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Improvement of pomegranate colorless arils using iron and zinc fertilization

Elahe Asadi [a], Ahmad Mohammadi Ghehsareh [a, *], Ebrahim Ganji Moghadam [b], Mehran Hoodaji [a], Hamid Reza Zabihi [c]

[a] Department of Soil Science, Faculty of Agriculture, Isfahan (Khorasgan) Branch, Islamic Azad University, Isfahan, Iran
[b] Department of Horticulture, Agricultural and Natural Resources Research Center of Khorasan Razavi, Mashhad, Iran
[c] Soil and Water Research Department, Khorasan Razavi Agricultural and Natural Resource Reaserch and Education Center, Agricultural Research, Education and Extension Organization (AREEO) Mashhad, Iran

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ABSTRACT

The purpose of waste reduction and cleaner production is achieved by properly managing crop production including efficient fertilization and subsequent crop marketing. In recent years, the issue of colorless arils has turned to a critical damage in the pomegranate orchards of Iran resulting in the probable loss of pomegranate export market. Accordingly, finding suitable methods, which may alleviate such a disorder, is of significance. The foliar application of Fe and Zn mineral fertilization as well as chelated Fe (total of 12 treatments) on pomegranate quality along with genotype effect (two genotypes) was hypothesized. The experiment was a factorial on the basis of a completely randomized block design with three replications, conducted in Bajestan city of Khorasan-Razavi province, Iran. Fruit Fe and Zn content, anthocyanin, sugar, titratable acidity (TitA), pH and colorless arils (PCA) disorder were determined. The results showed the significant interactions of fertilization treatments on Fe and Zn contents compared with the single effects. Fertilization treatments also increased anthocyanin concentrations. Fe fertilization affected fruit taste by significantly affecting fruit sugar, TitA and pH. Some fertilization treatments significantly alleviated the disorder of colorless arils (16.17–14.17%), compared with the other treatments. G1 (higher Fe concentration) was the more tolerant genotype to PCA than G2. The results confirms the necessity of Fe and Zn (both mineral and organic) for alleviating PCA disorder and enhancing pomegranate quality using Fe and Zn mineral and chlelated fertilization, along with the proper genotype. The results are promising and can be used for the further research in this respect.

1. Introduction

Pomegranate (*Punica granatum*) is one of the most consumed fruits in different parts of the world including Iran (Jamali, and Bonyanpour, 2017); among the European countries Spain is the main producer of pomegranate (Viuda-Martos et al., 2010). The importance of pomegranate fruit is because of its many desirable traits, taste, medicinal properties, and organic production (Melgarejo-Sánchez et al., 2015; Zinatloo-Ajabshir et al., 2018). The pomegranate fruit has a major role in human nutrition and health, because it is a source of minerals such as iron (Fe), zinc (Zn), and manganese (Mn), and it also contains vitamin C, A and antioxidants such as anthocyanin. The fruit is also of industrial significance as it is used for the production of different products including the favorable pomegranate juice. It must be noted that the efficient use of pomegranate requires the proper utilization of resources such as the macro and micronutrients required for the plant growth and fruit production (Ozlekci et al., 2013; Ben-Ali et al., 2017; Houshyar et al., 2017). Siebielec et al. (2018) indicated that the use of sewage sludge can enhance the fertility of soil by increasing the population and activities of soil microbes resulting in the higher rate of microbial enzyme production.

The essential nutrients such as Fe and Zn can significantly enhance pomegranate fruit quality, which is both of economical and marketing significance, and can considerably decrease the rate of waste in the environment resulting in a cleaner production. Iron and zinc deficiency, especially under calcareous conditions, causes

* Corresponding author.
E-mail addresses: mghehsareh@yahoo.com, amohammadi@khuisf.ac.ir (A.M. Ghehsareh).

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Notations

F1–F9	fertilization treatments
G1 and G2	pomegranate genotypes
TitA	titratable acidity
PCA	percentage of colorless arils

disorders in plant metabolic processes (Sabet and Mortazaeinezhad, 2018). Iron is a part of enzymatic structure controlling electron transfer and hence the redox reactions (Shourbalal et al., 2019). In addition, Fe has an effective role in the formation of cytochromes, and the processes of photosynthesis, respiration and nitrogen fixation (Miransari, 2011). Iron is a part of the metabolic enzyme coproporphyrinogen oxidase, which is essential for the biosynthesis of alpha-linolenic acid, and is a precursor for chlorophyll (Marschner, 1982; Kumawat et al., 2006).

Exterior fruit color is an important trait used for investigating pomegranate fruit quality affecting its marketing purposes. However, the molecular mechanisms, resulting in color difference in pomegranate, for example, between the red and the white ones, have been rarely investigated (Zhao et al., 2015). The authors were able to isolate the enzymes such as anthocyanidin synthase, UDP-glucose-flavonoid 3-*O*-glucosyltransferase, dihydroflavonol 4-reductase and flavanone 3-hydoxylase, which are essential for the biosynthesis of anthocyanin from fruit peels. The authors also indicated the transcription level of the anthocyanin biosyntheses genes and found the gene (not present in the white pomegranate), which is essential for anthocyanin biosynthesis in red pomegranate. Such investigations are definitely of medical and economical significance as they determine fruit quality and pave the way for the production of more healthy fruits.

