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Potential of sustained deficit irrigation to enhance biological and nutritional quality of pomegranate fruit during storage

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Abstract

Background While water availability is important for quality at harvest, it also continues to influence the quality of pomegranates during storage. Reducing the amount of irrigation, in addition to water saving has different effects on bioactive compounds of pomegranate during storage time. This study was conducted to determine the influence of irrigation level on fruit quality changes during storage period of two commercial Iranian pomegranate cultivars ('Shishecap' and 'Malas-Yazdi'). Sustained deficit irrigation (SDI) was applied to plants that received 75% (moderate stress) or 50% (severe stress) of their normal water requirement. A control group received 100% of their water requirement.

Results At harvest time and during storage period, fruit weight loss and some biochemical traits such as fruit total soluble solids (TSS), titratable acidity (TA), pH, total phenolic compounds (TPC), total anthocyanins content (TAC), antioxidant activity and vitamin C were measured in pomegranate fruits. Also, the quantity of the produced product was also measured at the time of harvesting. Results indicated that control fruits exhibited more weight loss than those produced under water deficit during the storage period in both years. According to results, fruit TSS, TAC, and antioxidant activity significantly increased during storage period but fruit TA and vitamin C significantly decreased throughout storage period. Also, reduction in irrigation level resulted in a decline in the yield.

Conclusions This study revealed a crucial link between irrigation level and the quality of pomegranate fruits, despite a reduction in the yield. This included affecting weight loss and the content of bioactive compounds, both at harvest and during storage.

Keywords *Punica granatum* L., Antioxidant activity, Phenolic compounds, Weight loss, Storage period, Vitamin C

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Introduction

Pomegranate (*Punica granatum* L.) belongs to the Lythraceae family is one the important tropical and subtropical climates fruit crops [1]. pomegranate is popular fruit tree in the world especially for poor soils regions due to its ability to grow up in these areas without a significant reduction in the amount of its yield [2]. Researchers propose that pomegranates originally come from the area between Iran and the Himalayas in northern India [3–5]. Pomegranate orchards are commercially grown in various regions around the world, including Iran, India, Mediterranean countries, Southeast Asia, parts of Africa, the United States, and even some areas of China, Japan, and Russia [6]. Iran, with an annual production of 1,009,000 tons of pomegranates from near 70,000 ha of own orchards is one of the biggest producers of pomegranate in the world [7, 8]. 'Shishecap' and 'Malas-Yazdi' are two of the main important commercial pomegranate cultivars in Iran. Despite growing in the arid regions of the country, growers face the challenge of limited water resources [9].

It has been reported that, pomegranate arils have high content of organic acids, sugars, polysaccharides, vitamins, and essential minerals [10]. Recently, the benefits of pomegranate fruit for human health, due to their abundance of antioxidant properties and its effect on degenerative diseases, have also been reported [11].

It has been reported that water scarcity has the greatest impact on agricultural products and their distribution in the world [12]. Pomegranate tree can tolerate drought stress through drought tolerance mechanisms such as avoidance and also high leaf relative apoplastic water content that they are the xeromorphic plant common characteristics [2]. Water management and water-saving strategy with a minimum influence on fruit quality and yield is very important in water scarcity condition. regulate deficit irrigation (RDI) and partial root-zone drying (PRD) are of these new techniques that expanded in arid and semi-arid areas [5]. Many factors including climate, soil condition, nutritional status and amount of fruit tree load and also interaction between water availability and mentioned factors can affect the fruit tree response to water deficit [13]. Considering the climatic conditions of Iran and as well as the pomegranate characteristics as a drought tolerant plant, it seems that deficit irrigation techniques can be used successfully for this plant.

Postharvest life of crops is influenced by their quality at harvest time that, numerous factors including harvest maturity, cultivar, growth climate, amount of light, tree age, flowering time, rootstock, chemical nutrients treatments and soil and water status are involved in this relationship [14]. It has been reported that, some physical and chemical processes occur in fruits and vegetables during storage period. Water loss of fruits and vegetables during storage time can influence on physicochemical quality that it is depend on the relative humidity status and temperature [15]. Water relations is very important for fruit quality at harvest time and in consequence for crops storability. It has been reported that, generally crops with higher water content have poorer storage ability than the other ones [14]. But optimum watering during the season can leads to production of fruit with good quality and marketable size. On the other hand, increasing the amounts of water during the growth season may have the negative effect on fruit quality because excessive watering leads to enhancement of vegetative growth and reduces the fruit productivity and quality [16].

As previously discussed, the reduction of irrigation can significantly impact the quality of fruits by altering some characteristics. By investigating the effect of decreased irrigation on pomegranate fruit quality at harvest and during storage, we can enhance its qualitative attributes, amplify its medicinal properties and optimize irrigation methods to grow pomegranate plants under water-deficient conditions. This research explored the impact of sustained deficit irrigation applied for two growing seasons on the weight loss and key quality parameters (TSS, TA, pH, TPC, TAC, antioxidant activity, vitamin C) of two Iranian pomegranate cultivars, assessed at harvest and during cold storage.

Materials and methods

Experimental plot conditions, plant material and irrigation treatments

The study took place over two consecutive years in Yazd province, Iran, on two commercially grown pomegranate cultivars: 'Shishecap' and 'Malas-Yazdi'. The experiment used eight-year-old pomegranate trees planted in sandy loam soil within the Yazd pomegranate collection. The research site is located at an altitude of 1230 m above sea level and experienced no rainfall during the two growing seasons (March to October). Trees were planted with a spacing of 4 m × 3 m and received the same fertilization and pest control throughout the season. The physicochemical properties of the orchard's soil and irrigation water are shown in Table 1. A randomized complete block design was employed with three replicates per treatment and two trees per replicate. Then, the fruits of each tree (including replication and observation in each replication) were harvested separately and transported to the laboratory to measure characteristics. Three irrigation treatments were applied including full irrigation (control, 100% of crop water requirement), moderate deficit (75% of crop water requirement) and severe deficit (50% of crop water requirement). Water requirement calculated as follow [17]:

Table 1 Physico-chemical properties of the soil and irrigation water used in the experiment

