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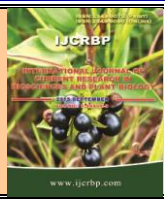

Original Research Article

Improving Physico–chemical Aspects of Wonderful Pomegranate Fruits through Fruit Thinning

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Abstract

This work was carried out to study the effect of fruit thinning on fruit quality of Wonderful pomegranate cv. in 2012 and 2013 seasons through evaluating the physiological effect of fruit thinning on the external and internal fruit quality. This work was conducted on 12 Wonderful pomegranate trees grown in sandy soil at 2.5 × 3m apart in a private orchard at Bilbees district, Sharkia Governorate, Egypt. Four thinning levels were studied in this experiment (four fruits/ cluster as a control treatment, beside three, two, and one fruit/cluster).The tested treatments significantly increased fruit weight, grains weight, total soluble solids, anthocyanin and ascorbic acid contents as well as total phenolic compounds and antioxidant activity, whereas yield and total acidity were decreased. Thinning pomegranate fruits to one fruit /cluster gave the most excellent physical and chemical abovementioned properties, but reduced yield /tree and total acidity percentage.

Keywords

Anthocyanin
Antioxidant activity
Fruit quality
Thinning
Wonderful pomegranate

Introduction

Pomegranate (*Punica granatum* L.) is a well-known fruit tree, especially in the Mediterranean countries basin (Spain, Tunisia, Turkey, Egypt and Morocco), beside Afghanistan, India and Iran (Jbir et al., 2008 and Melgarejo et al., 2009). Pomegranate in tropical and subtropical areas is considered an important fruit crop because the low cost to produce high quality fruits, economically to establish an orchard and good keeping quality for a long time (Indian Council of Agriculture Research, 2005).

Pomegranate is a deciduous fruit tree, requires high temperature in summer to fruit ripening. The cultivated area from pomegranate is increasing year to another into semi-arid and arid areas, especially where water deficiency and high concentrations of salts (Hernández et al., 2014). Last years, pomegranate cultivation has a wide expansion in several countries (FAO, 2012). Recently, its cultivation, production and consequently consumption have increased because of increasing the scientific knowledge concerned with its nutritional and medicinal value. Pomegranate fruits usually eaten directly as a fresh fruit, but nowadays after discovering

its functional properties there is an incredible increasing towards the industrial sector. In addition, it is used as a functional fruit, in health care because it contains a lot of diseases prevention substances (Fawole and Opara, 2013a; Hernández et al., 2014) and in producing cosmetic products (Al-Said et al., 2009). Many investigators *in vitro* and in vivo reported that pomegranate fruits contents play a role not only as anti-diabetic and antioxidants, but also it has an antibacterial activity (Lansky and Newman, 2007; Viuda et al., 2010). The healthy effect of pomegranate is due to its content from phytochemicals such as polyphenols and anthocyanin (Viuda et al., 2010).

Pomegranate is considered a non-climacteric fruit; so, it cannot complete its ripening process after separating from the tree (Kader, 2006). Fruit maturity is commercially depending on the color of the skin, juice acidity and grains color (Cristosto et al., 2000). The acceptable taste of pomegranate grains fresh or its juice mainly based on a combination of many factors such as total soluble solids, acidity and the percentage between them (Al-Said et al., 2009; Holland et al., 2009; Opara et al., 2009; Bchir et al., 2012). The required maturity minimum level for Wonderful pomegranate cv. based on titratable acidity is less than 1.85% and the juice color is more darker as compared with a references have been established. The concentrations of phytochemicals in pomegranate fruits and their useful activities differ according to ripening stage, cultivated variety, surrounded environmental factors, the used part of the fruit and finally ,the used method of juice extraction (Shwartz et al., 2009).