Iron is essential in anthocyanin formation because of its direct role in chlorophyll formation and photosynthesis process (Davarpanah et al., 2013). Anthocyanins, as bioactive compounds and a subgroup of flavonoids, determine pomegranate color. The concentrations of anthocyanins in fruits is a function of genotype, fruit growth stage, and climatic and agronomic practices (Mirdehghan and Rahemi, 2007; Sepúlveda et al., 2010; Chater et al., 2018). Sepúlveda et al. (2010) found that anthocyanin concentrations in pomegranate red genotypes (588–1328 mgL-1) were higher than the pink ones (197–913 mgL-1). The antioxidant activities of anthocyanins can prevent cancer (Li et al., 2006).

Zinc (Zn) is the other micronutrient, which can significantly enhance the quality and quantity of pomegranate fruit. Zinc regulates the metabolism of proteins and carbohydrates and has a role in enzymes reactions (Hasani et al., 2012). Zinc reduces the production of reactive oxygen species and their damages through participating in the metabolism and production of antioxidant enzymes. This nutrient is necessary to produce auxin from tryptophan amino acid and it can induce plant rooting (Hong and Jin, 2007; Zand et al., 2010). The application of zinc sulfate significantly affects the expression of genes in the phenolics biosynthesis pathways (Tang et al., 2015).

The edible parts of pomegranate are about 50% of the fruit including 40% arils and 10% seeds. However, the most important part of aril's juice is water (85%), followed by sugars (10%), pectin (1.5%), phenolic compounds and ascorbic acid (Viuda-Martos et al., 2010; Hmid et al., 2018).

Researchers (Mirdehghan and Rahemi, 2007; Mazza, 2018) have investigated the seasonal changes of anthocyanins and the concentrations of Fe, Zn, Mg and Ca in pomegranate. Mirdehghan and Rahemi (2007) found that the content of aril anthocyanins increased in the period of 10–50 days after bloom and then it decreased until the harvest. However, there was a sharp decrease in the aril concentrations of Fe and Zn in the period of 10–40 days after bloom and then the concentrations were almost constant until the harvest. It has also been indicated that application of 0.4% zinc sulfate and 0.4% ferrous sulfate solution with boric acid improved different properties of pomegranate orchards including canopy size, fruit weight and yield, chlorophyll *b* content, and number of arils, and it accelerated fruit ripening in the first and the second harvest (Yadav et al., 2014).

Research has also indicated that foliar and soil application of the chelated and mineral fertilization including chelated iron (Fe-EDTA) plus ferrous sulfate (FeSO4.7H2O) and zinc sulfate ($ZnSO_4.7H_2O$) significantly improved the qualitative and quantitative properties of pomegranate including the Brix index, fruit yield and diameter, and 100-seed weight (Mirzapour and Khoshgoftarmanesh, 2013). In another study application of zinc sulfate enhanced leaf photosynthesis and also the content of total dissolved solids, tannins, total amount of phenols, flavonols, flavonoids, and anthocyanins in red grapes (*Vitis vinifera* L.) (Song et al., 2015). It was also indicated that the use of chelated Fe and Zn enhanced the total amount of solids and anthocyanin in red grape under calcareous conditions (El-Razek et al., 2015).

Clean and economical production of pomegranate requires a balanced level of macro and micro nutrients including Fe and Zn, because according to the above mentioned details such nutrients affect both the quantity and quality of the fruit. The use of organic fertilization and nutrient spraying, especially for micro nutrients, is among the most usual methods of pomegranate fertilization. Such methods are not costly, are efficient and can make significant changes in the quality and quantity of pomegranate (Hamouda et al., 2016; Marathe et al., 2017). However, another interesting method, which can also be considered as a multipurpose method for both cleaning the environment from the extra amounts of heavy metals including Zn and Cu, and for fertilizing different crop plants including pomegranate, is the use of biotic and abiotic organic products (Zhou et al., 2019). For example, algae can be used for the removal of Zn from the polluted waters and it can then be used as a source of Zn fertilization (Badescu et al, 2017). The use of pomegranate pill has also been examined for the removal of Cu from the environment (Ben-Ali et al., 2017), which may also be used as a source of Cu fertilizer for pomegranate or other crop plants.

In recent years, the colorless arils of pomegranate, has changed to a critical damage in the pomegranate orchards of Iran, resulting in the higher production of waste, and likely loss of pomegranate export market. It is hence important to find suitable methods, which may result in the alleviation of such a disorder. Accordingly, it was hypothesized that the foliar application of Zn and Fe inorganic and chelated fertilizers may alleviate such a disorder. The theoretical (Whetten, 1989; Suddaby, 2014) and the practical aspects of producing pomegranate under calcareous conditions, using the efficient sources of Fe and Zn fertilization have been analysed and discussed in a way so that the production of waste may decrease and a cleaner productions is resulted. The objective of the study was to determine the qualitative and quantitative properties of pomegranate including the colorless aril disorder affected by: 1) different types of Zn and Fe fertilization (mineral and chelated), and 2) pomegranate genotypes.