Soil properties											
Soil depth (cm)	**Texture**	**ECe (dS m^{-1})**	**pH**	**$CaCO_3$%**	**OC %**	**P**	**K**	**Cu**	**Mn**	**Fe**	**Zn (mg kg^{-1} soil)**
0–30	SL	3.85	7.9	23.2	0.19	9.8	110	0.34	1.8	4.2	0.64
30–60	SL	4.9	7.8	22.6	0.09	11.3	150	0.86	3.4	5.8	0.7
60–90	SL	6.18	7.8	21.7	0.17	11.7	165	0.87	3.8	5.8	0.76
Irrigation water properties											
HCO^-_3 (meq L^{-1})	**pH**	**EC (dS m^{-1})**	**Cl (meq L^{-1})**	**SO_4^{-2} (meq L^{-1})**		**Ca^{2+} (meq L^{-1})**		**Mg^{2+} (meq L^{-1})**		**Na^+ (meq L^{-1})**	
2.7	7.35	3.99	24.5	13.9		13.3		10.3		17.5	

OC = Organic carbon, SL = Sandy loam, ECe = Saturated soil paste electrical conductivity, EC = Electrical conductivity

$$I_n = \frac{(\theta_{Fc} - \theta_i) \times D}{100}$$

Where:

I_n= Net irrigation depth (m), θ_{Fc}: Field capacity soil moisture (volumetric percentage), θ_i: pre-irrigation soil moisture (volumetric percentage) and D: effective root depth (m).

$$V_n = I_n \times A \times 1000$$

Where:

Net irrigation volume (litter/tree) and A wetted area (m^2/tree).

$$V_g = \frac{V_n \times T}{100 \times Ea}$$

Where:

V_g: Gross water requirement (litter/tree), Ea: application water efficiency (%) and T: water treatment (%).

Fruit

Pomegranates were harvested by hand when commercially mature and immediately taken to the lab for analysis. To harvest pomegranate fruits, the maturity index was determined using the following factors: total soluble solids (TSS) and brix-acid (TSS/TA) ratio, seasonal calendar in experiment site (mid-October) and fruit size and color. Initial measurements included: trees yield, fruit weight, sweetness (TSS), acidity (TA), maturity index, pH, vitamin C content, total phenolics, total anthocyanins, and antioxidant activity. Fruits were then stored at 5 °C with 90% humidity for 90 days. All measurements were repeated every 30 days. Five fruits per replicate were stored separately to track weight loss over time.

Weight loss

To assess weight loss during storage, the weight of pomegranates was recorded every 30 days for three months, while they were stored at 5 °C and 90% relative humidity. The weight loss for each pomegranate was calculated as a percentage of its initial weight, representing the cumulative weight loss over the storage period [18].

TSS, TA and pH

The TSS measurements were performed using a refractometer (ATC1, ATAGO, Japan) by placing 1–2 drops of clear pomegranate juice on the refractometer prism at room temperature that, was calibrated by distilled water and finally the results were reported as °Brix. Pomegranate juice TA was determined by titration to pH 8.1 with 0.1 mol L^{-1} NaOH solution and expressing the results as g of citric acid per 100 g of juice [19]. The fruit maturity index was determined by dividing the TSS content into TA (TSS/TA ratio). The pH in pomegranate juice samples were measured with a digital pH meter (Metrohm 601, Switzerland) at room temperature.

Vitamin C

The amount of vitamin C in the pomegranates was determined using 2,6-dichlorophenol indophenol method [20]. Briefly, 100 µL of pomegranate juice extract was first added to 10 mL of 2% metaphosphoric acid solution and then vortexed for 30 s. Subsequently, 1 mL of this mixture was transferred and combined with 9 mL of indophenol solution. This mixture was also vortexed for 30 s. After the final mixing, the light absorption of the solution was measured at a specific wavelength of 515 nm, and this value was used to calculate the vitamin C content, which was reported in milligrams per 100 g of juice.

Total phenolic content

The Folin-Ciocalteu method, with some modifications [18], was used to determine the total amount of phenolic compounds present in the pomegranates using a spectrophotometer. In short, a diluted pomegranate juice sample (900 µL) was added to a test tube containing specific volumes of sodium carbonate solution (900 µL) and Folin-Ciocalteu reagent (180 µL). After shaking, the mixture was incubated in the dark for 90 min at room temperature. The absorbance of the solution was then measured

at a wavelength of 760 nm using a UV-visible spectrophotometer (Bio Tek VT 05404−0998, USA). Finally, the results were expressed as milligrams of gallic acid equivalent per 100 g of fresh pomegranate weight (mg GAE/100 g FW).

Total anthocyanin content

The anthocyanin content was determined using the pH differential method [21]. This method involves measuring the absorbance of the extract at two different pH levels. In brief, pomegranate extract was mixed with two buffer solutions, one with a low pH (potassium chloride, pH 1 and 0.025 M) and another with a higher pH (sodium acetate, pH 4.5 and 0.4 M). The absorbance of the mixture was then measured at two specific wavelengths (510 nm and 700 nm) using a visible spectrophotometer, compared to a blank sample. Finally, a specific formula was applied to calculate the anthocyanin content, which was expressed as milligrams of cyanidin-3-glucoside (Cy-3-gluc) per 100 mL of pomegranate juice (mg Cy-3-gluc/100 mL). A = (A 510 −A 700) pH 1.0 − (A510 −A 700) pH 4.5

$$Total\ anthocyanin = \frac{A \times\ MW \times\ DF \times\ 1000}{MA\ \times\ 1}$$

Where A is the sample absorbance, MW is the molecular weight of anthocyanin (449.2), DF is dilution factor, and MA is the cyanidin −3- glucoside molar absorptive coefficient (26,900).

Antioxidant activity

Antioxidant activity was assessed using 2, 2-diphenyl1 picryl hydrazyl hydrate (DPPH) [22]. Briefly, 1 mL of pomegranate juice extract was mixed with 1 mL of Tris buffer solution and 1 mL of DPPH solution. The mixture was then incubated in the dark for 30 min at room temperature. The light absorption of the solution was measured at a wavelength of 517 nm using a UV-visible spectrophotometer. An ethanol solution served as a blank reference, and a separate control mixture without the juice extract was also measured. Finally, the antioxidant activity was calculated as a percentage using the formula provided, which compares the light absorption of the sample with the control mixture:

Antioxidant activity (%) = [1− (A Sample / A Control)] ×100.

