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Vegetative Growth, Enzymatic Activities, Fruit Yield, and Quality of 'Balady' Mandarins (*Citrus reticulata* Blanco) in Response to Deficit Irrigation Levels

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CITRUS is one of the most important fruit crops grown in the worldwide, especially in Mediterranean countries. Deficit irrigation (DI) is a proposed strategy many years ago to overcome the scarcity of water, especially in arid and semiarid regions. The vegetative growth, enzymatic activities, proline and phenolic content, fruit yield and quality in response to deficit irrigation levels (T1 (irrigation every 15 days as a control), T2 (irrigation every 20 days), T3 (irrigation every 25 days), and T4 (irrigation every 30 days), were evaluated. The results showed that the P, K, proline, phenols, vitamin C, SSC contents, and catalase and peroxidase activities were increased by T2 and T3. While the N content, fruit yield, weight, peel thickness, juice volume, and transpiration rate were increased by T1 and T2 compared to T3 and T4. The fruit yield, weight, and physical properties were not negatively affected by light and medium deficit irrigation (T2 and T3), which positively affected enzymatic activities, proline and phenol contents, and chemical fruit properties (SSC and Vitamin C).

Keywords: Deficit irrigation; Fruit quality; Enzymatic Activities; Water stress; Citrus.

1. Introduction

Citrus is one of the most important fruit crops grown in the worldwide especially in Mediterranean countries. The cultivated area of citrus crops is more than 10 million ha with annual production of more than 161 million tons worldwide (FAO, 2023). In Egypt, citrus crops represent 30% of total cultivated area with fruit trees, with annual production 4.4 million tons. Moreover, citrus is considered the 1st exportable agricultural crop in Egypt with two million tons. The mandarin is the 2nd main citrus crop after oranges with 46,055 ha and 988,105 tons (FAO, 2023). Since water is a natural resource which is increasingly scarce and expensive, with climate change in Mediterranean region, water management is extremely important for environmental sustainability (Torrecillas et al., 2018, Salama et al., 2021, Sari et al., 2024 and Toumi et al., 2024). By 2050, the world population is expected to increase by 30% and water deficiency is expected to increase with climate change especially in countries that depend on agriculture activities. Therefore, the optimizing of irrigation strategies is essentially over the world. The limited water resources in Egypt, that is well known, considers the main challenge for agriculture, where about 13.5 BCM/yr is the gap between the needs and availability of water (Omar and Moussa, 2016 and Gómez-Bellot et al., 2024). These facts led to promote research and development of irrigation methods to enhance the water use efficiency.

Deficit irrigation (DI) is a proposed strategy many years ago for fruit trees especially in arid and semiarid regions. The understanding of tree phenological stages and their responses to DI is essential for the correct practice (Gómez-Bellot et al., 2024). Geerts and Raes (2009) proposed the term of Crop water productivity (WP), that defined ''the

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ratio of the mass of marketable yield to the volume of water consumed by the crop'', which is considered the key term to study the benefits of DI. RDI strategy depends on decrease the irrigation during insensitive stages while watering the trees in the critical growth periods. The usefulness of deficit irrigation is not only saving water but also could improve fruit quality, reduction in the cost of trees pruning and watering by decreasing vegetative growth and irrigation times and quantities.