2. Materials and methods

2.1. Experimental site

The study was conducted during 2015 in Bajestan, Khorasan Razavi province, Iran, in a semi-arid area, at the eastern longitude of

58°11′9.73″, northern latitude of 34°31′25.09″, and altitude of 1250 m, with the yearly rainfall of 120 mm. The physical and chemical properties of the soil for the depths of 0–50 and 50–100 cm were determined using the standard methods (Table 1) (Miransari et al., 2008). The soil samples were sieved using a 2-mm mesh, the pH and salinity (EC) of a saturated paste as well as soil texture were determined (Page, 1986). Nitrogen was measured using the Kjeldahl method (Bremner and Mulvaney, 1982). The available phosphorous was determined using the ammonium molibdate method (Olsen et al., 1954). The available potassium was measured using the ammonium acetate method by the flame photometer (Model 7JENWAY PFP), soil Fe and Zn were determined using the DTPA extraction method (Lindsay and Norvell, 1978). Organic carbon of the soil was also determined (Walkley and Black, 1934).

2.2. Experimental design

The experiment was a factorial on the basis of a completely randomized block design with three replications. The experimental treatments including genotype (Khazar and Shisheh-Cap) and different types of fertilizer were tested. The fertilizer treatments were prepared and foliarly applied (two times, 22nd of June and 22nd of July) by dissolving the followings in 1000 L water, 1) ferrous sulfate ($FeSO_4$ 21%) (Konjalesaz Co., Iran) at three levels of 0 (control) (F1), 0.5 (1.05 kg of pure iron) (F2) and 1% (2.1 kg of pure iron) (F3), 2) zinc sulfate ($ZnSO_4$ 34%) (ParsAgro Co., Iran) at three levels of 0 (control) (F4), 0.5 (1.7 kg of pure zinc) (F5) and 1% (3.4 kg of pure zinc) (F6), 3) $FeSO_4$ + $ZnSO_4$ at three levels of 0 (control) (F7), 0.5% $FeSO_4$ + 0.5% $ZnSO_4$ (F8), and 1% $FeSO_4$ + 1% $ZnSO_4$ (F9), and 4) liquid Fe chelate 6% (Fe-EDTA 6%) at three levels of 0 (F10), 0.5 (F11) and 1% (F12). Each plot consisted of three 9-year-old pomegranate trees at a distance of 3 m between rows and 2.5 m on each row (Fig. 1).

2.3. Measurements and analyses

2.3.1. Sampling

Plant (leaf) sampling was conducted twice (June and August). The fruit sampling was done randomly during November from the four sides of each tree by collecting the healthy fruits. For the measurement of anthocyanin, acidity and Brix index the juice of the fruits samples was collected using a juicer.

2.3.2. Colorless aril percentage

The percentage of fruit colorless aril was measured by counting the number of colorless arils per three fruits and calculating the mean values based on the percentage (Fig. 2).

Table 1
The physical and chemical properties of soil.

depth (cm)	0–50	50–100
pH	8	7.9
Electrical conductivity (dS/m) (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 (mg/kg)	0.5	39.8
K (mg/kg)	29.9	70.9
Zn (mg/kg)	0.5	0.3
Fe (mg/kg)	4.5	4

2.3.3. Fruit color

The sample color was measured using the Hunter lab device (model Chroma Meter CR-410, Japan). The device was first calibrated with black and white calibration background and then the sample color was determined (Horuz et al., 2012).

2.3.4. Anthocyanin

The total anthocyanin (mgL-1) of the fruit juice was calculated based on cyanidin 3-glucoside as the dominant anthocyanins of pomegranate and via using the pH difference between the two buffer systems (Giusti and Wrolstad, 2003). The wavelength absorbance of fruit juice was determined at 510 and 700 nm using two different buffer solutions (pH of 1 and 4.5). The total anthocyanin was calculated using the following formula:

$$\text{Total anthocyanin} = (A \times MW \times DF \times 100)/MA$$
$$A = (A510 - A700)\ pH1.0 - (A510\text{-}A700)\ pH4.5$$

In which MW is the molecular weight of the dominant anthocyanin, DF is the dilution factor (10), MA is the coefficient of molar absorbance of cyanidin 3-glucoside (26.900), and “A” is the absorbance difference.

2.3.5. Iron and zinc content

Iron and zinc content of the samples were measured using atomic absorption spectrometer (Varian, model SpectrAA-200).

2.3.6. Acidity

The acidity (citric acid) of fruit juice was determined using the acidity meter device (model Metrohm 691).

2.3.7. Total soluble solids (TSS, Brix index)

Total soluble solids (TSS, Brix index) content, containing mainly sugars, was measured using the refractometer measurement device. The instrument was first calibrated by distilled water, and then TSS or the Brix index was measured using a few droplet of fruit juice, placed in the scaled glosbe (using sucrose) of the instrument and after the appearance of two equal light and dark phases on the scale the concentration of TSS or the Brix index was determined.