Statistical analysis

The software SAS version 9.1 was used to analyse the collected data. Analysis of Variance (ANOVA) was done to identify any significant differences between the groups being compared. If the ANOVA test revealed statistically significant differences (p-value less than or equal to 0.05), Duncan's multiple range test (also with a significance level of p-value less than or equal to 0.05) was used to further pinpoint which specific groups differed from each other. Factorial experiment splited in time was applied for measuring traits. Since the data were measured in two successive years, combined analysis of variance was used for analysing the data.

Results and discussion

Fruit weight loss

Analysis of the data from both years showed that fruit weight loss varied significantly between the two years (Table 2). This led the researchers to present the results for each year separately (Table 3). There was a significant interaction between storage time and both the year and the irrigation level (IRL) (Table 2). In both pomegranate cultivars and in both years, fruit weight loss increased with longer storage time at 5°C, regardless of the irrigation treatment. However, weight loss was lower in fruits produced under SDI condition. For example, in the 'Shishecap' cultivar, initial storage weight losses after 30 days were 5.36% (control), 4.20% (moderate stress), and 4.38% (severe stress). Similar trends were observed for 'Malas-Yazdi' in both years. These results align with prior findings by Pena et al. [23] on pomegranate fruit. Dehydration of fruit increases during storage and it is stated that, water exchange between fruit internal and external atmosphere and also amount of transpiration that increase via cellular breakdown are the two main reasons for fruit weight loss during storage time [24]. Pena et al. [23] reported that lower weight losses in SDI fruit throughout storage period is related to the partly thicker cuticle and closed crown area than the control fruit.

Total soluble solids

Table 2 shows the influence of various factors on TSS of pomegranates, including the year of the study, cultivar type, irrigation levels, storage duration, and their interactions. The combined statistical analysis revealed that the year the pomegranates were grown did not significantly affect their sweetness (p-value greater than 0.05). Consequently, the data from both years was combined for further analysis. (Table 4). In both cultivars, fruits TSS was significantly affected by applying severe water deficit whereas the moderate water deficit had no significant influence on this parameter in comparison with control treatment (Table 4). At harvest, control fruit had a lower TSS than stressed samples, although there was no a significant difference between control and moderate stress in both cultivars (Table 4). Similar observations have also been reported in other pomegranate cultivars that it is because of abiotic stress related to the irrigation strategy, as a similar trend was reported in previous research for similar condition [23, 25]. Also, water stress can increase

Table 2 The analysis of variance for the study year, storage stage, cultivar, irrigation level and their interaction effects on measured parameters (comparison of the means based on Duncan's multiple range test, $P \leq 0.05$)

Source of variance	DF	Weight loss	TSS	TA	pH	Phenolic compound	Anthocyanin	Antioxidant activity	Vitamin C
R	2	0.11ns	2.46***	0.002ns	0.04ns	1691.50ns	13.91***	16.39ns	1.03ns
Year	1	6.23**	1.42***	1.35***	0.76***	685.30ns	0.21ns	84.97*	321.51***
Error I	2	0.57	0.73	0.02	0.01	1918.66	2.61	273.10	2.85
CUL	2	16.68***	16.40***	4.15***	0.09ns	543646.44***	433.07***	694.84***	448.34***
Year × CUL	2	0.54ns	1.46ns	0.09ns	0.01ns	246.47ns	2.07ns	15.27ns	70.48**
IRL	1	0.51ns	1.38ns	0.35ns	0.39**	6417.08ns	229.80***	2.16ns	85.88**
Year × IRL	1	1.52ns	2.29ns	0.01ns	0.01ns	14481.30*	1.04ns	9.12ns	0.21ns
CUL × IRL	2	0.12ns	0.098ns	0.79**	0.09ns	209209.72***	54.30**	21.15ns	33.44*
Year × CUL × IRL	2	0.07ns	0.01ns	0.25ns	0.05ns	2854.24ns	2.53ns	3.89ns	0.28ns
Error II	10	0.81	0.97	0.1	0.02	2014.39	6.42	30.78	6.84ns
STS	3	1471.47***	9.99***	1.15***	1.99***	134565.20***	15.73***	144.41***	800.90***
CUL × STS	6	2.66**	0.07ns	0.42***	0.14***	9084.04***	1.11ns	6.06ns	67.76***
Year × STS	3	4.22***	0.04ns	0.49***	0.04*	2909.67ns	0.58ns	7.92ns	71.64***
STS × R	6	0.33ns	0.02ns	0.11ns	0.01ns	1121.90ns	1.11ns	8.68ns	10.85ns
Year × CUL × STS	6	0.42ns	0.06ns	0.13ns	0.06**	1837.89ns	0.55ns	11.99ns	42.30***
IRL × STS	3	0.64ns	0.27ns	0.003ns	0.03ns	15732.69***	1.70ns	2.92ns	156.60***
Year × IRL × STS	3	1.34ns	0.09ns	0.04ns	0.04*	1711.14ns	0.59ns	11.12ns	97.49***
CUL × IRL × STS	6	0.15ns	0.04ns	0.17ns	0.03ns	12830.51***	0.31ns	4.81ns	14.33ns
Year × CUL × IRL × STS	6	0.08ns	0.01ns	0.18ns	0.03ns	2042.02ns	0.20ns	3.72ns	16.02ns
Error III		0.69	0.10	0.08	0.01	1401.79	0.83	12.23	7.70
C.V		11.24	1.89	15.55	3.60	6.86	6.59	4.95	13.39

DF: degree of freedom. R: Replication, CUL: Cultivar, IRL: Irrigation levels and STS: Storage Stage. *, **, *** Significant at $P \leq 0.05$, 0.01 or 0.001, respectively

Table 3 Effect of different irrigation levels on weight loss (%) of two Iranian pomegranate cultivars during storage at 5 °C