Many previous studies had been done on the fruit trees to evaluate the effects of DI on trees vegetative growth, fruit yield and quality Ballester et al., (2014) and Nagaz et al., (2020) on navel oranges; Berríos et al., (2024) on mandarins; Intrigliolo and Castel, (2005) on plums; Soliman et al., (2018) on apples; Ezzat et al., (2021) on apricots. The regulated deficit irrigation (RDI) during pit-hardening and postharvest stages has no negative effects on fruit set and yield for stone fruits (Intrigliolo and Castel, 2005; Thakur and Singh, 2013). Deficit irrigation could advance fruit maturity, fruits of peaches and nectarines by increasing flavor, color and soluble solids content (SSC) and decreased total acidity without negative effect on fruit yield (Pérez-Sarmiento et al., 2016). Moreover, it increased the juice percentage and SSC in fruit compared to full irrigation or farmer irrigation of navel orange trees (Nagaz et al., (2020) and Berríos et al., (2024)). Ballester et al., (2014), found that the mid-summer RDI saved 20 % of water with a reduction in tree growth and without significant reduction on fruit yield, size and the economic return of 'Clementina de Nules' trees. Moreover, the vegetative growth (shoot length, diameter, leaf area and specific weight) and fruit yield of "Anna" apple trees negatively affected by DI rate compared with well irrigated trees (Soliman, et al., 2018), but the water productivity, fruit SSC, firmness and anthocyanin contents were improved. Under deficit irrigation conditions, many applications were tested to mitigate their negative effects such as; potassium silicate (El-Shafei et al., 2023), salicylic acid (Moustafa, et al., 2024), compost, biochar and sugar alcohols (El-Sherpiny et al., 2023).

The main goal of this study was to study the effects of DI levels on the vegetative growth, transpiration rate, total phenols content, enzymes activities, fruit yield, and quality of 'Balady' mandarins under north delta of Egypt conditions.

2. Materials and Methods

The experiment was conducted on 'Balady' mandarins cultivar (*Citrus reticulata* L.) grown in a private orchard at Desouk district, Kafr Elsheikh governorate, Egypt, during 2019 and 2020 seasons. Trees were 30 years old, planted at 6 x 6 m spacing in silty loam soil. A total of 32 trees, uniform in vigor as possible, divided into four treatments, so that, each treatment contained 8 trees in one row and neutral raw of trees (as control) was left between each two treatments, as follows: T1 (irrigation every 15 days as a control), T2(irrigation every 20 days), neutral raw as control, T3 (irrigation every 25 days), neutral raw as control, and T4 (irrigation every 30 days), no. of irrigations and amount of water for each treatment are showed in the Table 1.

Table 1. Number of irrigations and Amount of water during season.

	Season 2019				Season 2020			
	T1 (cont.)	T2	T3	T4	T1 (cont.)	T2	T3	T4
No. of irrigations	17	12	10	8	16	12	10	8
Basin dimensions (L*W*H) m	2*1.2*0.23 m							
Operation time (minutes)	357	452.2	523.6	571.2	357	452.2	523.6	571.2
m³ water/ha/irrigation[1]	303.17	383.91	444.45	484.95	303.17	383.91	444.45	484.95
Total m³ water/ha/year[2]	5153.98	4607	4444.5	3879.6	4850	4607	4444.5	3879.6

[1] was calculated by operation time * basin dimensions, [2] was calculated by multiplication of [1] * No. of irrigations

The total amount of water per each treatment and per hectare (ha) were calculated with the following equation:

$M3\ (\text{water/ha})/\text{irrigation}) =$ basin dimensions (L*W*H)* t_1*t_2

where L, W and H are the length, width and height of basin, t1 is the time to fill the basin which was 39 second, and t2 is the operation time to irrigate the treatment.

Vegetative growth parameters measurements

A total of 30 mature leaves 7 months old, representing the four directions of the tree, were collected, and directly transported to lab for estimating leaf area by planimeter (Licore 3100 area meter), (Teobaldelli et al., 2019), chlorophyll content (SPAD unit) by the CCM-200 plus Chlorophyll Content Meter (OPTI SCIENCES), (Xiong et al., 2015), fresh and dry weight, proline content, enzyme activities and NPK macronutrients.

Leaf chemical constituents

For determination the N, P and K, 0.2 g crude dried leaves sample was wet digested with a mixture of concentrated sulphoric acid and perchloric acid, then heated until become clear solution (Peterburgski, 1968), after digestion, the solution was quantitively transferred into 100 ml measuring flask with distilled water and kept for determinations. N, P and K% were determined according to Mertens (2005 a and b).