2.3.8. Titratable acidity (TitA)

The method by Hamouda et al. (2016) was used to measure TitA. First using a 50-ml beaker 2 ml of fruit juice was treated with 50 ml distilled water and a few drops of phenolphthalein, and the solution was then titrated with NaOH 0.1 N. The acidity was determined using the following formula.

$$TitA = (0.0064 \times \text{ml NaOH } 0.1\text{ N} \times 0.64)/(\text{sample weight}).$$

2.3.9. Microscopic images

The microscopic images were captured by the microscope (model Motic SFC-28 series) (Fig. 3).

2.4. Statistical analyses

Data were subjected to the analysis of variance using SAS. Means were compared using Duncan's multiple range tests at the level of 5%.

3. Results and discussion

3.1. Analysis of variance

The analysis of variance indicated the significant effects of the experimental treatments (genotype and fertilization) and their interactions on Zn, anthocyanin, and sugar concentration as well as

Fig. 1. Different stages of the experiment.

on the percentage of the colorless arils. Although the analysis of variance did not indicate the significant effects of experimental treatments on Fe concentration, the single and interactions effects indicated the positive effects of Fe on the quality of pomegranate including the concentrations of anthocyanin, and sugar and the decreased percentage of colorless arils (Table 2).

The cleaner production of healthy pomegranate fruits (free of colorless arils) under calcareous conditions is an important research topic, which must be addressed by research. In such circumstances the rate of waste decreases resulting in a more efficient use of resources such as water and energy. If optimum conditions including the required amounts of nutrients such as Fe and Zn are presented for plant growth production, a more sustainable production of fruits may be achieved. Such ideas are of medical, environmental and economical significance. The selected methods for the production of optimum fruits must be economically explainable and agriculturally sustainable (Tampio et al., 2017; Liu et al., 2019; Siebielec et al., 2018). Accordingly, it was hypothesized that the disorder of colorless arils can be controlled by the use of Fe and Zn mineral and/or organic fertilization under calcareous conditions. The use of compost and the proper genotype of corn resulted in significantly higher amounts of yield (Ulm et al., 2019).

4. Single effects

4.1. Fe and Zn

The highest Fe concentrations were resulted by the F3

Fig. 2. The micro-macroscopic image of colorless arils of pomegranate (Shishe-cap) (control treatment, not treated).

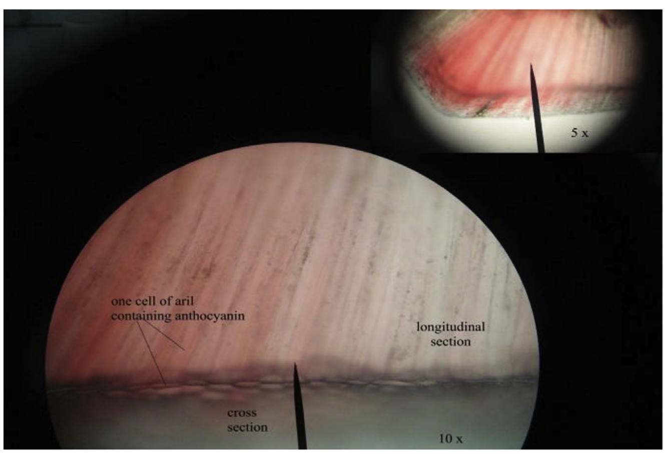

Fig. 3. The two-dimensional microscopic image of pomegranate aril (Shishe-cap) treated by the foliar application of Fe chelated1%. The arils are colored containing anthocyanin and contain more juice compared with the control treatment.

(0.08 mgL-1), F5 (0.14 mgL-1) and F6 (0.13 mgL-1) treatments, not significantly different from the other treatments. Interestingly, Zn treatment at 1% (F6) resulted in the highest concentration of Fe. There was not also any significant difference between the two genotypes in terms of Fe concentration. The treatments including F1 (0.81), F2 (0.82), F4 (0.81), F6 (0.79), F7 (0.81), F9 (0.83) and F10 (0.81 mgL-1) resulted in significantly higher Zn concentrations, compared with the other treatments. Although not significantly different from the other treatments, F9 (Fe + Zn at 1%) resulted in the highest concentration of Zn. The concentration of Zn in G2 (0.78 mgL-1) was significantly higher than that of G1 (0.73 mg/l) (Table 3).

Means followed by the same letters in each column are not significant (Duncan's multiple rang test 5%). G1: Khazar, G2: Shisheh-cap, F1 (mgL-1): Ferrous sulfate 0, F2: Ferrous sulfate 0.5, F3: Ferrous sulfate 1, F4: Zinc sulfate 0, F5: Zinc sulfate 0.5, F6: Zinc sulfate 1, F7: Ferrous sulfate + Zinc sulfate 0, F8: Ferrous sulfate + Zinc sulfate 0.5, F9: Ferrous sulfate + Zinc sulfate 1, F10: Liquid iron chelate 0, F11: Liquid iron chelate 0.5, F12: Liquid iron chelate 1.