Year	Cultivar	Storage time (day)	Irrigation level			Mean of cultivar	Mean of time			
			Control	Moderate stress	Severe stress		0	30	60	90
1	Shishecap	0	0.00 h	0.00 h	0.00 h	7.78 A	0.00 D	4.71 C	10.40 B	15.38 A
		30	5.36 g	4.20 g	4.38 g					
		60	11.49 d	9.82 ef	10.83 de					
		90	17.35 a	14.77 bc	15.22 b					
	Malas-Yazdi	0	0.00 h	0.00 h	0.00 h					
		30	5.43 g	4.20 g	4.69 g	7.46 B				
		60	10.73 de	9.16 f	10.38 def					
		90	16.39 a	13.81 c	14.74 bc					
	Mean of irrigation level		8.35 A	6.99 C	7.53 B					
	Cultivar	Storage time (day)	Irrigation level			Mean of cultivar	Mean of time			
			Control	Moderate stress	Severe stress		0	30	60	90
2	Shishecap	0	0.00 g	0.00 g	0.00 g	7.16 A	0.00 D	4.84 C	9.98 B	14.00 A
		30	5.24 f	4.67 f	4.96 f					
		60	10.26 de	9.21 e	9.31 e					
		90	15.32 a	13.46 bc	13.54 bc					
	Malas-Yazdi	0	0.00 g	0.00 g	0.00 g	7.25 A				
		30	5.41 f	4.04 f	4.74 f					
		60	11.23 d	10.18 de	9.74 de					
		90	14.80 ab	13.04 c	13.82 abc					
	Mean of irrigation level		7.78 A	6.82 B	7.01 B					

For main effects, different capital letters show significant differences at $P<0.05$ and for each interaction effects, different lowercase letters show significant differences at $P<0.05$

the conversion of starch to sugar and as a consequence sugars accumulate in fruit [26]. The data in this experiment indicated that fruit TSS increased significantly during storage at 5 °C in both cultivars (Table 4). Similar results were reported by previous study who proposed that increased TSS in fruit juice at the end of the storage time is related to the concentrate of the juice due to dehydration and hydrolysis of polysaccharides during the

Table 4 Effect of different irrigation levels on TSS (Brix) of two Iranian pomegranate cultivars during storage period at 5 °C

Cultivar	Storage time (day)	Irrigation level			Mean of cultivar	Mean of time			
		Control	Moderate stress	Severe stress		0	30	60	90
Shishecap	0	16.65 hij	16.85 g-j	17.58 d-f	17.55 A	16.83 D	17.28 C	17.64 B	18.06 A
	30	16.93 f-i	17.33 c-g	17.93 bc					
	60	17.24 d-h	17.61 cde	18.35 ab					
	90	17.48 c-g	17.96 bc	18.71 a					
	0	16.23 j	16.46 ij	17.20 e-h	17.35 A				
	30	16.66 hij	16.91 f-i	17.90 cbd					
	60	16.91 f-i	17.36 c-g	18.40 ab					
	90	17.54 c-f	17.98 bc	18.72 a					
Mean of irrigation level		16.95 B	17.31 B	18.10 A					

For main effects, different capital letters show significant differences at $P<0.05$ and for each interaction effects, different lowercase letters show significant differences at $P<0.05$

Table 5 Effect of different irrigation levels on TA (%) of two Iranian pomegranate cultivars during storage period at 5 °C

Year	Cultivar	Storage time (day)	Irrigation level			Mean of cultivar	Mean of time			
			Control	Moderate stress	Severe stress		0	30	60	90
1	Shishecap	0	1.76 d-g	2.13 c-f	3.03 a	2.03 A	2.28 A	2.13 A	1.81 B	1.64 B
		30	1.69 efg	2.19 b-e	2.78 abc					
		60	1.36 g	1.56 efg	2.81 ab					
		90	1.66 efg	1.66 efg	1.70 efg					
	Malas-Yazdi	0	1.66 efg	2.40 a-d	2.73 abc	1.91 A				
		30	1.93 d-g	2.16 b-f	2.06 d-g					
		60	1.66 efg	1.86 d-g	1.63 efg					
		90	1.73 d-g	1.46 fg	1.66 efg					
	Mean of irrigation level		1.68 C	1.93 B	2.30 A					
	Cultivar	Storage time (day)	Irrigation level			Mean of cultivar	Mean of time			
			Control	Moderate stress	Severe stress		0	30	60	90
2	Shishecap	0	1.55 e-i	1.67 d-i	2.48 a	1.82 A	1.83 A	1.81 A	1.81 A	1.66 B
		30	1.40 ghi	1.81 c-g	2.33 ab					
		60	1.60 d-i	1.84 c-g	2.00 b-e					
		90	1.56 e-i	1.55 e-i	2.03 bcd					
	Malas-Yazdi	0	1.43 f-i	1.80 c-g	2.05 bcd	1.73 A				
		30	1.76 d-h	1.33 hi	2.23 abc					
		60	1.56 e-i	1.86 c-f	2.00 b-e					
		90	1.78 d-g	1.31 i	1.72 d-i					
	Mean of irrigation level		1.58 B	1.64 B	2.10 A					

For main effects, different capital letters show significant differences at $P<0.05$ and for each interaction effects, different lowercase letters show significant differences at $P<0.05$

storage period [24]. According to results, at the end of the storage time, fruit from severe water deficit and moderate water deficit had the higher TSS than control fruit in both cultivars which is may be linked to higher initial value of TSS in water deficit fruit in both cultivars or may be related to the higher respiration rate in these fruits. The increase in TSS of the pomegranate fruit throughout storage period is favourable characteristic, because it can increase the sweetness and general flavour of the fruit.

Titratable acidity

The year in which the pomegranates were grown significantly affected their acidity levels (Table 2). This led to analysis and reporting of the data for each year individually (Table 5). Acidity is a crucial aspect of pomegranate juice quality [27]. In the first year of the experiment, severe water deficit resulted in the highest fruit acidity, while control irrigation yielded the lowest acidity. In the second year, there was no significant difference in acidity between the control and moderate deficit treatments. However, severe water deficit still led to increased acidity in both pomegranate varieties. This finding that reduced irrigation increases pomegranate acidity aligns with prior studies by Laribi et al. [26] and Parvizi and Sepaskhah [27]. According to the results, in the first year of experiment, there was no statistically significant difference between first and second stage of measurement with respect to fruit TA. But, the TA of fruit significantly

decreased after 60 days of storage time (1.18%) when compared to the initial values, with slightly higher TA values for the moderate and sever water deficit treated fruit. Also, there was no statistically significant difference between third and fourth stages in the first year (Table 5). In the second year of experiment, TA of fruit remained unchanged throughout the storage period until the third stage of measurement when compared to the initial values, but fruit TA significantly decreased after 90 days of storage time (Table 5). Our results were in agreement with Peña-Estévezet al. [28] who reported that TA of pomegranate fruit reduced during storage time, but Pena et al. [23] reported that there was no significant difference throughout storage period for the same cultivar as result of deficit irrigation. The TA is one of the main parameters that shows the quality of fruit and it links to the content of organic acids and emphasizes this fact that organic acids decrease during storage period due to consumption of organic acids in respiration process [24].

