0.03200 b	0.8083 b-g	0.7839 b-h
$S_{Zc\ 0.5\%}$	0.06667a-b	0.03650 b	0.6967 d-i	0.5822 i-j
$S_{Zc\ 1\%}$	0.02667 b	0.03650 b	0.6583 f-i	0.8828 a-c

Mean followed by the same letters in each column are not significant (Duncan's multiple range test 5%).

V_1 = cv. *Khazar*, V_2 = cv. *Shisheh-cap* F_{Fs} = Foliar application of Ferrous sulfate F_{Zs} = Foliar application of Zinc sulfate F_{Fs+Zs} = Foliar application of Ferrous sulfate + Zinc sulfate, F_{LFc} = Foliar application of Liquid Fe chelate, S_{Fc} = Soil application of Fe chelate, S_{Zc} = Soil application of Zn chelate.

Table 5. Mean comparison for interaction effect between fertilizer treatments, cultivars and year on fruit juice Fe content.

Trait	Fe content of fruit juice (mg/L)			
Treatments	First year V_1	Second year V_1	First year V_2	Second year V_2
$F_{Fs\ 0}$	0.03667 c-e	0.03200 c-e	0.03333 c-e	0.03200 c-e
$F_{Fs\ 0.5\%}$	0.1667 b	0.1020 b-e	0.01567 d-e	0.03167 c-e
$F_{Fs\ 1\%}$	0.2667 a	0.1300 b-d	0.1100 b-e	0.08100 b-e
F_{Zs0}	0.03667 c-e	0.03200 c-e	0.03333 c-e	0.03200 c-e
$F_{Zs\ 0.5\%}$	0.04667 c-e	0.03533 c-e	0.1100 b-e	0.04333 c-e
$F_{Zs1\%}$	0.05667 c-e	0.03870 c-e	0.0341 c-e	0.06400 b-e
F_{Fs+Zs0}	0.03667 c-e	0.03200c-e	0.03333 c-e	0.03200 c-e
$F_{Fs+Zs\ 0.5\%}$	0.01667 c-e	0.04540 c-e	0.04667 c-e	0.04200 c-e
$F_{Fs+Zs\ 1\%}$	0.006667 e	0.06000 b-e	0.06667 b-e	0.06867 b-e
$F_{LFc\ 0}$	0.03667 c-e	0.03200 c-e	0.03333 c-e	0.1310 b-c
$F_{LFc\ 0.5\%}$	0.05667 c-e	0.05200 c-e	0.05333 c-e	0.05733 b-e
$F_{LFc1\%}$	0.06667 b-e	0.07533 b-e	0.04333 c-e	0.08800 b-e
$S_{Fc\ 0}$	0.03667 c-e	0.03200 c-e	0.03667 c-e	0.03200 c-e
$S_{Fc\ 0.5\%}$	0.07333 b-e	0.05133 c-e	0.03667 c-e	0.04033 c-e
$S_{Fc\ 1\%}$	0.08667 b-e	0.07333 b-e	0.04667 c-e	0.06667 b-e
$S_{Zc\ 0}$	0.03667 c-e	0.03200 c-e	0.03333 c-e	0.03200 c-e
$S_{Zc\ 0.5\%}$	0.04667 c-e	0.03867 c-e	0.08667 b-e	0.03433 c-e
$S_{Zc\ 1\%}$	0.0490 c-e	0.03900 c-e	0.05333 c-e	0.03400 c-e

Mean followed by the same letters in each column are not significant (Duncan's multiple range test 5%).
V_1 = cv. *Khazar*, V_2 = cv. *Shisheh-cap*, F_{Fs} = Foliar application of Ferrous sulfate, F_{Zs} = Foliar application of Zinc sulfate, F_{Fs+Zs} = Foliar application of Ferrous sulfate + Zinc sulfate, F_{LFc} = Foliar application of Liquid Fe chelate, S_{Fc}= Soil application of Fe chelate, S_{Zc} = Soil application of Zn chelate.

The result of analysis of variance revealed the interaction effect between year and cultivar was not significant (Table 2). Nevertheless, the mean comparison of data showed Zn obtained from the fruit juice in V_2 was 11.57% and 10.95% higher than V_1 respectively in the first and second year.

Regardless of cultivar treatments, applied fertilizer treatments had no significant positive effect on increasing the amount of fruit juice Zn content in the first year.But, supplying $F_{Zs1\%}$ cuased to enhanced fruit juice Zn content (1.019mg/L) in the second year (Table 4).