The large demand from the market request is obtaining high quality pomegranate fruit (physical and chemical characteristics) to be economically valuable (Hernández et al., 2014). Generally, setting too many fruits on the trees may have a negative effect on the yield in the following year and will affect fruit size and quality in the current season. If there is a cluster or group of fruits develop, it is recommended to remove the contacting fruits because, the contacting place has a favorable conditions for the development of insects and diseases. Generally, the main reasons for fruit thinning is to improve fruit quality, size and at the same time help in the preventing alternate bearing habit as well as, maintaining tree health. In addition, removing damaged, immature or malformed fruits allow the retention fruits to develop perfectly. There is a negative correlation between the number of fruits and their size and quality. The aim of this experiment was to characterize the suitable fruit thinning level on Wonderful pomegranate cv. to obtain high quality fruits with high economic value.

Materials and methods

The present investigation was carried out during two successive seasons of 2012 and 2013 on 10-year-old Wonderful pomegranate trees grown in a private orchard at Bilbees district, Sharkia Governorate, Egypt. The selected trees were grown in sandy soil under drip irrigation system and planted at 2 x 3 m apart. The experimental trees were selected to be healthy and nearly similar in growth vigor and uniform, it received the normal cultural practices, except for the tested thinning treatments. Generally, after fruit set all experimental trees were adjusted to 30 fruit cluster/tree then, adjusted to the following four thinning treatments:(a) Four fruits/ cluster, without any thinning as a control treatment, (b) three fruits/cluster, (c) two fruits/cluster and (d)one fruit/cluster. Each thinning treatment was conducted on three trees representing three replicates for each. All thinning treatments were carried out by the end of May when the average fruit diameter reached about 2.0cm (about 26.5% from that of the ripened fruits of control treatment). This experiment was set in a completely randomized block design with 4 treatments; each treatment was applied on three trees (three replicates).

At harvest time when the fruits reached the commercial ripening stage, fruits were picked and the yield / tree (kg) was recorded. After that, randomly fruit samples (10 fruits) from each replicate were taken and transported to the laboratory of fruit research of Horticulture Department, Faculty of Agriculture, Zagazig University, to evaluate the following fruit physical and chemical characteristics.

Fruit physical characteristics estimation

Fruit weight (g), fruit length without calyx length (cm), fruit diameter (cm),then the fruit shape index, i.e. length/width was calculated, grains (arils+seeds) weight (g) and its percentage to the whole fruit weight, flesh prominences weight (g) (weight of all fruit parts, except grains+skin) and its percentage to the whole fruit weight, skin weight plus flesh prominences weight(g) and its percentage to the whole fruit weight and skin thickness (mm).

Fruit chemical parameters determination

Pomegranate grains of each replicate were hand-separated and pressed for 3 min using a Moulinexblinder (Type 716, France) at the maximum speed to extract juice. Prior to analysis and further processing, the juice was centrifuged at 10000 xg (4°C, for 10 min) to separate the supernatant and stored at -20°C until analysis for the following chemical parameters.Juicecolour absorbance, pH, titratable acidity percentage, anthocyanin pigment and ascorbic acid contents.Titrable acidity was calculated as percentage of citric acid by titrating 10 ml of the pomegranate juice with NaOH (0.1 mol/l) reaching pH 8.1 and expressed as g 100 ml^{-1} citric acid (AOAC, 2006) and pH was assessed by pH meter (pH 211 HANNA instruments Inc. Woonsocket- USA made in Romania).

Samples of pomegranate grains were lyophlized until a constant weight was obtained. The anthocyanin content was colorimetrically determined (OD_{535} nm) according to the method described by Caleb et al. (2013).

Ascorbic acid was determined according to Klein and Perry (1982) with slight modifications (Barros et al., 2007). Briefly, juice sample (0.5 ml) was mixed with 14.5 ml of meta phosphoric acid (1%), the resulting mixture was vortexed and then sonicated in ice for 5 min followed by centrifugation at 10000 rpm for 5 min at 4°C. The supernatant (1 ml) was mixed with 2, 6-dichloroindophenol endophenol (9 ml) and the absorbance was measured within 30 min at 515 nm against a blank using a UV–vis spectrophotometer (Helios Omega, Thermo Scientific technologies, Madison, USA). Ascorbic acid content was calculated using the calibration curve of authentic L-ascorbic acid (0.01–0.1 mg/ml), and the results were expressed as ascorbic acid equivalent (AAE) per milliliter crude juice (mg AAE/ml juice).