Fruit pH

According to results, as the effect of year on pH of fruit was significantly different (Table 2), the results of two years were analysed separately (Table 6). In the first season, there was no significant difference in fruit pH between control, moderate water deficit and severe water deficit strategy, thus in this year deficit irrigation did not have any significant effect on the pH of pomegranate fruit. In the second season of experiment, with increasing the irrigation level, the fruit pH gradually increased (Table 6). In this year, the maximum and the minimum of pH were resulted from trees which irrigated with highest and lowest irrigation level, respectively (The main effect that shown with capital letters). The pH of pomegranate fruit juice is an indicator of its acidic taste that there is an inverse correlation between pH value and TA of pomegranate fruit [27]. In both years, at harvest time there was no significant difference between the irrigation levels in fruit pH in both cultivars (The interaction effects that shown with lowercase letters). These results are in line with the results of previous researches on pomegranate fruit affected by deficit irrigation [25, 27, 28].

With regard to the results, there was a significant difference in the fruit pH between two cultivars in the second year. In both years, the fruit pH increased during the storage period, regardless of the cultivar when compared to the initial values at the end of the storage period. In this case, the fruit pH increased until third stage and then declined clearly during next stage in both years (Table 6). Our finding was in line with previous report of Agbemafle et al. [29] who reported that the minimum of fruit pH was recorded in the first day of storage and the highest of it was observed in last stage of storage. It has been reported that citric acid is the main titratable acidity in pomegranate fruit [27] and it has been proposed that fruit pH increased during storage period as a result of conversion of the citric acid into sugars [29]. Also Peña-Estévezet al. [28] proposed that, increasing the fruit pH

Table 6 Effect of different irrigation levels on pH of two Iranian pomegranate cultivars during storage period at 5 °C

Year	Cultivar	Storage time (day)	Irrigation level			Mean of cultivar	Mean of time			
			Control	Moderate stress	Severe stress		0	30	60	90
1	Shishecap	0	3.42 fgh	3.37 gh	3.33 h	3.67 A	3.38 C	3.72 B	4.02 A	3.73 B
		30	3.79 b-e	3.65 d-g	3.60 e-h					
		60	4.09 ab	3.95 bcd	3.70 c-f					
		90	3.67 def	3.82 b-e	3.70 c-f					
	Malas-Yazdi	0	3.51 e-h	3.32 h	3.36 gh	3.76 A				
		30	3.80 b-e	3.76 cde	3.74 cde					
		60	4.08 ab	4.00 bc	4.35 a					
		90	3.72 c-f	3.74 cde	3.75 cde					
	Mean of irrigation level		3.76 A	3.70 A	3.69 A					
	Cultivar	Storage time (day)	Irrigation level			Mean of cultivar	Mean of time			
			Control	Moderate stress	Severe stress		0	30	60	90
2	Shishecap	0	3.33 i-l	3.30 jkl	3.23 l	3.51 B	3.31 D	3.51 C	3.82 A	3.63 B
		30	3.77 bcd	3.47 f-j	3.24 kl					
		60	3.98 a	3.66 c-f	3.60 d-h					
		90	3.40 i-l	3.52 e-i	3.61 d-g					
	Malas-Yazdi	0	3.41 h-l	3.32 jkl	3.31 jkl	3.63 A				
		30	3.63 def	3.67 cde	3.31 jkl					
		60	4.00 a	3.90 ab	3.77 bcd					
		90	3.43 g-k	3.84 abc	3.99 a					
	Mean of irrigation level		3.62 A	3.58 A	3.51 B					

For main effects, different capital letters show significant differences at $P<0.05$ and for each interaction effects, different lowercase letters show significant differences at $P<0.05$

during the storage period is a normal consequence of fruit ripening.

Total phenolic compounds

Analysis of the data showed that the year the pomegranates were grown significantly impacted their total phenolic content (TPC) (Table 2). Due to this variation, it was decided to analyse the data from each year separately (Table 7). In both cultivars and years, the fruit TPC increased significantly by reducing the plant water supply. Previously, an increase in fruit TPC under SDI when compared to the full irrigation reported in another Iranian pomegranate 'Shahvar' cultivar [7] and they reported that, the increase in TPC under deficit irrigation condition may be due to the induction of enzymes involved in phenolic compounds biosynthesis. It has been proposed that, water stress leads to change in secondary metabolites content in plant tissues [30].

As shown in this study (Table 7), there was no significant difference between 'Shishecap' and 'Malas-Yazdi' cultivars (551.16 and 544.46 mg GAE/100 mL, respectively) in the first season of experiment, but in the second season, 'Malas-Yazdi' cultivar showed the higher TPC than the 'Shishecap' cultivar that statistically, these differences in the fruit TPC were significant ($P \leq 0.05$). It has been reported that genotype is the primary source of variation in plants TPC [31, 32]. According to results that presented in Table 6, fruit TPC increased significantly during storage period in both years for all water levels when compared to the initial values in both years. Accordingly, in the first year, TPC of fruit was significantly different between the first stage (463.50 mg GAE g-1 FW) and fourth stage (579.82 mg GAE g-1 FW) of measurement. In the second season of experiment, similar trend was observed during storage time (Table 7). It has been reported that, the activity of phenylalanine ammonia-lyase enzyme that involved in the biosynthesis of phenolic compounds increases during the storage period which leads to an increase in phenolic compounds in the post-harvest period [28]. Previously, Sayyari et al. [33] reported an increase in phenylalanine ammonia-lyase enzyme activity for one of the Iranian pomegranates ('Malas Saveh') that stored at 2 °C for 3 months. Furthermore, Galani et al. [32] reported that in addition to increase in the activity of phenylalanine ammonia-lyase enzyme (that occurs in response to the adverse climate conditions like chilling injury) that lead to increasing the synthesize of the polyphenolic phytoalexins during cold storage, decrease in the activity of polyphenol oxidase enzyme during cold storage can lead to the diminished oxidation of phenolic substrates to quinones.

