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Influence of pre-harvest spraying of gibberellic and boric acids on the growth and fruit characteristics of pomegranate fruit cv. Shishe-Kab

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ABSTRACT

Influence of foliar application of gibberellic and boric acids on fruit growth and characteristics of pomegranate cv. Shishe-Kab was carried out in the research orchard of Birjand University during 2017 and 2018 seasons. The experiment conducted in a randomized complete block design with six replications. Experimental treatments were control (spray with distilled water), gibberellic acid at 50 and 150 mg L^{-1} and boric acid at 200 and 600 mg L^{-1}. Gibberellic acid and boric acid sprayed three times at one-month interval started from late May, to July during fruit growth and developmental stages. At mid-October fully mature fruits were harvested and fruit quality and quantity were assessed in the laboratory of Department of Horticultural Science, University of Birjand. Results of this study indicated that foliar application of gibberellic acid and boric acid decreased chroma of pomegranate peel in 2017 and 2018, while it increased hue of pomegranate peel. Foliar application of boric acid significantly increased length, volume, fresh weight, and juice content of pomegranate fruit. However, peel thickness and sunburned were significantly increased by application of gibberellic acid in both studied years. Total acidity was significantly increased by application of gibberellic acid in 2018 season. Leaves chlorophyll content increased by application of 150 mg L^{-1} gibberellic acid and 200 mg L^{-1} boric acid in both seasons. Application of 50 mg L^{-1} of gibberellic acid significantly increased total soluble solid of pomegranates in 2017 and 2018. This result showed that foliar application of gibberellic acid and boric acid had positive effects on quality and quantity properties of pomegranate fruits cv. Shishe-Kab cultivar and it may be used to improve fruit production. However, further studies need to determine the exact application time and concentration for practical application.

Keywords: Acidity, boron, chlorophyll, gibberellin, sunburned

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1 Introduction

Pomegranate (*Punica granatum* L.) with hundreds of known varieties is native to central Asia and due to high adaptively to a wide range of climates and soil conditions cultivated in worldwide (Holland et al., 2009). Pomegranate widely used as fresh fruit and juice. Also, it has been used extensively in the tradition of many cultures. Various organic acids, phenolic compounds, sugars, water-soluble vitamins, and minerals are some of pomegranate valuable compounds (Aviram et al., 2000). It is known as one of the most important commercial fruits in Iran (Anonymous 2015). For example 'Shishe-Kab' is a commercial cultivar in the South Khorasan province that has delicious fruits with high marketability (Ebtedaei and Shekafandhe, 2016).

Boron is necessary as a micronutrient for the growth and development of vascular plants (Rasheed, 2009). Because of its role in the growth of the pollen tube during flower pollination and thus fruit and seed production, adequate B nutrition is crucial for crop production (Camacho-Cristóbal et al., 2011). Growth and development of floral structures require a higher rate of B than do vegetative structures (Dell and Huang, 1997). So, to supply sufficient amounts of B for reproductive development boron often used as the foliar application (Solar and Stampar, 2001). Similarly, gibberellins (GAs) are essential for many developmental processes in plants. Gibberellins can increase the organ ability to work as a nutrient sink (Addicott, 1982) and control fruit development in various ways. Also, they have known to affect cell division and cell enlargement (Adams et al., 1975). Increasing fruit production is one of the main functions of foliar application of gibberellic acid (Lombardo et al., 1978). Gibberellins influenced fruit growth in species such as citrus (El-Sese, 2005), litchi (Chang and Lin, 2006), guava (Horvitz et al., 2003), and pear (Zhang et al., 2007). Optimizing pre-harvest condition is necessary to achieve a higher fruit quality and yield (Antunes et al., 2007). Foliar application by improving carbohydrate and mineral storage content has been shown to have positive effects on fruit quality (Vajari et al., 2018). Hence, the application of phytohormone and micronutrient are considered the most effective way to improve physical properties of fruits.