Also the mean comparison of data showed the treatments application had no significant effect on increasing fruit juice Zn content of V1 and V2 in the first year of experiment (Table 5). But, in the second year, the $F_{Zs\ 1\%}$ was the superior treatment for significantly increasing the Zn of the fruit juice on V_2 (1.083 mg/L) (Table 5). Also, the utilizing of $F_{Fs+Zs\ 0.5\%}$ (1.026 mg/L) and $F_{Fs+Zs\ 1\%}$ (1.015 mg/L) enhanced significantly Zn content of fruit juice as well as $F_{Zs\ 1\%}$ on V_2 in the second year (Table 5).

In this experiment, totally the foliar application of Fe and Zn either separately or combined as well as soil application had a positive effect on enhancement the anthocyanin of fruit juice and reduced the PWA disorder in both pomegranate cultivars. Treatments of Fe and Zn as a foliar application had more effects on studied traits which can be due to less Fe and Zn uptake in calcareous soils or the inhibitor effects of Fe and Zn in root zone to attract them (Mirzapoura and KhoshGoftarmanesh, 11). The results of this experiment are consistent with the results of Mirdehghan and Rahemi (10), Hamouda *et al.* (7) also announced application of 0.5% and 1% Fe fertilizer as a foliar significantly increases the anthocyanin content of fruit juices. Zabihi and Rezayan (15) reported that foliar application of 0.6% Zn sulfate and 0.3% Ferrous sulfate increased the color of pomegranate arils cv. *Shisheh-cap*. Abd El-Razek *et al.* (1) also announced that foliar application of 1% Fe and 1.5% Zn fertilizers increased anthocyanin content of red grape. Some researchers reported that Fe and Zn directly increase the anthocyanin content of fruit juice via the enhancement of photosynthesis and sugar supply involved in the production of anthocyanin (Davarpanah *et al.,*4; Khaksar *et al.*, 9), also Zn significantly influence gene expression of the phenolics biosynthetic pathway (Chang-Zheng *et al.*, 3).

The other studies also showed that there is a significant correlation between the concentrations of Fe, Zn and anthocyanin in pomegranate fruit juice. Also, the several researchers reported the Fe and Zn content in the juice of PWA is less than the normal and red juice of pomegranate arils (Zabihi and Rezayan, 15; Mirdehghan and Rahemi, 10). So it can be concluded the increment of fruit juice anthocyanin concentration via enhancement of iron and zinc supplement. In the present study, $F_{Fs\ 0.5\%}$ and $F_{Fs\ 1\%}$ had the maximum significant effect on increasing the Fe content of fruit juice of V_1 in the first year. Hamouda *et al.* (7) reported similar results in cv. *Manfalouty*. Low mobility of Fe in the plant is due to its deposition as the insoluble oxides and phosphates, or formation of a complex with protein in the leaf (phytoferritin) that it limits Fe movement in to the phloem and thus the whole plant (Taiz and Zeiger, 13). Hence, Fe foliar application can have an effective role in increasing the Fe content of leaves and fruit. Some researchers also reported that using Zn leads to more plant growth, thereby uptake more elements such as Fe by plants. Soil application of Fe chelate at all levels also had no positive effect on the amount of leaf Fe content. This is probably due to lack of suitable uptake of Fe in calcareous soils (Mirzapoura and KhoshGoftarmanesh, 11). Also in this study, the application of $F_{Zn\ 1\%}$ and $F_{Fs+Zs\ 0.5\%\ and\ 1\%}$ increased significantly Zn of the fruit juice on V_2. Zare *et al.* (16) reported increased Zn of pomegranate juice by foliar application of Zn sulfate on four cultivars. Ghayekhloo and Sedaghatpour (6) also declered that the use of Zn fertilizer lead to increase Zn content in (*Citrus reticulata* L.) and the highest value was obtained in Zn and Fe combination treatment.

In present experiment, the use of fertilizers containing Fe and Zn on V_1 as well as the use of fertilizer containing Fe on V_2 increased juice anthocyanin and thus reduced PWA in both cultivars (Fig. 5 and 6). The micro-macro scopic image of colourless arils (Fig. 7) and two dimensional microscopic image of pomegranate aril related to the effect of Fe chelated 1% foliar application (Fig. 8) showed arils are coloured having arthocyanin & more juice compared to control. Hamouda *et al.* (7) announced that foliar application of ferrous sulfate 0.5% and 1% and zinc sulfate 1% and 2% increased anthocyanin concentrations and reduced the PWA disorder in cv. *Manfalouty*. The other researchers also reported the fundamental role of environmental and climatic factors in the production of anthocyanin and change in the number of PWA (Zabihi and Rezayan, 15; Khaksar *et al.*, 9). The PWA disorder has been reported in thermal and salinity stress conditions (Faragi and Basaki, 5). The researchers believed that it was due to decreased expression of a gene (which called *PgUFGT*) in environmental stress conditions. This gene is the