Anthocyanin content

Since combined analysis of variance showed that the influence of year on anthocyanin content of fruit was not significant (Table 2), the average results of two years

Table 7 Effect of different irrigation levels on total phenolic compounds (mg GAE 100 ml^{-1}) of two Iranian pomegranate cultivars during storage period at 5 °C

Year	Cultivar	Storage time (day)	Irrigation level			Mean of cultivar	Mean of time			
			Control	Moderate stress	Severe stress		0	30	60	90
1	Shishecap	0	334.52 j	428.64 hi	646.94 cd	551.16 A	463.50C	548.92 B	599.03 A	579.82 A
		30	358.27 j	613.52 cde	714.01 b					
		60	501.21 fg	576.35 e	784.32 a					
		90	377.17 ig	474.62 fgh	804.45 a					
	Malas-Yazdi	0	416.27 hi	456.62 gh	497.98 fg	544.46 A				
		30	503.52 fg	590.72 de	513.48 fg					
		60	472.40 fgh	662.09 bc	597.78 de					
		90	520.65 f	661.03 bc	641.02 cd					
	Mean of irrigation level		435.50 C	557.95 B	650.00 A					
	Cultivar	Storage time (day)	Irrigation level			Mean of cultivar	Mean of time			
			Control	Moderate stress	Severe stress		0	30	60	90
2	Shishecap	0	279.22 j	421.05 i	665.00 ab	526.74 B	463.80 C	520.37 B	595.14 A	594.50 A
		30	327.94 j	571.86 c	680.34 ab					
		60	464.78 e-i	531.58 c-f	736.22 a					
		90	429.27 hi	508.39 c-h	705.35 ab					
	Malas-Yazdi	0	440.34 ghi	455.19 f-i	522.04 c-f	560.15 A				
		30	477.85 d-i	540.09 cde	524.12 c-f					
		60	510.53 c-g	684.86 ab	642.88 b					
		90	554.42 cd	678.49 ab	691.09 ab					
	Mean of irrigation level		435.54 C	548.94 B	645.88 A					

For main effects, different capital letters show significant differences at $P<0.05$ and for each interaction effects, different lowercase letters show significant differences at $P<0.05$

were used for analysis (Table 8). Based on our results, at harvest time, control fruit had anthocyanin concentration of 12.47 and 8.61 mg 100 ml^{-1} in 'Shishecap' and 'Malas-Yazdi' cultivars, respectively (Table 8), which was lower than the deficit irrigation treatments in both cultivars. Initially, in 'Shishecap' cultivar, the moderate and severe deficit irrigated fruit had 1.15- and 1.37-times greater anthocyanin concentration than control samples, respectively. In 'Malas-Yazdi' cultivar it was 1.14 and 1.85 times greater than the control sample, respectively that probably was due to abiotic stress linked to the irrigation treatments. Previously, a similar trend was reported by others for similar conditions [7, 25, 26]. It has been reported that, decrease in available water will be responsible for significantly increased the expression of some of the genes of anthocyanin synthesis pathway [34]. Also it has been reported in previous study that, anthocyanin biosynthesis as secondary metabolism increases by water stress as part of drought stress response [35]. It has been reported that, sugars play an important role in anthocyanin synthesis and furthermore, SDI treatment increases the TSS and total sugar content, which describe the highest anthocyanin content in fruit by applied DI [26]. As a response to drought stress, a significant difference in TAC level was detected between two cultivars that may be due to different genotype of two cultivars.

Generally, anthocyanin concentration increased to 9% during the shelf-life, regardless of the irrigation treatment when compared to the initial values in both cultivars. According to results, the percentage of increases in anthocyanin concentration of the 'Shishecap' cultivar during the storage period with respect to the different water application were, 11.78%, 8.13% and 2.56% for control, moderate and severe water deficit, respectively. Increases percentage for 'Malas-Yazdi' cultivar were 26.94%, 17.25 and 10.06% for control, moderate and severe water deficit, respectively. Our findings were in agreement with those previously reported about increasing the anthocyanin concentration during cold storage period in other pomegranate cultivars [22, 23, 36]. It is well known that among phenolic compounds, pomegranate anthocyanins have the most important value due to their role in fruit red colour, and also their antioxidant properties [37]. It has been reported that, content of fruit anthocyanin augment throughout shelf life because of biosynthesis of these compounds during the cold storage [23]. Also in another study, authors suggested that the release of membrane bounded anthocyanins during cold storage is the main reason for increasing the total anthocyanin content during cold condition [32].

Antioxidant activity

As the influence of year on antioxidant activity of fruit was not significant (Table 2), averages of two years were used for statistical analysis (Table 9). Based on our results, the antioxidant activity of the pomegranate showed a trend of increasing with respect to deficit irrigation level with mean values ranging from 66.4% for control samples, 71.12% for moderate water deficit and 73.98% for sever water deficit fruit. At harvest time in 'Shishecap' cultivar, fruit antioxidant activity was increased 5.73% and 12.94% in moderate and sever water deficit respectively compare with control and increasing percentage for 'Malas-Yazdi' cultivar were 9.12% and 10.51% for moderate and sever water deficit respectively compare with control fruit treatment. According to results there was no significant difference between control and moderate water deficit as well between moderate and severe water deficit in 'Shishecap' cultivar but there was a significant difference between control and deficit water treatments.