Review of literature shows positive effects of these chemicals on different pomegranate cultivars and improved the quantity and quality of fruit. Khalil and Aly (2013) reported gibberellic acid and boric acid application increased fruit weight, fruit peel, fruit diameter and fruit length of pomegranate Manfalouty cultivar. Also, pre-harvest application of 50- 200 ppm at full bloom, 45 and 90 days after full bloom increased aril weight and fruit juice content and also resulted in the highest fruit yield in pomegranate cv. Ganesh (Venkatesan and Koder Mohideen, 1994). In addition, foliar application of boric acid significantly increased fruit yield in pomegranate cv. Ganesh (Balakrishnan et al., 1996). GA spray improved total soluble solid of pomegranate cv. Ruby (Ghosh et al., 2009). However, little information is available about physiological response of Shishe-kab cultivar to gibberellic acid and boric acid foliar application during growth and developmental stages. Therefore, the purpose of the present investigation was to study the effects of pre-harvest application of gibberellic acid and boric acid on the growth and quality properties of pomegranate fruit cv. Shishe-kab during 2017 and 2018 growing seasons.

2 Materials and Methods

The pomegranate trees were selected from the research orchard of the Faculty of Agriculture, the University of Birjand during 2017 and 2018 seasons. All trees were similar regarding age (9 years) and orchard management. One-year-old shoots with the same length (45±5 cm), diameter and flowering were tagged for future treatments. There were five treatments as follows: control (spray with distilled water), gibberellic acid (50 and 150 mg L^{-1}) and boric acid (200 and 600 mg L^{-1}). The foliar spray was given three times at one-month interval started from late May, to July during fruit growth and developmental stages in the morning. Fruits were picked at maturity stage in mid-October. This experiment was designed in a randomized complete block design with six replications for each treatment and three plants for each replication. All measurements were carried out in the laboratory of Department of Horticultural Science, University of Birjand.

2.1 Growth characters and quality

Color measurements Color measurements were performed using a color meter (TES-135A, Taiwan). The hue and chroma of pomegranate fruits were estimated with the following equations as suggested by Voss (1992) and Erguneş and Tarhan (2006), respectively.

$$h = \tan^{-1}\left(\frac{b}{a}\right) \tag{1}$$

$$C = \left(a^2 + b^2\right)^{0.5} \tag{2}$$

where, h = hue angle, C = chroma, a = redness, and b = yellowness.

Fruit specifications Fruit length and diameter were measured with a caliper with 0.1 mm sensitivity. Fruit volume was obtained from the water displacement method (Mohsenin, 1986; Storshine and Hamann, 1994). Fruits weight was weighted using a Scale with 0.01 g sensitivity. Arils were weighted using a scale with 0.01 g sensitivity.

Juice volume Juice volume was measured using a graduated cylinder.

Peel thickness Peel thickness was determined with the help of caliper with 0.1 mm sensitivity.

Sunburned The ratios of the sunburned fruits were determined by dividing the affected fruits to the total number of fruits (Hegazi et al., 2014).

2.2 Total chlorophyll

Total chlorophyll in leaves was determined in late September. 0.025 g of leaves were homogenized in 5 mL 80% acetone. The samples kept for 24 h in the fridge and then absorbances were read at 470, 645 and 663 nm by Unico 2100 spectrophotometer (Lichtenthaler, 1987).

2.3 Total acidity

Total acidity was determined by the titration using sodium hydroxide (Cochran et al., 1986).

2.4 Total soluble solids

Total soluble solid in the extracted juice of fruit was measured by a hand-held refractometer (Extech Co., Model RF 10, Brix, 0–32%, USA), and the results were expressed as °Brix.

2.5 Data analysis

Obtained data were analyzed by Genstat (Discovery Edition, Version 9.2, 2007, VSN International Ltd., UK) and mean values were compared at the level of 5% probability according to LSD test.

3 Results and Discussion

3.1 Color attributes

Analysis of results in Table 1 reveals that the application of gibberellic acid and boric acid significantly affected the hue of pomegranate fruit. Foliar application of gibberellic acid and boric acid at two different concentrations decreased chroma and increased hue of pomegranate peel in 2017 and 2018. Application of 600 mg L^{-1} boric acid significantly decreased lightness, redness and yellowness of pomegranate peel compared to control. Also, spraying 150 mg L^{-1} gibberellic acid decreased yellowness of pomegranate peel. The decrease in chroma and increase in the hue of pomegranate peel might be a consequence of the absence in some phytochemical compounds such as antioxidants and anthocyanin in fruits peel.