The physiological disorder of colorless arils is an important issue in the pomegranate orchards of Iran affecting the quantity and quality of the fruit, and its exporting market. Accordingly, this research work was conducted to find a suitable method, which may result in the alleviation of such disorder. In this research it was determined how mineral Fe and Zn fertilization as well as chelated Fe (organic fertilization) may affect pomegranate Fe and Zn content, and its qualitative properties including anthocyanin, sugar, TitA, pH and PCA.

The results indicated that the two genotypes were not significantly different in terms of Fe concentration. Accordingly, if the increased concentration of Fe in pomegranate fruit is favorable, higher concentrations of Fe fertilization or other methods of fertilization or other genotypes may be tested and used. Fe is immobilized in the plant due to iron deposition in cells as an insoluble oxide or phosphate. Moreover, Fe combines with phytoferritin (the protein in the leaf), and this complex limits Fe mobilization into phloems, and hence the other parts of the plant (Marschner, 1982).

Fertilization negatively affected fruit Zn concentration, and G2 contained significantly higher concentration of Zn than that of G1. This may indicate that the type of fertilization, which tested in this research, may contribute to the organic structures of Zn in plant rather than its single presence. Different types of fertilization significantly affected the anthocyanin concentration of pomegranate juice, in the following order: chelated Fe > Zn fertilization > Fe fertilization = Fe + Zn fertilization. Hamouda et al. (2016) also found that foliar application of Fe and Zn fertilizers at 0.5, 1 and 2%, using ferrous Fe and Zn, significantly increased anthocyanin content of pomegranate juice.

The positive effects of Fe application on the concentrations of anthocyanin of other fruits have been also indicated by research work. For example, Shi et al. (2017) indicated that Fe treatment significantly affected the presence of anthocyanins and the expression of the related genes in *Vitis vinifera*. El-Razek et al. (2015) also indicated that the foliar application of Fe 1% and Zn 1.5% increased anthocyanin content in red grape. Researchers also found that Fe and Zn fertilizer could increase fruit juice

Table 2
Analysis of variance indicating the effects of genotype and fertilization treatments on the pomegranate juice traits.

S.V.	d.f.	M.S.						
		Fruit juice content						PCA
		Fe	Zn	Anthocyanin	Sugar	TA	pH	
Block	2	0.005 ns	0.008[a]	2268.978ns	3.783[b]	0.024ns	0.259[b]	49.231 ns
Genotype	1	0.007 ns	0.176[b]	37515.128[b]	5.787[b]	0.019ns	0.045ns	1459.343[a]
Treatment	11	0.007 ns	0.045[b]	10843.842[b]	2.556[b]	0.115[b]	0.112ns	3539.597[b]
T*G	11	0.008ns	0.038[b]	10573.697[b]	3.627[b]	0.064[b]	0.044[a]	1326.735[b]
Error	7	0.004	0.003	906.328	0.139	0.002	0.012	36.231
CV%		131.6	6.3	19.6	3.2	6.4	3.1	15.4

[a] Significant at 5%.
[b] Significant at 1%; ns: non-significant.

Table 3
Mean comparison of the pomegranate juice traits affected by the single effects of genotype and fertilization treatments.

	Fe (mgL-1)	Zn (mgL-1)	Anthocyanin (mg/L)	Sugar (%)	TitA (%)	pH	PCA (%)
Genotype							
G1	0.07 ± 0.01a	0.73 ± 0.13b	111.51 ± 65.46b	13.00 ± 1.23b	0.86 ± 0.17a	3.64 ± 0.13a	34.44 ± 26.80b
G2	0.05 ± 0.04a	0.78 ± 0.89a	161.92 ± 58.11a	13.58 ± 0.66a	0.84 ± 0.21b	3.68 ± 0.15a	37.58 ± 23.76a
Fertilization							
F1	0.04 ± 0.01	0.81 ± 0.08a	111.50 ± 66.39ef	13.18 ± 0.80cd	0.80 ± 0.08c	3.71 ± 0.12 ab	55.50 ± 4.18b
F2	0.03 ± 0.02	0.82 ± 0.03a	126.54 ± 41.41de	14.03 ± 0.62b	1.09 ± 0.31a	3.68 ± 0.24abc	16.17 ± 2.64e
F3	0.08 ± 0.03	0.66 ± 0.05cd	100.70 ± 62.10ef	12.13 ± 1.52f	1.09 ± 0.07a	3.75 ± 0.09a	30.50 ± 22.84d
F4	0.04 ± 0.01	0.81 ± 0.08a	111.50 ± 66.39ef	13.18 ± 0.80cd	0.80 ± 0.08c	3.71 ± 0.12 ab	55.50 ± 4.18b
F5	0.14 ± 0.05	0.64 ± 0.01d	147.20 ± 8.13cd	13.15 ± 1.01cd	0.68 ± 0.05d	3.58 ± 0.13bc	43.33 ± 42.01c
F6	0.13 ± 0.28	0.79 ± 0.02a	191.41 ± 21.42b	12.75 ± 0.38de	0.98 ± 0.10b	3.650.11abc	10.00 ± 6.23e
F7	0.04 ± 0.01	0.81 ± 0.08a	111.50 ± 66.39ef	13.18 ± 0.80cd	0.80 ± 0.08c	3.71 ± 0.12 ab	55.50 ± 4.18b
F8	0.03 ± 0.02	0.63 ± 0.16d	86.73 ± 72.32f	13.88 ± 0.67b	0.98 ± 0.25b	3.58 ± 0.13bc	15.67 ± 9.99e
F9	0.04 ± 0.03	0.83 ± 0.16a	136.22 ± 65.66cde	14.75 ± 0.64a	0.67 ± 0.04d	3.63 ± 0.16abc	14.17 ± 5..08e
F10	0.04 ± 0.01	0.81 ± 0.08a	111.50 ± 66.39ef	13.18 ± 0.80cd	0.80 ± 0.08c	3.71 ± 0.12 ab	55.50 ± 4.18b
F11	0.06 ± 0.01	0.72 ± 0.17bc	161.83 ± 57.98bc	12.48 ± 0.92ef	0.72 ± 0.11d	3.68 ± 0.06abc	66.67 ± 21.11a
F12	0.06 ± 0.02	0.73 ± 0.09b	243.95 ± 5.37a	13.58 ± 0.26bc	0.83 ± 0.09c	3.54 ± 0.21c	13.67 ± 6.09e