Transpiration rate

Leaf transpiration rate was dignified directly on the leaf. It signifies the sum of the rates for the adaxial and abaxial surfaces according to (Sicher and Barnaby 2012).

Leaf proline content, phenols, peroxidase, and catalase enzymes activities

For determination the leaf proline content, a sample of 0.5 g of fresh leaf was used according to methods of Bates et al., (1973). Total phenols were estimated according to (Martínez-Esplá et al., 2014), by the Folin–Ciocalteu reagent and external calibration with gallic acid. The Peroxidase (POD) and Catalase (CAT) enzymes activity were estimated by 0.2 mg samples of fresh leaves that homogenized in ice-cold 0.1 M sodium phosphate buffer pH 7.0, at 4 °C then centrifugated at 11200 g for 10 min at 4 °C. The enzymes activities were assayed at clear supernatant. Peroxidase (POD) activity was determined with the final 3 mL reaction mix containing: 50 mM potassium phosphate buffer (pH 7.0), 20 mM guaiacol, 40 mM H2O2 and 0.1 mL enzyme extract. The absorbance of the reaction solution at 470 nm was recorded every second for 3 min., (Chance & Meahly, 1955). As well as the catalase (CAT) activity was determined in the final 3 mL reaction mix containing: 50 mM Phosphate buffer (pH 7.0), 5.9 mM H2O2 and 0.1 mL enzyme extract was used. The absorbance of the reaction solution at 240 nm was recorded per second for 3 min, according to (Chance & Meahly, 1955).

Fruit yield

The fruits were harvested at the mid-November and yield was calculated as Kg per tree.

Fruit quality properties

Random samples of 10 fruits were taken from each tree (80 fruits each treatment) and brought to Physiology and Breeding of Horticultural Crops Lab (PBHC), a certified lab in determining of SSC and acidity in fruits, for measuring the physical and chemical fruit properties. Fruit physical properties: Average fruit weight (g) was estimated by digital balance. Peel thickness (mm) was measured by Vernier clipper. Average fruit juice volume (cm^3) was determined by graduated cylinder. Firmness (Newton (N)/cm2) was measured by penetrometer pressure tester (Push-full Dynamometer) equipped with probe (8 mm diameter) for 30 fruits per treatment at the equatorial area of both sides of each fruit.

Chemical fruit properties including soluble solids contents (SSC) were determined with a digital refractometer Atago PR-101 (Atago Co. Ltd., Tokyo, Ja-pan) at 20°C. Total acidity was determined also in the same juice by automatic titration (785 DMP Titrino, Metrohm) with 0.1 N NaOH up to pH 8.2 and results expressed as % of citric acid equivalent.

Vitamin C. (ascorbic acid): was determined in filtered juice samples and expressed as mg/100ml juice according to AOAC (2005).

Statistical analyses

The statistical significance was evaluated by one-way analysis of variance (ANOVA) using Costate and the individual comparisons were obtained by L.S.D multiple range test (DMRT). Values were considered statistically significant when $p<0.05$.

3. Results

Effects on leaf Chlorophyll (SPAD value), Leaf area and leaf dry weight

Data in Table 2, illustrated that Chlorophyll (SPAD value), and Leaf area were affected by irrigation treatments through both seasons of the trial. The highest overall means were recorded under trees irrigated every 30 days (T4) and the lowest values were recorded under trees irrigated every 20 days (T3). While the dry weight of leaves wasn't mostly affected by the irrigation treatments.

Effects on leaf macronutrients and transpiration rate

Data in Table 3 cleared that leaf N, P and K contents as well transpiration rate were significantly affected by the irrigation treatments. With decreasing the irrigation period and increasing irrigation times (T1 and T2 treatments), the leaf N Content and transpiration rate increased, and at the same time, decreased mostly leaf P and K contents as compared to the T3 and T4 treatments.