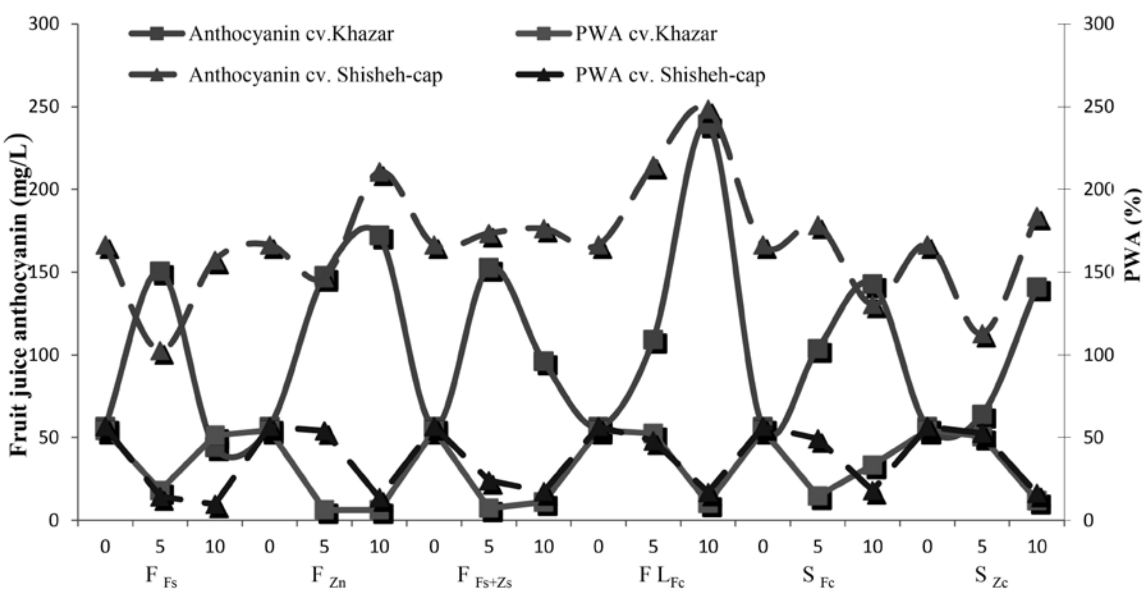

Fig. 5. The relationship between anthocyanin content of fruit juice and pomegranate white arils under Fe and Zn fertilizer application in the first year of experiment.

F_{Fs} = Foliar application of Ferrous sulfate F_{Zs} = Foliar application of Zinc sulfate F_{Fs+Zs} = Foliar application of Ferrous sulfate + Zinc sulfate F_{LFc} = 3 Foliar application of Liquid Fe chelate S_{Fc} = Soil application of Fe chelate S_{Zc} = Soil application of Zn chelate.

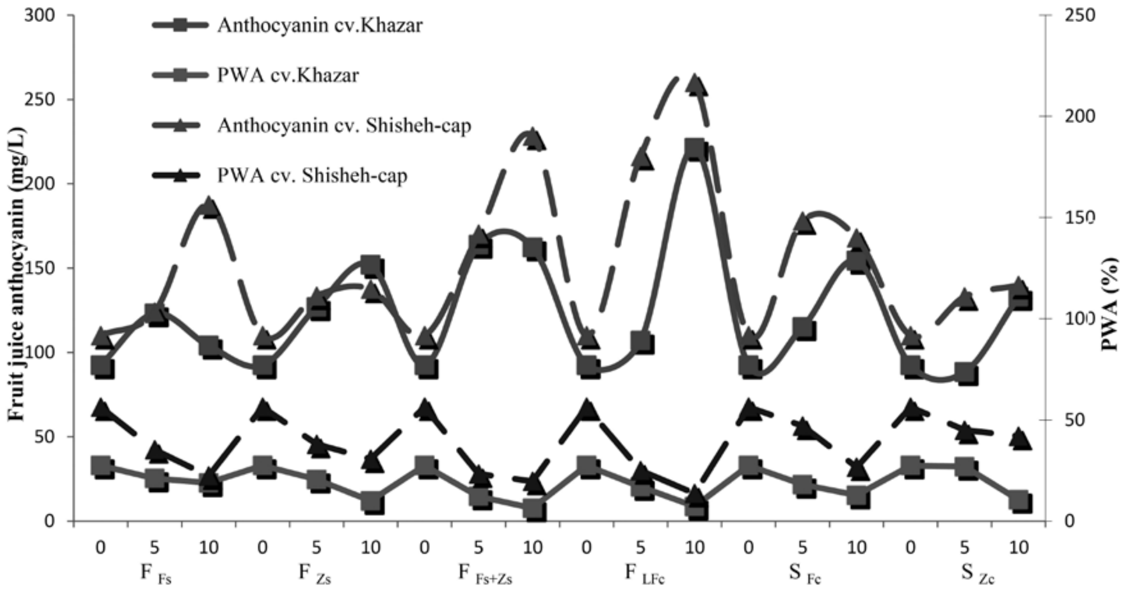

Fig. 6. The relationship between anthocyanin content of fruit juice and pomegranate white arils under Fe and Zn fertilizer application in the second year.