anthocyanin content through the direct enhancement of photosynthesis and providing sugars essential for anthocyanin production (Davarpanah et al., 2013; Kiani et al., 2014). Our results also indicated there is a direct relation between fruit juice anthocyanin and sugar as the use of Fe and Zn fertilization significantly increased both factors. Accordingly, this may contribute to a healthier and sweater fruit for the use of consumers and hence it can be of more exportable quality. The increased concentrations of anthocyanins by Zn may be attributed to the improved activities of carbohydrate metabolic enzymes (Zhang et al., 2016).

The red color (not present in the colorless arils) is due to the presence of anthocyanins in the arils (Zhao et al., 2015). According to the results of this study the physiological disorder of colorless arils is a result of Fe and Zn deficiency under calcareous conditions. However, the use of mineral Fe and Zn as well as chelated Fe improved such a disorder by significantly affecting the fruit quality traits, such as anthocyanin, sugar concentrations, titratable acidity and pH. Mirzapour and Khoshgoftarmanesh (2013) found that the use of Fe and Zn mineral fertilization can improve the quantity and quality of pomegranate fruits under calcareous conditions.

4.2. Anthocyanin and sugar

Although significantly increased by all fertilization treatments, the highest concentration of anthocyanin was resulted by F12 (243.95), followed by the F6 (191.41) and F11 (161.83 mgL-1) treatments. This indicates that the fertilization treatments, tested in this research, can considerably increase pomegranate anthocyanin, contributing to the production of healthier fruits. For example, anthocyanins are considered as an important source of antioxidants, which are essential for people health. Genotype G2 (161.92 mgL-1) contained significantly higher anthocyanin, compared with G1 (111.51 mgL-1). Genotype G2 may have more genes, essential for the production of anthocyanins, however this requires further investigation. Regardless of genotype, application of fertilizer treatments significantly affected anthocyanin content of fruit juice ($P < 0.01$), and the foliar application of F12 was the most effective treatment as it increased the anthocyanin concentration by 119% compared to the control treatment (Table 3). It can be interpreted that because anthocyanins are organic molecules, the use of organic fertilization (Fe-EDTA) may be more effective on the increased concentrations of fruit anthocyanins, compared with mineral fertilization. Treatment F9, (14.75%), F2 (14.03) and F8 (13.88) had the highest concentration of sugar, significantly higher than the other treatments. This indicates that the presence of both Zn and Fe are essential for the production of sugar in pomegranate, with respect to the functions of the two nutrients in plant. Genotype G2 (13.58%) resulted in significantly higher sugar than G1 (13.00%) (Table 3). Accordingly and as also previously mentioned, genotype G2 may be a more suitable genotype than genotype G1, in terms of some of the biochemical properties including Zn, anthocyanin and sugar.

4.3. TitA and pH

Treatments F2 (1.09), F3 (1.09), F6 (0.98) and F8 (0.98%) resulted in significantly higher TitA, than the other treatments indicating that the presence of the two nutrients in plant can significantly affect fruit quality in terms of health by increasing the concentration of some organic acids such as ascorbic acid. Genotype G1 (0.86%) resulted in significantly higher TitA than G2 (0.84%). The higher pH values were resulted by treatments F3 (3.75), F1 (3.71), F4 (3.71), F7 (3.71) and F10 (3.71) significantly different from the other treatments. There were no significant differences between the two genotypes in terms of pH (Table 3). The increased concentration of TitA by Fe and Zn fertilization are similar to the results by other researchers (Mirzapour and Khoshgoftarmanesh, 2013; El-Shewy and Abdel-Khalek, 2014; Ghayekhloo and Sedaghatpoor, 2015). Hamouda et al. (2016) found that Fe and Zn fertilization enhances juice TitA by increasing the juice ascorbic and citric acid. Such effects can also accordingly affect the juice pH.