Effects on leaf proline and phenol contents, catalase, and peroxidase activities

According to data in Figure 1, water-stressed trees had higher leaf proline values in the first season compared to control, while in the second season belonged to the T4 treatments (more water-stressed trees. As for leaf phenols weren't affected by the irrigation treatments except only in the second season, with higher values in the T3 and T4 treatments as compared to the control and T2. Regarding to leaf Catalase and peroxidase contents; the control and T2 treatments decreased catalase values than T3 and T4. Leaf peroxidase control recorded the lowest value as compared to remaining treatments except T3 in the first season as well as T2 in the second one. Briefly, stressed trees except T2 mostly increased proline and phenol contents, as well as catalase and peroxidase activities comparing to the control.

Table 2. Effect of the irrigation treatments on Chlorophyll content (SPAD value), Area, Dry weight of leaves Balady mandarin trees in 2019 and 2020 seasons.

Treatments	Chlorophyll (SPAD value)		Leaf area (cm2)		Dry weight (g)	
	2019	2020	2019	2020	2019	2020
T1 (the control)	96.92b	98.32b	59.83a	56.35a	96.29	86.66b
T2	99.70b	99.44b	57.50ab	58.58a	98.63	89.34ab
T3	88.28c	88.23c	56.08b	51.05b	92.13	93.99a
T4	107.87a	110.88a	52.00c	50.00b	96.31	93.71a
F- test	**	**	**	*	NS	*

Table 3. Effect of the irrigation treatments on leaf macro mineral content and its transpiration rate of Balady mandarin trees in 2019 and 2020 seasons.

Treatments	N, %		P, %		K, %		Transpiration rate, %	
	2019	2020	2019	2020	2019	2020	2019	2020
T1 (the control)	1.89a	2.085a	0.783c	0.600b	1.40b	1.12b	41.71a	42.46a
T2	2.04a	1.965b	0.805c	0.675ab	1.41b	1.14b	42.21a	41.35a
T3	1.65b	1.72c	0.878b	0.703ab	1.48b	1.15ab	40.15b	39.62a
T4	1.42c	1.51d	0.943a	0.778a	1.60a	1.21a	39.35b	34.99b
F- test	**	**	**	*	**	*	*	*

T1: Control trees were irrigated every 30 days = 17 times/season & T2; trees were irrigated every 20days= 12 times/season & T3: trees were irrigated every 20 days=10 times/season & T4: trees were irrigated every 30 days =8 times/season.

Effects on some fruit physical characters and the yield

Table 4 showed that fruit weight, fruit firmness, fruit peel thickness and fruit size were significantly affected by the irrigation treatments in both seasons, with greater values of fruit weight in the control treatment as well as T2 in the second season. As for fruit firmness, there is no constant trend, in the first season T2 and T4 recorded higher values than the control and T3, while in the second season T4 and control provided the highest value, followed by T2. Regarding peel thickness the control had the higher values than the rest treatments, whereas the T3 gave the lowest values in both seasons. As for fruit juice, in the first season, the control had the highest juice volume but in the second season it was only higher than T4.

The irrigation treatments had a substantial effect on the Balady mandarin trees' productivity in both seasons; control trees produced the maximum yield, followed by T2 trees. While, as can be shown in Fig 2, the T4 reported the lowest yield. under water deficit.

Effects on some chemical physical characters

Data in Table 5 cleared that TSS and Vitamin c of Balady mandarin fruit trees were affected by the irrigation treatments in both seasons as well as acidity only in the second season. For TSS, the T2 recorded the highest content than the other treatments except for the T3 in the first season and T4 in the second one. Regarding to acidity, the highest acidity was in the control and the other irrigation treatments had the same acidity. As for the vitamin C content in the first season, the T4 and T3 gave the highest value, while in the second season T3 gave the lowest value.