One ml of crude pomegranate juice sample was extracted with 29 ml of cold aqueous methanol 50%. The resulting mixture was vortexed, and then sonicated in ice for 20 min in a cold water bath followed by centrifugation at 10000 rpm for 5 min at 4 °C. The supernatant was subsequently collected and assayed for antioxidant capacity and phenolic components.

The DPPH (1,1- diphenyl-2-picrylhydrazyl) radical scavenging activity was determined by the method of Gocer and Gulcin (2011) with some modification. One milliliter of aqueous methanolic juice extract was mixed with 4 ml of 0.15 mM DPPH (in 95% methanol). The mixture was then shaken vigorously using a mixer. The reaction mixture was incubated for 60 min in the darkness at room temperature. The absorbance of the resulting solution was measured at 517 nm with a spectrophotometer. Ethanol was used as a control. Percentage of antioxidant activity of free radical DPPH was calculated as follow:

$$\text{Antioxidant activity (\%)} = [(A_{control} - A_{sample})/A_{control}] \times 100$$

Where $A_{control}$ is the absorbance of the control reaction and A_{sample} is the absorbance in the presence of sample. The IC_{50}value was defined as an effective concentration of sample required to scavenge 50% of radical activity (Bursal and Gulcin, 2011). All experiments were carried out in triplicate.

For the ABTS [2,2-azino-bis(3- ethylbenzothiazoline-6-sulphonicacid)] assay the method of Re et al. (1999) was adopted. The stock solutions were 7 $mmoll^{-1}$ ABTS solution and 2.4 $mmoll^{-1}$ potassium persulfate solution. The solution was prepared by mixing the two stock solutions in equal quantities and allowing them to react for 12–16 h at room temperature in the dark. One ml of the resulting $ABTS^{\bullet+}$ solution was diluted with 60 ml of methanol at 7 mmol l^{-1}. $ABTS^{\bullet+}$ solution was freshly prepared for each assay. One milliliter of aqueous methanolic extract of Pomegranate juice were allowed to react with 5 ml of $ABTS^{\bullet+}$ solution for 7 min, then the absorbance at 734 nm was recorded. A control with no added extract was also analyzed. Scavenging activity was calculated as follows:

$$\text{ABTS radical-scavenging activity (\%)} = [(Abs_{control} - Abs_{sample})/Abs_{control}] \times 100$$

Where $Abs_{control}$ is the absorbance of ABTS radical+methanol and Abs_{sample} is the absorbance of ABTS radical + extract/synthetic antioxidant. Total phenolic compounds were determined using the Folin-Ciocalteu method (Singleton and Rossi, 1965). Briefly, 1 ml of pomegranate juice was exactly diluted to 10 ml with distilled water, then 200 ml of the diluted juice was mixed with 0.5 ml of Foline-Ciocalteu reagent, alkalinized with 1 ml of 20 g/100 g sodium carbonate solution and taken to 10 ml with distilled water. The mixture was allowed to stand for 60 min at room temperature, then the absorbance was measured by a UV/Vis spectrophotometer (JENWAY 6405 UK) at 760 nm. Total phenolics were calculated against a calibration

curve obtained with gallic acid and results were reported as gallic acid equivalents (mg/l).

Statistical analysis

The obtained data were subjected to analysis of variance (ANOVA) according to Snedecor and Cochran (1980) using SPSS program. The individual comparisons between the obtained means were carried out using LSD at 5% level.

Results and discussion

Yield/tree

The obtained data in Table 1 reveal significant effects of the tested thinning treatments on the different parts of pomegranate fruit. Regarding yield /tree, the highest significant yields were recorded for unthinned (control) trees and those thinned to three and two fruits/cluster without significant differences between them in both seasons. The lowermost yield/tree in both seasons came from the highest thinning rate treatment (one fruit / cluster). However, two fruits /cluster thinning treatment increased yield/tree by 33.7 and 47.1% compared to those thinned to one fruit /cluster in both seasons, respectively.