4.4. PCA

The most effective treatments on the alleviation of colorless arils were F2 (16.17), F6 (10), F8 (15.67), and F9 (14.17%) with significant differences from the other treatments. Genotype G1 was significantly more tolerant to the disorder of colorless arils compared with genotype G2 (Table 3). It concludes both Zn and Fe are essential for the increased tolerance of pomegranate to colorless arils, which according to the presented results is a function of pomegranate biochemical properties including anthocyanin, sugar, and titratable acidity.

5. Interaction effects

5.1. Fe and Zn

Treatments G1F5 (0.17), G1F6 (0.27) and G2F5 (0.11 mgL-1) resulted in the highest concentrations of juice Fe indicating that the G1 genotype was the more efficient genotype in terms of having Fe by fertilization. However, G2 genotype was the more effective treatment on Zn concentration compared with G1 as G2F2, G2F9, and G2F11 resulted in the highest Zn concentrations (Table 4). Accordingly, it may be interpreted that if the proper combination of genotype and fertilization is used, pomegranate fruits may contain the optimum concentration of Fe and Zn, and hence with a higher quality for health and export.

5.2. Anthocyanin and sugar

G1F2, G1F6, G1F12, G2F6, G2F9, G2F11 and G2F12 treatments resulted in the highest concentrations of anthocyanin compared with the other treatments. However, according to the results, the effects of fertilization on anthocyanin concentration were more evident in G2 than G1. The fruits of G1F8, G1F9, G2F2 and G2F9 treatments contained the highest percentage of sugar, compared with the other treatments (Table 4).

The sugar content of fruit juice is the product of photosynthesis, and Fe is an effective nutrient for chlorophyll formation. Accordingly, under Fe deficient conditions, the chlorophyll content, and photosynthesis process is negatively affected (Marschner, 1982) decreasing fruit quantity and quality. Researchers found that supplying Zn lead to photosynthesis enhancement via increasing the chlorophyll *a* and *b* contents, and LAI, and hence the subsequent increases of total sugar in plant and fruit juice (Hasani et al., 2012; Davarpanah et al., 2013).

5.3. TitA and pH

The highest percentage of TitA was resulted by G1F3, G1F8, G2F2 and G2F6. The pH's were in the range of 3.40–3.75, and treatments G1F12 and G2F8 resulted in the least pH values and treatments G1F3, G1F11, G2F1, G2F3, G2F4, G2F6, G2F6 and G2F10 resulted in the highest pH values (Table 4) indicating that the trend of the treatments tested in this research was more toward the higher pomegranate values.

5.4. PCA

The least percentage of PCA was resulted by treatments G1F5, G1F6, G1F8, and G2F3; however, treatments G1F11, G2F4, and G2F7 resulted in the highest percentage of PCA (Table 4). This indicates G1 is the more tolerant genotype to the PCA disorder, and the mineral fertilization may be more effective on the alleviation of PCA disorder than organic fertilization (FE-EDTA).

In brief, the single effects of Fe fertilization and its combined effects with Zn fertilization resulted in significantly higher percentage of sugar compared with the other treatments. G2 also contained significantly higher percentage of sugar than G1. Fe, Zn and their combined fertilization resulted in significantly higher TitA than the other treatments. G1 also contained higher TitA than G2. Fe fertilization affected fruit taste by significantly affecting fruit sugar, TitA and pH. The single and the combined effects of Fe and Zn fertilization significantly alleviated the physiological disorder of colorless arils.

6. Conclusion

There is little data, to our knowledge, about the physiological disorder of colorless arils in pomegranate. Such a disorder can considerably affect the fruit quality, and hence its exporting market. The results of this study indicated that the use of Zn and Fe (mineral and chelated) spraying is a useful and applicable method, which can both provide the plant with its essential Zn and Fe, and significantly improve pomegranate quantity and quality. It can considerably improve the colorless aril disorder and its other marketing traits including the taste and the appearance. Compared with the increased rate of pomegranate yield, Fe and Zn spraying is not costly, it is quick and efficient and does not result in the contamination of the environment. Such results are of medical, environmental, and economical significance, and decrease the production of waste resulting in a cleaner production of pomegranate under calcareous conditions. The proper use of Fe and Zn fertilization for the removal of colorless arils in pomegranate orchards, tested in this research, can enhance the efficiency of resources such as water and energy required for a more sustainable production of pomegranate. It has been tried to relate the theoretical aspects of producing healthy pomegranate orchards (free of colorless arils) with the practical aspects so that the obtained

Table 4
Mean comparison of the interaction effects of genotype and fertilizer on pomegranate traits. 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.