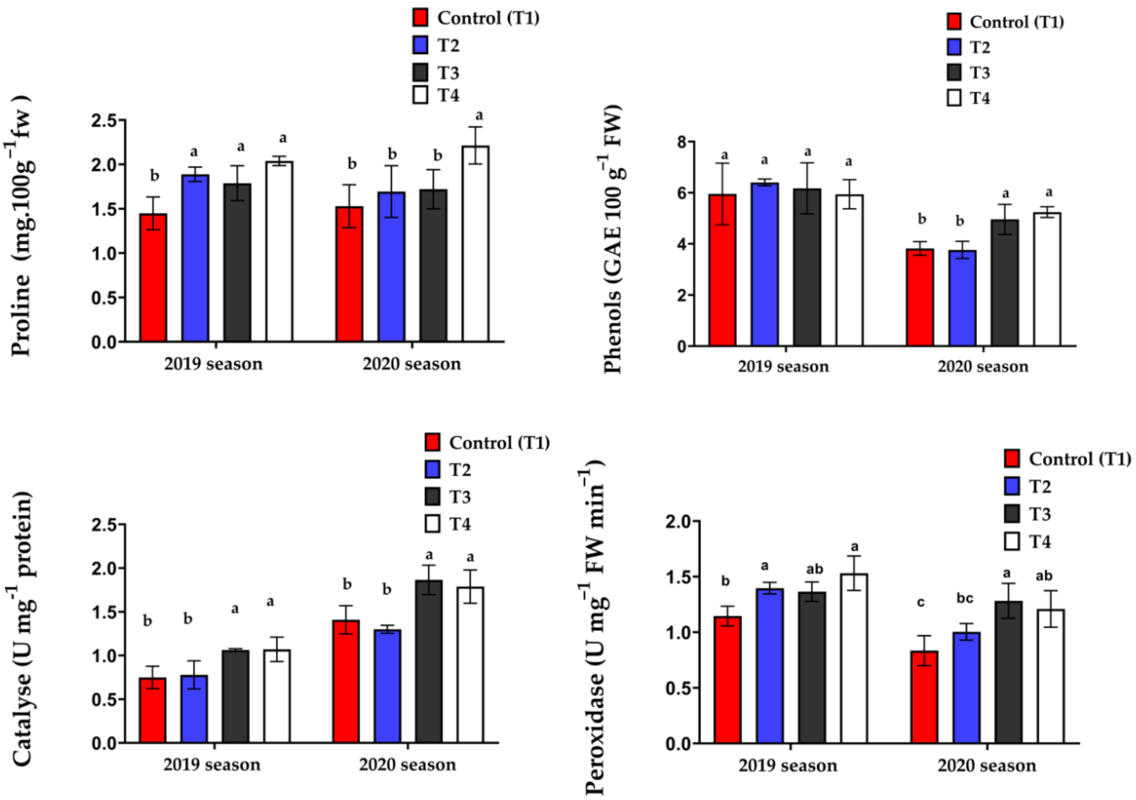

Fig. 1. Effect of the irrigation treatments on leaf proline and phenol Contents, as well as catalase and peroxidase activities of Balady mandarin trees in 2019 and 2020 seasons.

T1: Control trees were irrigated every 30 days = 17 times/season & T2; trees were irrigated every 20days= 12 times/season & T3: trees were irrigated every 20 days=10 times/season & T4: trees were irrigated every 30 days =8 times/season.

Table 4. Effect of the irrigation treatments on some fruit physical characters of Balady mandarin trees in 2019 and 2020 seasons.

Irrigation Treatments	Fruit weight (g)		Fruit firmness (cm3)		Fruit Peel thickness (cm)		Fruit Juice size (%)	
	2019	2020	2019	2020	2019	2020	2019	2020
T1 (the control)	115.22a	114.77a	1.54b	1.76a	3.00a	2.68a	44.17a	40.63a
T2	102.07b	116.48a	1.92a	1.67b	2.61c	2.41b	37.67bc	34.79ab
T3	103.15b	109.81b	1.39c	1.61c	2.41d	1.99c	39.04b	34.17ab
T4	98.93b	110.74b	1.90a	1.79a	2.79b	2.32b	36.44c	30.00b
F- test	**	*	***	***	***	***	***	*

Table 5. Effect of irrigation treatments on some fruit chemical characters of Balady mandarin trees in 2019 and 2020 seasons.