Fruit weight

All rates of fruit thinning significantly increased average fruit weight in the two seasons. The uppermost fruit weight (624.5 and 607.3 g) was recorded for one fruit /cluster thinning treatment in the first and second season, respectively. On the other hand, the lowermost fruit weight (200.9 and 211.0 g), was gained by control treatment (four fruits/cluster) in both seasons, respectively. It means that thinning the fruit cluster increased fruit weight by 28.1 to 210.9 % in the first season and 28.8 to 187.8 % in the second one. This result is in line with that of Greene et al. (1990). As an average of both seasons, thinning fruits to three fruits/cluster increased fruit weight by 128.4 %. The corresponding fruit weight increments for two and one fruit/cluster treatments were 216.4and 299.0% respectively.

Table 1. Effect of different levels of fruit thinning on yield characteristics of Wonderful pomegranate fruits in 2012 and 2013 seasons.

Thinning level	Yield/tree (kg)		Fruit weight (g)		Grains weight (g)/fruit		Grains weight %		Skin+flesh prominences (g) weight		Skin+flesh prominences weight %		Flesh prominences weight(g)/fruit		Flesh prominences weight %	
	2012	2013	2012	2013	2012	2013	2012	2013	2012	2013	2012	2013	2012	2013	2012	2013
(Control) without thinning	25.1	25.5	200.9	211.0	81.0	84.4	41.0	40.4	119.9	128.4	60.0	61.4	50.3	53.9	25.1	25.7
3 fruits/cluster	24.0	26.1	257.3	271.8	111.8	117.6	43.5	43.8	145.5	154.8	56.5	57.0	59.8	64.5	23.3	23.7
2 fruits/cluster	26.2	27.8	432.6	458.7	219.1	232.8	50.7	50.9	213.5	219.0	49.6	47.8	84.0	88.7	19.5	19.5
1 fruit/cluster	19.6	18.9	624.5	607.3	358.5	354.6	57.4	58.5	266.6	252.3	42.7	41.6	99.3	92.2	15.9	15.2
LSD 0.05	2.23	2.24	36.7	35.8	24.7	28.5	8.69	10.6	16.8	15.5	6.61	8.21	5.61	7.66	2.52	3.33

Light cropping trees in all cases produce fruits with larger cells and consequently larger weight and size than do small fruits produced from heavy cropping ones (Ouma, 2010). Mainly because thinning treatments changed the leaf /fruit ratio, thus, there was more number of leaves per each fruit, which support fruit growth and reduce the competition between the remainder fruits for the available photo assimilates (Palmer et al., 1997). At the same line**,** thinning treatments increases the available carbohydrates which are responsible for increasing fruit weight and size (Agusti et al., 2000). Removing small immature fruits early in the season can lead to increase fruit weight and size by minimizing the number of fruits that remain growing to harvest time permitting the fruits to ripe more quickly (Kitren and Louise, 2008). Also, thinning decrease and/or prevent alternate bearing habit and eliminate damaged or malformed fruits.

Grains weight

All thinning rates significantly increased grains weight/fruit in both seasons (Table 1). The highest grains weight/fruit (358.5 and 354.6 g) was gained by one fruit/ cluster treatment in the first and second season, respectively. On the other hand, the lower most values were recorded for control treatment (81.0 and 84.4 g in the two seasons, respectively). Comparing with control treatment, grains weight/fruit was increased by 138.7, 273.3 and 431.2% as a result of three, two and one fruit/cluster thinning treatments (average of both seasons), respectively.

The percentage of grains weight against the total fruit weight was increased significantly with two and one fruit/cluster thinning treatments.This increment ranged between50.7- 57.4% in the first season and 50.9 to 58.5% in the second one. Three fruits/cluster thinning treatment insignificantly increased grain weight percentage compared to control in the two seasons. It could be concluded that thinning treatments increased the edible part of pomegranate fruits (grains). The obtained result is in line with those of Akbarpour et al. (2009).