3.2 Fruit length

Spraying with gibberellic acid and boric acid significantly increased fruit length of pomegranate in 2017 and 2018 (Table 2). The maximum fruit length (93.9 mm) was recorded under 200 mg L^{-1} boric acid over control in the two studied seasons. Similar results have been reported by Kumar et al. (2017), and Khalil and Aly (2013). Gibberellin promotes growth by increasing plasticity of the cell wall and also by hydrolysis of starch into sugars reduces the cell water potential, that results in the entry of water into the cell and causing elongation thus affect fruit size (Richard, 2006). Aulakh et al. (2005) reported that increasing fruit length by application of gibberellic acid might be related to the role of growth regulators in cell division, cellular expansion and also increasing intercellular spaces in mesocarp cells. Similarly, boric acid affects cell elongation and cell division (Rath et al., 1980). Also, boron increase sugar translocation that may increase fruit physical characters (Shalan, 2013). Boric acid by affecting cell division and nucleic acid synthesis during fruit development resulted in fruit growth. O'Kelley (1957) also reported that the use of boron increased oxygen uptake and transfer of sugars that increase fruit size. Boron is one of the nutrients that contribute to cell division, cells expansion, and sugar translocation.

3.3 Fruit diameter

Data in Table 2 reveals that various concentrations of gibberellic acid and boric acid affected the fruit diameter of pomegranate in 2017 and 2018. Application of 150 mg L^{-1} gibberellic acid and 200 mg L^{-1} boric acid resulted in maximum fruit diameter of pomegranate in 2017 and 2018, respectively. Khalil and Aly (2013) reported spraying 80 mg L^{-1} gibberellic acid and 3000 mg L^{-1} boric acid increased diameter in pomegranate fruit Manfalouty cultivar. In another study, Kumar et al. (2017) observed that spraying of 6000 mg L^{-1} boric acid 30 days after fruit setting increased fruit diameter of pomegranate cv. Jodhpur Red. Increasing fruit diameter can be related to the role of boric acid and gibberellic acid in cell growth (Lal et al., 2011). There is various evidence that gibberellin effect on cell division and ovule growth (Ranganna, 1995). Increase in fruit diameter can be attributed to this fact that gibberellin has been used during fruit development. Shukla et al. (2011) reported that boric acid treatment increased the size of anola fruit, which could be due to the role of micronutrients in accelerating photosynthetic activity.

3.4 Fruit volume

The results showed that fruit volume was influenced by the pre-harvest application of gibberellic acid and boric acid in 2017 and 2018. In 2017, the maximum fruit volume (336 cm^3) was obtained in treated fruits with 150 mg L^{-1} gibberellic acid, although statistically there was no significant difference with 200 mg L^{-1} of boric acid. Mean comparison in 2018 showed that the maximum fruit volume with 320 cm^3 was observed in treated fruits with 600 mg L^{-1} of boric

Table 1. Effect of different treatments on color attributes of pomegranate peel in 2017 and 2018 seasons

Treatment	Lightness (*L*)	Redness (*a*)	Yellowness (*b*)	Hue (*h*)	Chroma (*C*)
2017					
Control	40.3c	53.9a	22.1c	22.2c	58.3a
BA 200	41.4bc	49.0b	22.1c	24.3c	53.7b
BA 600	46.8a	41.3e	24.3b	30.5ab	47.9d
GA_3 50	42.6b	43.6d	27.0a	31.7a	51.3c
GA_3 150	40.9c	46.8c	25.4ab	28.5b	53.3bc
2018					
Control	43.1a	48.5a	23.1ab	30ab	53.7a
BA 200	39.6b	47.6a	23.2ab	27.3b	52.9a
BA 600	21.1d	32.6c	22.0bc	16.6c	39.3c
GA_3 50	30.2c	38.5b	21.1c	24.8bc	44.0b
GA_3 150	38.7b	43.9a	24.3a	39.1a	50.2a

Table 2. Effect of different treatments on growth characteristics and quality of pomegranate fruits in 2017 and 2018 seasons