Irrigation Treatments	TSS %		Acidity %		Vitamin c	
	2019	2020	2019	2020	2019	2020
T1 (the control)	9.81b	8.68b	1.74	2.745a	11.22c	22.83a
T2	10.32a	9.82a	2.13	2.30b	11.63bc	23.4a
T3	9.97ab	8.98b	1.84	2.42b	12.97ab	15.87b
T4	9.34c	9.51a	2.26	2.38b	13.08a	22.47a
F-test	**	***	NS	**	*	**

T1: Control trees were irrigated every 30 days = 17 times/season & T2; trees were irrigated every 20days= 12 times/season & T3: trees were irrigated every 20 days=10 times/season & T4: trees were irrigated every 30 days =8 times/season.

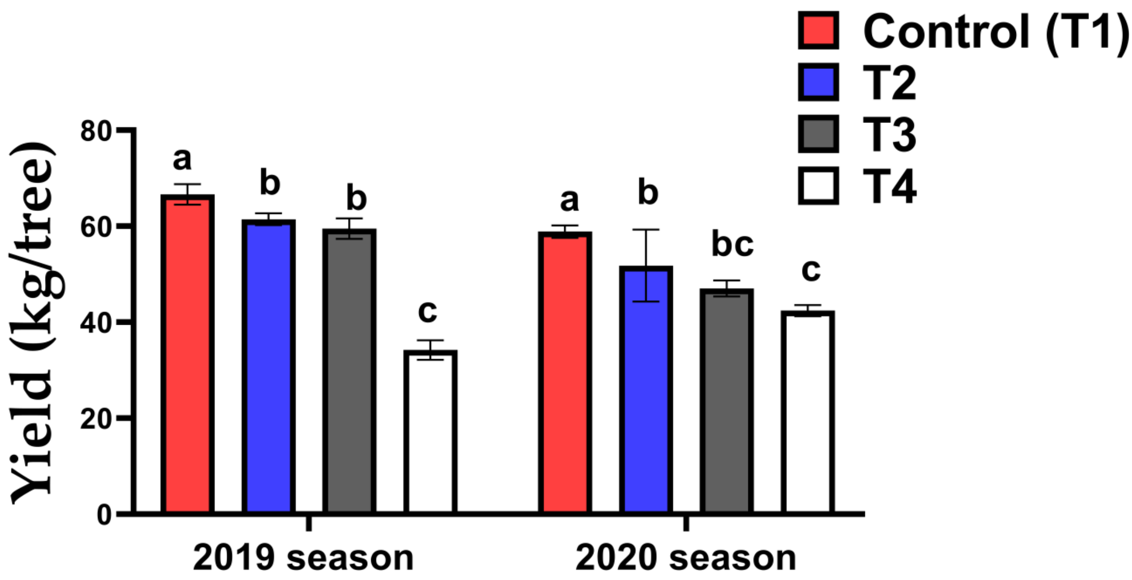

Fig. 2. Effect of irrigation treatments on the yield of Balady mandarin trees in 2019 and 2020 seasons.
T1: Control trees were irrigated every 30 days = 17 times/season & T2; trees were irrigated every 20days= 12 times/season & T3: trees were irrigated every 20 days=10 times/season & T4: trees were irrigated every 30 days =8 times/season

2. Discussion

The increase in Chlorophyll (SPAD value) in the most stressful treatment (T4) may be associated with smaller leaf area or a lower leaf water content which was confirmed by (Forest et al., 2018), observed a negative correlation between SPAD values and either specific leaf area or the leaf water content. A significant decrease of leaf area caused by water deficit may be related to slower rates of cell division and elongation which may result in smaller leaf area, this explanation observed by (Okba et al., 2022), found that the leaf area decreases as the soil moisture content lowers. Similar results were confirmed by (Ballester et al., 2011) on Clementine trees and (Pérez-Pastor et al., 2014) on apricot, where the vegetative growth was reduced by water deficit treatments.