This experimental trend was in concordance with previous researches indicated that differences between the deficit irrigation and control fruit were statistically significant at harvest time [7, 23, 26]. Pomegranate fruit antioxidant activity depends on some compounds including anthocyanins, punicalagins, phenolic and ascorbic acids, alone or in combination [28]. It has been shown

Table 8 Effect of different irrigation levels on total anthocyanin content (mg 100 ml^{-1}) of two Iranian pomegranate cultivars during storage period at 5 °C

Cultivar	Storage time (day)	Irrigation level			Mean of cultivar	Mean of time			
		Control	Moderate stress	Severe stress		0	30	60	90
Shishecap	0	12.47 ghi	14.38 c-f	17.13 ab	15.13 A	13.07 C	13.59 B	14.29 A	14.52 A
	30	12.70 fgh	14.34 c-f	17.08 ab					
	60	13.75 efg	15.27 cde	17.41 a					
	90	13.94 d-g	15.55 bcd	17.57 a					
	0	8.61 l	9.85jkl	16.00 abc	12.61 B				
	30	9.17 kl	10.60 jk	17.67 a					
	60	10.50 jk	11.27 hij	17.52 a					
	90	10.93 ij	11.55 hij	17.61 a					
Mean of irrigation level		11.51 C	12.85 B	17.25 A					

For main effects, different capital letters show significant differences at $P<0.05$ and for each interaction effects, different lowercase letters show significant differences at $P<0.05$

Table 9 Effect of different irrigation levels on antioxidant activity (%) of two Iranian pomegranate cultivars during storage period at 5 °C

Cultivar	Storage time (day)	Irrigation level			Mean of cultivar	Mean of time			
		Control	Moderate stress	Severe stress		0	30	60	90
Shishecap	0	64.82 hi	68.54 e-h	73.21 a-e	70.63 A	68.33 C	70.00 B	70.56 B	73.16 A
	30	64.85 hi	70.15 c-g	75.18 abc					
	60	66.68 f-i	70.19 c-g	74.42 a-d					
	90	69.47 d-h	73.67 a-d	76.45 a					
	0	63.61 i	69.48 d-h	70.30 c-g	70.39 A				
	30	65.25 hi	72.19 a-e	72.43 a-e					
	60	66.17 ghi	71.49 a-f	74.42 a-d					
	90	70.69 b-g	73.20 a-e	75.44 ab					
Mean of irrigation level		66.44 C	71.12 B	73.98 A					

For main effects, different capital letters show significant differences at $P<0.05$ and for each interaction effects, different lowercase letters show significant differences at $P<0.05$

Table 10 Effect of different irrigation levels on vitamin C (mg 100 ml^{-1}) of two Iranian pomegranate cultivars during storage period at 5 °C

Year	Cultivar	Storage time (day)	Irrigation level			MC	MT			
			Control	Moderate stress	Severe Stress		0	30	60	90
1	Shishecap	0	30.69 b	31.60 b	44.67 a	22.94 A	29.49 A	22.61 B	21.53 B	15.19 C
		30	20.33 efg	21.67 efg	24.63 cde					
		60	17.33 gh	28.57 bc	14.63 hi					
		90	10.39 i	16.76 gh	14.03 hi					
	Malas-Yazdi	0	17.00 gh	25.00 cde	28.01 bcd	21.47 B				
		30	20.09 efg	23.72 cde	25.24 cde					
		60	18.15 fgh	27.51 bcd	23.03 def					
		90	11.00 i	21.91 efg	17.06 gh					
	Mean of irrigation level		18.12 B	24.59 A	23.91 A					
	Cultivar	Storage time (day)	Irrigation level			Mean of cultivar	Mean of time			
			Control	Moderate stress	Severe stress		0	30	60	90
2	Shishecap	0	22.06 b-e	23.42 a-d	28.27 a	20.03 A	23.22 A	21.42 A	17.20 B	15.03 C
		30	21.00 c-f	20.36 d-g	27.36 a					
		60	17.21 e-i	16.79 f-i	20.20 d-h					
		90	12.82 i	14.91 hi	15.98 f-i					
	Malas-Yazdi	0	15.69 f-i	23.73 a-d	26.15 ab	18.41 B				
		30	14.03 i	19.85 d-h	25.95 abc					
		60	14.91 hi	16.18 f-i	17.93 e-i					
		90	13.91 i	15.22 ghi	17.37 e-i					
	Mean of irrigation level		16.45 C	18.81 B	22.40 A					

For main effects, different capital letters show significant differences at $P<0.05$ and for each interaction effects, different lowercase letters show significant differences at $P<0.05$. MC and MT are mean of cultivars and mean of times, respectively

that water deficiency, acting as a form of oxidative stress, can enhance the antioxidant system in plants. This leads to variations in the levels of bioactive compounds and antioxidant activity, influenced by factors such as cultivar, harvest date, tree age, and the severity of drought stress [7]. Also other author mentioned that phenolic compounds that based our results increase by application deficit irrigation has major role in antioxidant activity in different pomegranate cultivars [7, 19, 38].

Vitamin C

As the influence of year on vitamin C content of fruit was significant (Table 2), the results of each year were analysed separately (Table 10). Based on our results, at harvest time, the effect of irrigation treatment on vitamin C content of both pomegranate fruit cultivars was significant in both seasons. The highest vitamin C content of pomegranate fruit was observed in severe water deficit treated fruit in both 'Shishecap' (44.67 and 28.27 (mg 100 ml^{-1}) in first and second year, respectively) and in 'Malas-Yazdi' (28.01 and 26.15 (mg 100 ml^{-1}) in first and second year, respectively. However, at harvest time, the differences in the vitamin C content of the fruit for the deficit irrigation treatments were significant in the first year of experiment only for 'Shishecap' cultivar. In the second year of experiment, the differences were not

significant in both cultivars. Generally, the results indicated that the fruit from trees grown under severe water deficit showed the highest vitamin C content and there was no significant difference between deficit water treatments in the first year. In this sense, significant difference was observed among all irrigation levels (Table 10). This results are in compromise with the previous findings by other researcher, who reported that increasing the rate of water deficit can lead to an increase in the vitamin C content of 'Mollar de Elche' pomegranate [23]. The higher vitamin C content of pomegranate fruit with reduction in water application compared to control fruit samples may play a protective role against drought damage [23]. According to results (Table 7), there was significant difference between 'Shishecap' and 'Malas-Yazdi' cultivars in both years. It has been well documented that climatic conditions such as temperature and light and cultural factors during growth season including growth regulators, nitrogen fertilization, thinning, pruning, and pesticides can affect fruits and vegetables vitamin C content [39]. It is observed that the vitamin C content decreased during the storage period in both years. In first year of experiment, the decrease in the vitamin C content of the pomegranate from day 0 to day 90 of storage time was significant. The vitamin C content of the pomegranate fruit (mg 100 ml^{-1}) is in the order 29.49 > 22.61 > 21.53 > 15.19 from first to the fourth measurement stage, respectively. In the second year of experiment, the percentage of decreases in vitamin C content of fruit with the respect to the first storage time were 8.40%, 14.43% and 24.53% for the 30, 60 and 90 days after harvest time, respectively. Generally, based on our results, storage period had a negative effect on vitamin C content of the pomegranate fruit regardless of irrigation strategies.