Treatment	Fruit len. .(mm)	Fruit dia (mm)	Fruit vol. (cm^3)	Fruit wt. (g)	Arils wt. (g)	Juice vol. (mL)	Peel thick. (mm)	Sunbur-ned (%)
2017								
Control	75.7d	73.7c	194.8c	247.7c	115.8d	74.1c	3.2b	49c
BA 200	93.9a	79.8b	335.0a	320.2a	137.0c	114.6a	3.2b	52c
BA 600	73.8d	72.4c	190.5c	245.8c	156.8b	72.1c	3.2b	48c
GA_3 50	84.7c	73.6c	285.2b	268.5b	119.8d	74.8c	4.3a	66b
GA_3 150	90.0b	86.5a	336.0a	265.1c	186.8a	94.1b	4.2a	79a
2018								
Control	80.6c	81.2b	254.5c	313.4c	156.7c	114.2c	3.7b	50c
BA 200	96.3a	92.2a	316.7ab	370.0a	176.8a	137.2a	3.7b	50c
BA 600	89.4b	82.1b	320.8a	299.7c	170.5b	121.0bc	3.7b	47c
GA_3 50	93.4ab	81.8b	304.2b	320.2bc	151.1c	103.3d	3.6b	65b
GA_3 150	89.9b	80.5b	277.5c	329.2b	174.0ab	122.8b	4.6a	79a

Table 3. Effect of different treatments on TA and TSS of pomegranate fruits and total chlorophyll, chlorophyll a and chlorophyll b of pomegranate leaves in 2017 and 2018 seasons

Treatment	TA (g 100 mL^{-1})	TSS (%)	Total chlorophyll ($\mu g\ mL^{-1}$)	Chlorophyll a ($\mu g\ mL^{-1}$)	Chlorophyll b ($\mu g\ mL^{-1}$)
2017					
Control	26.6a	18.5c	15.5bc	12.0b	3.4ab
BA 200	25.8a	19.8b	27.7a	20.4a	7.2a
BA 600	23.6ab	17.9c	11.1c	7.7c	3.4ab
GA_3 50	19.9b	22.6a	19.4abc	14.8b	4.6ab
GA_3 150	26.1a	19.0bc	23.8ab	21.2a	2.6b
2018					
Control	23.1ab	17.5c	12.6b	9.8b	3.0b
BA 200	22.8b	19.0b	24.9a	18.5a	7.5a
BA 600	23.1ab	17.9bc	8.3b	8.8d	3.5b
GA_3 50	20.1c	23.3a	16.6ab	12.4c	3.8b
GA_3 150	24.3a	18.6bc	21.0a	15.8b	2.3b

Control (distilled water), BA 200 = Boric acid 200 mg L^{-1}, BA 600 = Boric acid 600 mg L^{-1}, GA_3 50 = Gibberellic acid 50 mg L^{-1} and GA_3 150 = Gibberellic acid 150 mg L^{-1}, respectively.
In each column, means with the same letter are not significantly different at 5% level of probability using LSD.
TA = titratable acidity, TSS = total soluble solids

acid, although statistically there was no significant difference with 200 mg L^{-1} boric acid. Application of 75 mg L^{-1} gibberellic acid and 3000 mg L^{-1} boric acid significantly increased volume of pomegranate fruit cv. Bhagwa (Digrase et al., 2016). These results are similar with findings of Khalil and Aly (2013) on pomegranate.

Boron improves physical properties of fruit not only by contributing to cell elongation and cell division but also transporting organic food (Wojcik et al., 2008). Likewise, growth regulators increase sugar transfer to flowers and fruits, resulting in sugar accumulation and developing these organs (Nawaz et al., 2008). Shukla et al. (2011) stated that an increase in fruit diameter and volume could be related to the higher accumulation of food material and increase in size of fruits. Gibberellin affects fruits size by its role in cell division and prolongation.

3.5 Fruit weight

Results in Table 2 revealed that maximum fruits weight (320.2 and 370 g in 2017 and 2018, respectively) were observed by foliar application of boric acid at 200 mg L^{-1} and minimum fruits weight (245.8 and 299.7 g in 2017 and 2018, respectively) by application of 600 mg L^{-1} boric acid. These findings are in line with those observed by Digrase et al. (2016) and Khalil and Aly (2013). As mentioned before, boric acid and gibberellic acid has an important role in cell elongation and cell division that could result in increasing fruit weight. Rokaya et al. (2016) stated Increase in fruit growth with GA_3 treated fruits might be due to the mediating process for faster translocation and mobilization of photosynthates from the source. Marschner (1995) reported that the role of boron in enhancing many metabolic processes such as carbohydrate transport might increase fruit yield.