The increase in transpiration rate may be due to higher stomatal conductance was produced by a much better water supply (Mafakheri et al., 2010; Petridis et al., 2012) or may attributed to the decrease in permeability of plasma membrane which aids in water retention in the cells during conditions of water deficit (Rodríguez-Gamir et al., 2011) for inducing citrus tolerance to water stress. The reduction in Leaf N content may be linked to a slower rate of the transpiration in water-stressed mandarin trees while, the increase in Leaf K and leaf P contents may be related to nutrients-antagonism relationship with leaf N (Smith, 1966; Rosen and Carlson, 1984).The results agree with those obtained by (Rangel et al., 2021), transpiration rate in orange leaves decreased under water deficit.

The stressed-trees except T2 mostly increased proline and phenol contents, as well as catalase and peroxidase activities comparing to the control, the accumulation of such compounds and enzymes is thought to have main roles in plant stress tolerance (Grace, 2005; Vendruscolo et al., 2007; Verbruggen and Hermans, 2008), through counteracting the oxidative damage due to ROS production under various stresses (Scandalios, 1997; Faghih et al., 2021). Similar results were obtained by (Faghih et al., 2019) confirmed leaf and leaf and fruit total phenolic content (TPC) of apple trees were increased by water-deficit treatments. Also, (Petridis et al., 2012) disclosed Oleuropein and other phenolic compounds accumulated in olive leaves as a result of water stress, indicating their potential as antioxidants. Moreover, (Okba et al., 2022) found that the endogenous proline concentration in "Taifi" pomegranate leaves decreased the greater the irrigation level.

The decrease in the fruit's water status (Torrecillas et al., 2000) and growth as a result of the highly l levels of ABA produced under such conditions (Kobashi, 1997; Brookbank et al., 2021) might resulted in reducing fruit weight and consequently fruit juice. These results agree with those registered by (Faghih et al., 2021) apple fruit weight decreased under water restriction treatments. Severe water-stress treatment (T4) and control led to increase fruit firmness more than the moderate water-stress treatments (T2 &T3) which has been reported by (Faghih et al., 2021) revealed that the firmness of the severe water- deficit trees was the highest. The reduction in yield as a result of water shortage treatments may be due to the reduction in fruit weight (Faghih et al., 2021).

Pectinase and polygalacturonase enzymes are inhibited by reduced irrigation, where the water stress encourages ethylene production and thereby glucose, fructose, and sucrose were accumulated (Ebel et al., 1993; Rangel et al., 2021), as a mean to modify the osmosis of cells under such conditions, and an improvement in fruit TSS or this improving in TSS content may be as a result of fruit’s water content. Similar results were obtained by (Wang et al., 2019) on apple, stated that water stress increased the contents of TSS and reduced fruit acidity and also (Kobashi, 1997) found that peach fruit TSS increased under water deficit. It was observed that the concentration of vitamin c was induced under water deprivation, indicating the ascorbic acid's beneficial interactions in the defense of chloroplasts and other cell compartments (Zhu, 2001).

3. Conclusions

The scarcity of water is the most important challenge facing agriculture in arid and semiarid regions. Thus, deficit irrigation is a proposed strategy to face this challenge. Most chemical fruit quality attributes (SSC, vitamin C), enzymatic activities (catalyse and peroxidase), phenols, and proline contents were improved by light and medium deficit irrigation (T2 and T3) compared to control (T1) and severe deficit irrigation (T4). As well as the physical fruit quality attributes and yield, were not significantly affected by light and medium-deficit irrigation compared to control.

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