Weight of skin and flesh prominences /fruit

Data in Table 1, clear that the weight of fruit skin and flesh prominences(all fruit parts except grains) were significantly increased with increasing thinning level in both seasons. The highest weight of skin and flesh prominences was recorded for one fruit/cluster thinning treatment. Whereas, the lower most value was gained by control treatment. Generally, this increment may be attributed to the increase in fruit weight and size.

The percentage of weight of skin and flesh prominences/fruit was significantly decreased with increasing thinning rate, especially the highest two rates (two and one fruit /cluster). This decrement ranged between 42.7 to 49.6 % in the first season and 41.6 to 47.8 % in the second one. This result clear the positive effect of fruit thinning on decreasing the percentage of skin and flesh buttresses weight /fruit significantly. This means also that thinning treatments increased average fruit weight through increasing grains weight than increasing other fruit parts. In other words, the main effect of fruit thinning on increasing weight of pomegranate fruits is scoped or focused mainly on increasing grains weight compared to other fruit parts.

Flesh prominences weight

Data shown in Table 1 clear that, all thinning treatments significantly increased flesh prominences weight with increasing thinning level in the two seasons. This increment reached the highest value with the highest thinning level (one fruit/ cluster) compared to control treatment in the two seasons. The percentage of flesh prominences weight followed an opposite trend, since it was significantly decreased with all thinning treatment to reach its minimum value with one fruit / cluster thinning treatment in both seasons.

Fruit dimensions

Data presented in Table 2 shows significant effect of the tested thinning treatments on different physical parameters of pomegranate fruit, especially the highest two thinning rates. Regarding fruit dimensions (length and diameter), the highest significant values were recorded for the highest thinning treatments (two and one fruit/cluster) without significant differences between them. Whereas, the lowermost values were gained by control treatment in both seasons. The positive effect of thinning treatments on fruit dimensions supported those of Westwood (1993). Fruit shape index was insignificantly affected by the studied thinning treatments in both seasons.

Skin thickness

Data in Table 2 show that, all thinning treatments increased skin thickness in both seasons, but the significance was realized only with the highest two thinning levels. However, the thickest (0.75 and 0.78 mm) and the thinnest (0.45 and 0.47 mm) peel thickness were recorded for the highest thinning rate treatment (one fruit/cluster) and control treatments in the first and second seasons, respectively.

Table 2. Effect of different levels of fruit thinning on fruit length, fruit diameter, fruit shape index , peel thickness of Wonderful pomegranate fruits in 2012 and 2013 seasons

Thinning level	Fruit length (cm)		Fruit diameter (cm)		Shape index		Peel thickness (cm)	
	2012	2013	2012	2013	2012	2013	2012	2013
(Control) without thinning	6.20	5.90	7.71	7.40	0.82	0.86	0.45	0.47
3 fruits/cluster	6.93	6.40	8.20	8.60	0.85	0.77	0.48	0.51
2 fruits/cluster	8.41	8.20	9.75	10.0	0.87	0.82	0.59	0.56
1 fruit/cluster	9.50	10.6	10.8	11.2	0.88	0.99	0.75	0.78
LSD 0.05	1.33	2.02	1.40	2.85	NS	NS	0.05	0.09

Chemical fruit characteristics

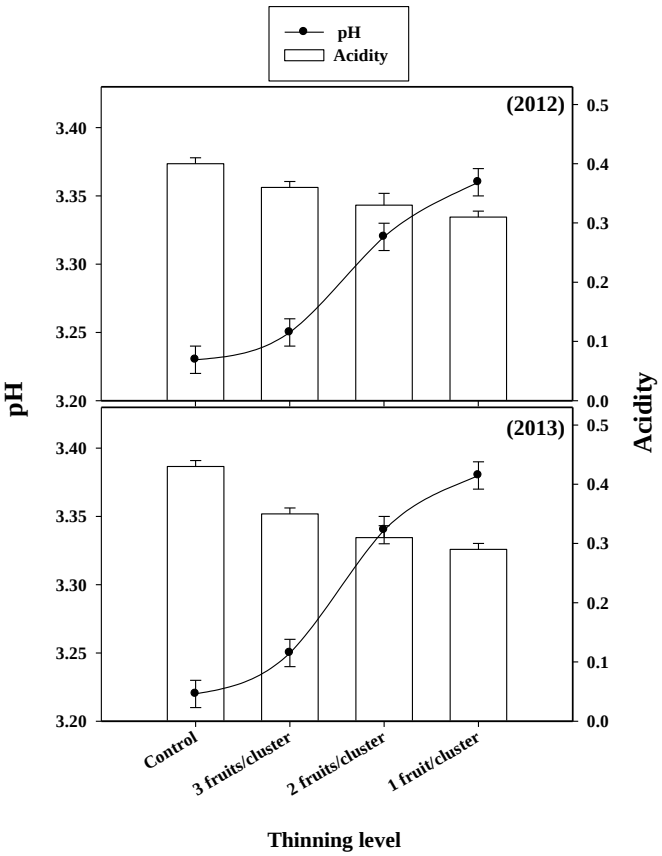
Total soluble solids percentage (TSS%)