Trt	Fe (mgL-1)	Zn (mgL-1)	Anthocyanin (mgL-1)	Sugar (%)	TitA (%)	pH	PCA (%)
G1F1	0.04 ± 0.01	0.83 ± 0.12	56.53 ± 42.92	12.57 ± 0.31	0.85 ± 0.07	3.67 ± 0.14	54.33 ± 5.86
G1F2	0.05 ± 0.01	0.79 ± 0.00	150.48 ± 50.65	13.57 ± 0.47	0.81 ± 0.05	3.74 ± 0.05	18.00 ± 2.65
G1F3	0.06 ± 0.01	0.62 ± 0.00	44.19 ± 3.90	10.80 ± 0.60	1.15 ± 0.03	3.75 ± 0.09	51.33 ± 1.15
G1F4	0.04 ± 0.01	0.83 ± 0.12	56.53 ± 42.92	12.57 ± 0.31	0.85 ± 0.07	3.67 ± 0.14	54.33 ± 5.86
G1F5	0.17 ± 0.02	0.64 ± 0.00	147.74 ± 2.53	13.53 ± 0.36	0.67 ± 0.03	3.58 ± 0.09	6.33 ± 4.93
G1F6	0.27 ± 0.38	0.80 ± 0.00	172.24 ± 6.36	12.93 ± 0.42	0.89 ± 0.04	3.57 ± 0.06	6.33 ± 4.93
G1F7	0.04 ± 0.01	0.83 ± 0.12	56.53 ± 42.92	12.57 ± 0.31	0.85 ± 0.07	3.67 ± 0.14	54.33 ± 5.86
G1F8	0.02 ± 0.01	0.48 ± 0.02	152.74 ± 2.27	14.47 ± 0.31	1.21 ± 0.05	3.70 ± 0.02	7.33 ± 5.03
G1F9	0.01 ± 0.001	0.68 ± 0.01	96.11 ± 52.70	15.23 ± 0.38	0.68 ± 0.05	3.56 ± 0.14	11.00 ± 3.00
G1F10	0.04 ± 0.01	0.83 ± 0.12	56.53 ± 42.92	12.57 ± 0.31	0.85 ± 0.07	3.67 ± 0.14	54.33 ± 5.86
G1F11	0.06 ± 0.01	0.56 ± 0.02	109.20 ± 8.74	11.73 ± 0.39	0.62 ± 0.05	3.72 ± 0.05	85.33 ± 8.08
G1F12	0.07 ± 0.01	0.81 ± 0.01	239.33 ± 0.17	13.53 ± 0.25	0.90 ± 0.02	3.40 ± 0.17	10.33 ± 3.21
G2F1	0.03 ± 0.01	0.78 ± 0.03	166.48 ± 10.50	13.80 ± 0.60	0.76 ± 0.07	3.75 ± 0.11	56.67 ± 2.31
G2F2	0.02 ± 0.001	0.84 ± 0.06	102.61 ± 1.77	14.50 ± 0.30	1.37 ± 0.04	3.62 ± 0.36	14.33 ± 0.58
G2F3	0.11 ± 0.01	0.70 ± 0.01	157.20 ± 18.09	13.47 ± 0.35	1.03 ± 0.05	3.74 ± 0.12	9.67 ± 0.58
G2F4	0.03 ± 0.01	0.78 ± 0.03	166.48 ± 10.50	13.800.60	0.76 ± 0.07	3.75 ± 0.12	80.33 ± 16.74
G2F5	0.11 ± 0.05	0.65 ± 0.01	146.61 ± 12.56	12.77 ± 0.55	0.69 ± .07	3.59 ± 0.18	13.67 ± 5.68
G2F6	0.00 ± 0.00	0.77 ± 0.01	210.59 ± 1.98	12.57 ± 0.31	1.07 ± 0.05	3.73 ± 0.07	56.67 ± 2.31
G2F7	0.03 ± 0.01	0.78 ± 0.03	166.48 ± 10.50	13.80 ± 0.60	0.75 ± 0.07	3.75 ± 0.11	80.33 ± 16.74
G2F8	0.05 ± 0.01	0.77 ± 0.00	20.73 ± 0.91	13.30 ± 0.10	0.75 ± 0.05	3.47 ± 0.05	13.67 ± 5.69
G2F9	0.07 ± 0.01	0.97 ± 0.01	176.34 ± 56.33	14.27 ± 0.42	0.65 ± 0.04	3.69 ± 0.17	56.67 ± 2.31
G2F10	0.03 ± 0.01	0.78 ± 0.03	166.48 ± 10.50	13.80 ± 0.60	0.75 ± 0.07	3.75 ± 0.11	24.00 ± 4.00
G2F11	0.05 ± 0.01	0.87 ± 0.02	214.46 ± 4.39	13.23 ± 0.25	0.81 ± 0.05	3.64 ± 0.06	48.00 ± 2.00
G2F12	0.04 ± 0.01	0.65 ± 0.02	248.57 ± 2.84	13.63 ± 0.32	0.74 ± 0.056	3.67 ± 0.17	17.00 ± 7.00

results can be a buildup for the future research. The production of more quality pomegranate orchards can enlarge their exporting markets, which is both useful for the gardeners and the country.

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