3.6 Arils weight

Foliar application of gibberellic acid at 150 mg L^{-1} concentration and boric acid at 200 mg L^{-1} concentration resulted in the maximum arils weight of pomegranate Shishe-Kab cultivar in 2017 and 2018, respectively (Table 2). Khalil and Aly (2013) reported that spraying 3000 mg L^{-1} boric acid and 80 mg L^{-1} gibberellic acid increased arils weight in pomegranate cultivar 'Manfalouty'. The increase in arils weight is due to the effect of these treatments (boric acid and gibberellic acid) on the physical properties of the pomegranate fruit.

3.7 Juice volume

The effect of boric acid on juice volume showed significant differences (Table 2). The maximum juice volume (114.6 and 137.2 mL in 2017 and 2018, respectively) was recorded in fruits sprayed with 200 mg L^{-1} boric acid. The improvement of the length, diameter and weight under boric acid application caused an increase in arils and that resulted in an increase in juice volume. Similar results were reported by Sahu and Sahu (2018) and Venkatesan and Koder Mohideen (1994). Spraying time should be chosen optimally since the time interval between spraying and harvesting can affect the increase in fruit juice (Fidelibus et al., 2002).

3.8 Peel thickness

Results in Table 2 showed gibberellic acid at 150 mg L^{-1} concentration significantly increased peel thickness in the two studied seasons. Also, 50 mg L^{-1} gibberellic acid significantly affected peel thickness in 2017. The maximum and minimum peel thickness was observed in treated fruits with 150 mg L^{-1} gibberellic acid and 200 mg L^{-1} boric acid, respectively. Similar to our results Kishor et al. (2017) reported that pre-harvest application of gibberellic acid at 50 and 75 mg L^{-1} significantly increased peel thickness in pomegranate fruit cv. Bhagwa. In another study, Korkmaz and Aşkın (2017) reported that the foliar application of gibberellic acid at 75 mg L^{-1} in late July increased the accumulation of calcium in pomegranate peel of Hicaz Nar variety. Gibberellic acid application increased calcium accumulation in fruits peel and increased peel thickness.

3.9 Sunburn

Sunburn is a physiological disorder that skin color change at high temperatures and intensity of sunlight. Arils usually are dehydrated and colorless in the sunburned area (Weerakkody et al., 2010). The percentage of sunburned fruits is shown in Table 2. Spraying gibberellic acid at both concentrations significantly increased the percentage of sunburned in 2017 and 2018 seasons. The highest percentage of sunburned (79%) was recorded in fruits treated with 150 mg L^{-1} gibberellic acid. Karami (2016) reported that spraying with 30 and 60 mg L^{-1} of gibberellic acid did not have any significant effect on the percentage of sunburn in pomegranate fruit Malase- Saveh cultivar. However, Ehteshami et al. (2011) reported that application of 150 mg L^{-1} of gibberellic acid in mid-June decreased the percentage of sunburn in pomegranate fruit Robab Nairez cultivar. This inconsistency can be due either to the difference in the number of spraying, timing or difference among pomegranate cultivars.

3.10 Titratable acidity

Application of gibberellic acid and boric acid at different concentrations significantly affected the titratable

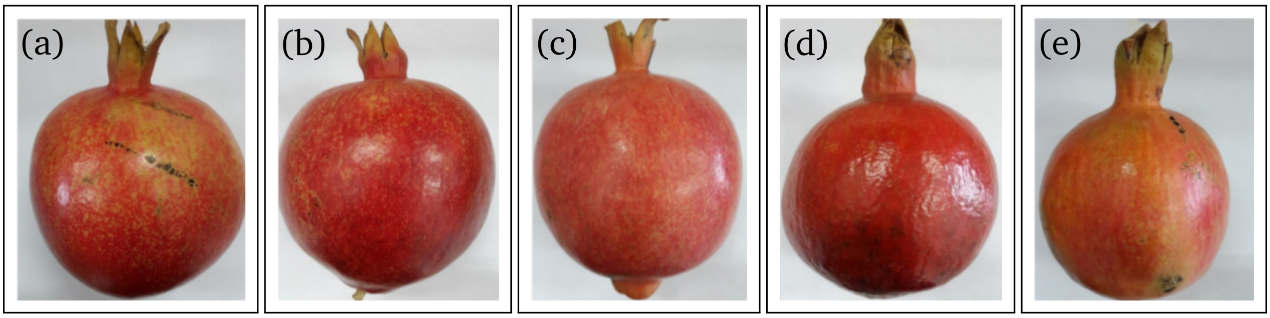

Figure 1. Effect of chemicals sprays on the visual appearance of pomegranate fruits cv. Shishe-Kab in 2018. Treatments were: (a) control (distilled water), (b) boric acid 200 mg L^{-1}, (c) boric acid 600 mg L^{-1}, (d) gibberllic acid 50 mg L^{-1}, and (e) gibberllic acid 150 mg L^{-1}