Data in Table 3 clears that, the tested thinning treatments significantly affected TSS percentage in both seasons. The highest TSS percentage (17.0 and 17.4 %) was found in the fruit juice of one fruit/cluster thinned trees, while the lowermost percentages (15.0 and 14.4 %) were found in control treatment in the two seasons, respectively. The same trend was recorded with pH value in both seasons. Whereas total acidity percentage followed the opposite trend, since the highest and lowest total acidity percentages were recorded for unthinned (control) fruits and those thinned to one fruit/cluster, respectively in both seasons (Fig. 1).

Table 3. Effect of different levels of fruit thinning on TSS and TSS/acid ratio of Wonderful pomegranate fruits in 2012 and 2013 seasons.

Thinning level	TSS		TSS/ acid ratio	
	2012	2013	2012	2013
Control (Without thinning)	15.0	14.4	37.5	33.5
3 fruits/cluster	15.5	15.2	43.6	43.5
2 fruits/cluster	16.0	16.0	48.0	51.6
1 fruit/cluster	17.0	17.4	54.3	60.1
LSD 0.05	0.76	1.78	3.33	5.41

Fig. 1: Effect of different levels of fruit thinning on pH value and acidity percentage of Wonderful pomegranate fruits in 2012 and 2013 seasons.



The obtained results concerning TSS and acidity percentages are in line with those obtained by Gariglio et al. (2003). Pomegranate fruit ripening time is closely associated with the increase in the total soluble solids content (Fawole and Opara, 2013b). This result could be attributed to the grand received amount of nutrients and photosynthesis assimilates by the fruit, mainly because the higher number of leaves per each fruit which support fruit growth and ripening which subsequently increasing of sugars content and decreasing the acids percentage.

In both seasons, it is clear that thinning treatments significantly increased TSS/ acid ratio in the fruit juice (Table 3). The highest TSS/acid ratio was recorded for one fruit/cluster treatment (54.3 and 60.1) followed in descending order by those thinned to two fruits/cluster (48.0 and 51.6) and three fruits/cluster (43.6 and 43.5) in the two seasons, respectively. Fruits on unthinned trees gained the lowest TSS/acid ratio (37.5 - 33.5) in both seasons, respectively. These results are in agreement with those obtained by Greene et al. (1990) on several apple cultivars. Chemical attributes like TSS, acidity and TSS/acid ratio are used to describe the taste in terms of sourness or sweetness. Total acidity as citric acid greatly contribute to the flavour of pomegranate juice which vary more often in cultivars of different taste profiles compared to TSS and sugars (Dafny-Yalin et al., 2010).