The results of vitamin C content agreed with the findings of previous research, who found similar decrease in vitamin C content during cold storage [40]. It has been reported that solubility in water, thermic degradation and enzymatic oxidation are three main reasons of decreasing the content of vitamin C during the storage period [32, 41]. Also it has been reported that vitamin C might be used as antioxidant compound against oxidative stress that occur through storage in low-temperature condition [32]. By Using some treatments such as chelating factors and decreasing the activity of ascorbic acid oxidase enzyme by applying the inhibitor factors can maintain the initial value of vitamin C during the storage period against oxidation [42].

Table 11 Findings from the study of analysis of variance on year, irrigation, cultivar and their interactions on yield of pomegranate trees (Comparison of averages based on Duncan's test, P ≤ 0.05)

	DF	yield of pomegranate trees	
		F value	*P* value
Year	1	2.59	> 0.05
(CUL)	1	0.01	> 0.05
(IRR)	2	35.74	< 0.05
Year × CUL	1	0.51	> 0.05
Year × IRR	2	0.23	> 0.05
CUL × IRR	2	0.25	> 0.05
Year × CUL × IRR	2	0.01	> 0.05
Error		4.45	
C.V		12.35	

DF: degree of freedom, CUL: Cultivar, IRR: Irrigation

Yield

Table 11 displays the impact of year, water stress, cultivar and their interactions on the yied of the two pomegranate cultivars ($P \leq 0.05$). The results showed that pomegranate yield was not significantly affected by the year and cultivar, whereas it was significantly affected by irrigation treatments (Table 11). During the both years, all the water stress levels in both cultivars led to a decrease in pomegranate yield (Fig. 1). However, 'Malas-Yazdi' showed no significant difference between the fully irrigation and moderate stress treatments but did between moderate and severe stress in both years. 'Shishecap' showed significant difference between control and both deficit irrigation levels (Fig. 1). These findings were consistent with previous research on pomegranate, that restricted water accessibility had a negative impact on pomegranate yield, accordingly. Studies on cultivars such as 'Rabab' [5], 'Mollar de Elche' [38], 'Manfalouty' [43] and eleven Mediterranean pomegranate cultivars (except 'Zheri Automne' [44] support this finding. Conversly, another study showed that neither 'Mollar de Elche' [45] nor 'Wonderful' experiences a significant decrease in yield when exposed to severe water stress [46]. According to reports, certain fruits on water stressed fruit trees behave as potent sinks for photosynthates before dropping, likely contributing to overall yield reduction due to fruit loss [47]. Furthermore, maximum fruit set and fruit retention percentages were obtained with adequate water in the soil, however a significant drop in fruit retention was seen with decreased water in the soil [43]. A decrease in fruit weight may be linked to water stress, which may ultimately result in a reduction in the quantity of the product [44].

Conclusion

Regardless of yield reduction, SDI strategy can be used as an effective approach to improve the pomegranate fruits quality at harvest time, and maintain their quality during the storage period. SDI strategy in addition to water saving, reduced the pomegranate fruit weight loss in both cultivars. Furthermore, SDI strategy had a positive effect on fruit TSS and other bioactive compounds of pomegranate like TPC, TAC, antioxidant activity and

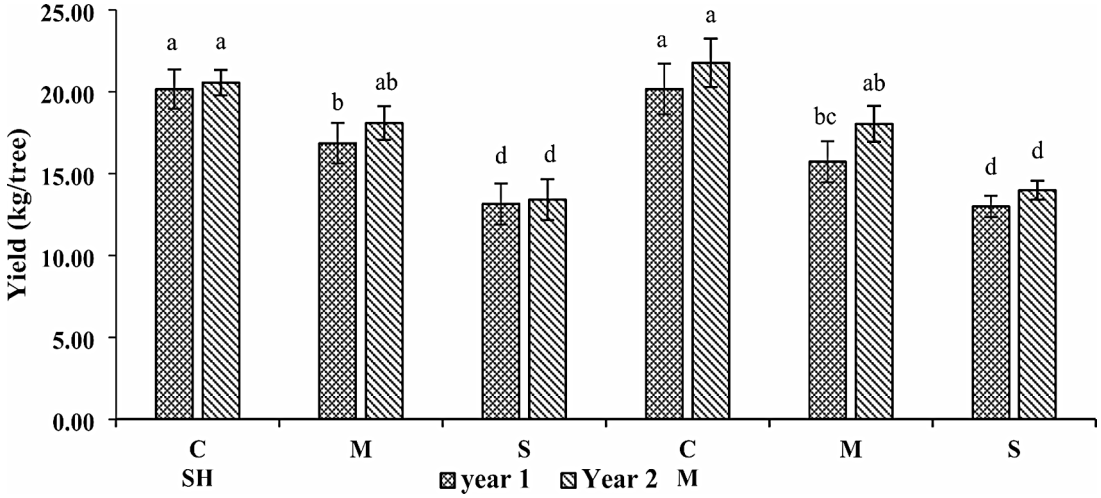

Fig. 1 Effect of different irrigation levels (C = control, M = moderate stress, and S = severe stress) on yield of two Iranian pomegranate cultivars (SH = 'Shishecap' and M = 'Malas-Yazdi'). Similar letters above the columns indicate non-significant differences among the irrigation levels at $P \leq 0.05$

vitamin C at harvest time and throughout storage period in two cultivars in two years. Overall, the application of SDI results in increased levels of bioactive compounds in both cultivars, underscoring its significance in optimizing fruit quality and nutritional value.

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M. N. Conducting Research, Formal analysis, Writing - original draft. A. R. Conceptualization, Project administration, Resources. D. V. Advice - review & editing.

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Data availability
All data are available upon reasonable request.

Declarations

Ethics approval and consent for publication
In accordance with institutional, national, and international guidelines and legislation governing experimental research and field studies on plants, the procedures described in this study were conducted in full compliance with the requisite ethical and legal standards. All authors declare their consent for publication of this manuscript.

Consent for publication
Not applicable.

Competing interests
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