acidity of the pomegranate fruit. Foliar application of 50 mg L^{-1} gibberellic acid decreased titratable acidity compared to the control in 2016 and 2017. Similarly, foliar application of 40 mg L^{-1} gibberellic acid decreased titratable acidity of pomegranate fruit cv. Yousefkhani (Jafari, 2014). These results are in accordance with the results of Yadav et al. (2010) in aonla. They stated that mineral compounds decreased the acidity in fruits since it is neutralized in parts during metabolic pathways and/or used in the respiratory process as a substrate. Also, Shukla et al. (2011) reported that the acid content of the fruits after application of plant growth regulator has either been changed into sugar and their derivatives by, response involving the reversal of glycolytic pathway or been used in respiration or both.

3.11 Total soluble solids

The data (Table 3) indicate that foliar application of 50 mg L^{-1} gibberellic acid and 200 mg L^{-1} boric acid increased total soluble solids of pomegranate fruits. The highest total soluble solids was obtained in fruits sprayed with 50 mg L^{-1} gibberellic acid (22.6 in 2017 and 23.3 in 2018). Khalil and Aly (2013) reported that spraying gibberellic acid and boric acid increased total soluble solids of pomegranate cv. Manfalouty. Soluble solids reflect the amount of sugar in the fruit. Gibberellins effect activation of the amylase enzyme that is responsible for the change of starch into sugars which could increase TSS (Shukla et al., 2011). Yadav et al. (2010) reported that the high amounts of soluble solids in aonla fruits treated with boric acid and gibberellic acid relate to the rapid transformation of complex carbohydrates to soluble sugars, as well as the rapid mobilization of metabolites from source to the reservoir.

3.12 Total chlorophyll

Chlorophylls are green pigments that present in all higher plants and are responsible for photosynthetic (Hosikian et al., 2010). Application of 200 mg L^{-1} boric acid significantly increased leaf total chlorophyll in 2017 and 2018 seasons. The maximum total chlorophyll was observed in fruits treated with 200 mg L^{-1} boric acid. Similar to our results, Sahu and Sahu (2018) observed that foliar application of boric acid at 2000 and 4000 mg L^{-1} increased leaf chlorophyll in pomegranate cv. Kandhari. In another study spraying 200 mg L^{-1} boric acid increased chlorophyll in olive leaves but the application of higher concentration of boric acid (400 and 600 mg L^{-1}) decreased chlorophyll content (Hegazi et al., 2018). Boron application increases the chlorophyll content and photosynthesis intensity in leaves and improves the transfer of photosynthetic materials (Nikkhah et al., 2013). It has also been proved that boron delay senescence in leaves by affecting the amount of leaves chlorophyll and increasing the synthesis of indole acetic acid that prolonged photosynthesis period and improved carbohydrate production and its transmission to growing organs like leaves (Brown et al., 2002). Similarly, application of gibberellin increases the synthesis of photosynthetic pigments (Sardoei et al., 2014). Spraying 200 mg L^{-1} boric acid and 150 mg L^{-1} gibberellic acid significantly increased chlorophyll compared to the control. But the application of boric acid at 600 mg L^{-1} decreased the leaf chlorophyll content in 2017 compared to the control. Chlorophyll b content in pomegranate leaves significantly increased by application of 200 mg L^{-1} boric acid. Similar results were observed by Shamsadini et al. (2016) that foliar application of 200 mg L^{-1} boric acid increased chlorophyll a content in walnut leaves, but did not affect the amount of chlorophyll b. In another study, Zang et al. (2016) reported that application of 500 mg L^{-1} gibberellic acid increased chlorophyll a in blueberry.

4 Conclusions

Physical characteristics such as diameter, length, weight, the volume of fruit, arils weight and juice volume significantly increased by application of gibberellic acid and boric acid. Also, leaves chlorophyll was increased under these treatments. The TSS and TA were affected by different treatments. In conclusion, foliar application of boron and gibberellin could be adopted in commercial pomegranate orchards as a practice to increase fruit production, however, further studies needed.

Conflict of Interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

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