F_{Fs} = Foliar application of Ferrous sulfate F_{Zs} = Foliar application of Zinc sulfate F_{Fs+Zs} = Foliar application of Ferrous sulfate + Zinc sulfate F_{LFc} = 3 Foliar application of Liquid Fe chelate S_{Fc} = Soil application of Fe chelate S_{Zc} = Soil application of Zn chelate.

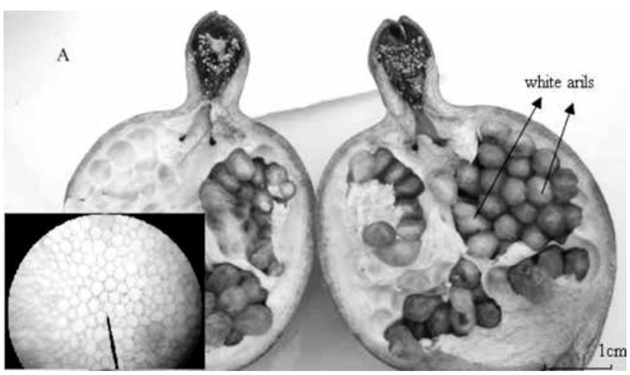

Fig. 7. The micro-macroscopic image of colourless arils of pomegranate cv. *Shisheh-cap* (control treatment).

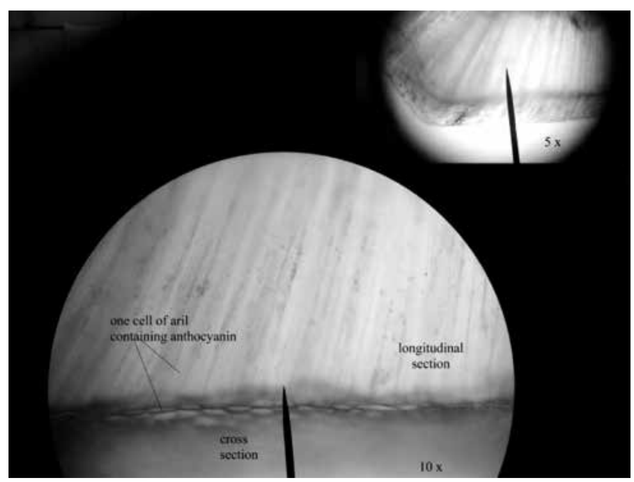

Fig. 8. The two-dimensional microscopic image of pomegranate aril related to the effect of Fe chelated 1% foliar application on cv. *Shisheh-cap*. The arils are colored having Anthocyanin and more juice comparing to the control treatment.

capture agent of glucose molecules to anthocyanidin and formation of anthocyanin which is the reason for red color of arils and peel in pomegranates. In response to environmental factors such as stresses, the expression of *PgUFGT* thus the formation of anthocyanin is affected by a series of controllers. In fact, colour production leads to understanding the environmental changes and amount of the plant flexibility in response to stressful conditions (Khaksar *et al*., 9). Hence, according to presented arguments, it seems that the intervention of factors controlling the expression of *PGUFGT* is decreased on the reduced environmental stresses conditions. It leads to more anthocyanin production and the reduced PWA. On the other hand, numerous researchers have reported the reduced effects of environmental stresses via the use of Fe and Zn fertilizer in different plants (Amiriy Nejad *et al*., 2). In this study, with regard to environmental and climatic stresses within the site of our experiment, especially in recent years, it seems probably the use of Fe and Zn fertilizer lead to increased anthocyanin and reduced PWA disorder through the improvement the effects of environmental stresses such as drought and high temperatures. According to the results obtained in presented study, foliar application of Zn sulfate and ferrous sulfate 1% either separately or combined and liquid Fe chelate 1% as superior treatments were recommended to improve the physiological disorder of pomegranate white arils in cv. *Khazar* and *Shisheh-cap.*

ACKNOWLEDGEMENTS

The authors would like to thank Khorasan Razavi Agricultural and Natural Resource Research and Education Center, Mashhad, Iran, for scientific support and providing facilities.

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Received : February, 2018; Revised : March, 2019; Accepted : May, 2019.