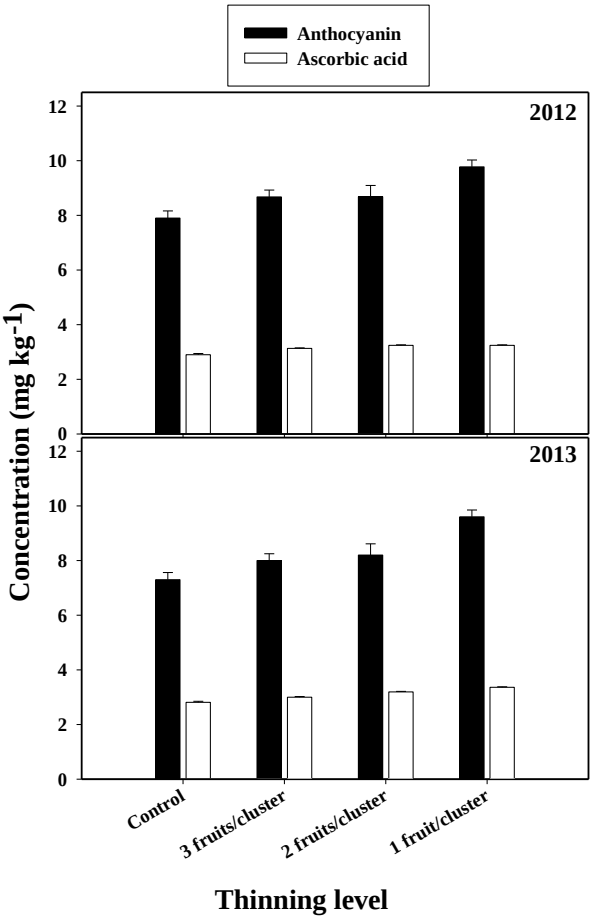
The effects of different levels of fruit thinning on anthocyanin pigment and ascorbic acid contents of Wonderful pomegranate fruits in 2012 and 2013 seasons are shown in Fig. 2. It is clear that, the concentration of anthocyanin pigment was gradually increased by all thinning treatments (8.67, 8.68 and 9.77 mg/kg^{-1} and (8.0, 8.2 and 9.6 mg/kg^{-1}by the treatment of three, two and one fruit /cluster, in the first and second season, respectively) compared to

control (7.90 and 7.30 mg/kg^{-1} in the two seasons, respectively). Anthocyanin pigment was found in pomegranate fruit juice and cause the red colour (Noda et al., 2002) and antioxidant activity of pomegranate juice (Drogoudi et al., 2005; Tzulker et al., 2007). Fig. 2 also reveals insignificant effect of the studied thinning treatments on juice ascorbic acid content in both seasons.

Table 4. Effect of different levels of fruit thinning on total phenolic compounds (TPC) and antioxidant activity (DPPH and ABTS assays) of Wonderful pomegranate fruits in 2012 and 2013 seasons.

Thinning level	TPC (mg GAE l^{-1})		Antioxidant activity (%)			
			DPPH		ABTS	
	2012	2013	2012	2013	2012	2013
Control (without thinning)	387.43	388.7	78.39	76.43	81.06	82.80
3 fruits/cluster	387.4	388.90	80.37	79.03	85.30	86.12
2 fruits/cluster	388.2	389.30	82.54	81.21	87.44	87.06
1 fruit/cluster	390.5	392.10	85.10	83.52	89.11	90.33
LSD 0.05	1.96	2.26	0.72	1.25	0.50	1.17

Fig. 2: Effect of different levels of fruit thinning on anthocyanin pigment and ascorbic acid contents of Wonderful pomegranate fruits in 2012 and 2013 seasons.



Effect of different levels of fruit thinning on total phenolic compounds (TPC) and antioxidant activity (DPPH and ABTS assays) of Wonderful pomegranate fruits in 2012 and 2013 seasons are shown in Table 4. The total phenolic content was only significantly affected when the fruits were thinned to one fruit/cluster in both seasons (390.5 and 392.10 mg GAE l^{-1}) compared to control (387.43 and 388.7 mg GAE l^{-1} in the first and second seasons, respectively). DPPH radical scavenging ability is widely used as an index to evaluate the antioxidant potential of medicinal plants. The antioxidant activity of the control treatment was 78.39 and 76.43 % in the two seasons, respectively. The extent of DPPH radical scavenging activity was significantly and gradually increased by all thinning treatments. So, this increase reached its highest value with one fruit/cluster treatment in the two seasons (390.5 and 392.10% in the first and second seasons, respectively). The same trend was observed when their ABTS was assayed (Table 4).

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