

## Effects of Growth Regulators Ethephon and 2,4-D Isopropyl Ester on Fruit Ripening and Quality of Fig under Rain-Fed Conditions

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### ABSTRACT

Fig (*Ficus carica* L. cv. Sabz) fruit growth and development under rain-fed conditions differs from fig grown under irrigated conditions and responds differently to the growth regulators application. This study aimed to reveal how ethephon (0, 50, 100, 150, 200 mg L<sup>-1</sup>) and 2,4-D isopropyl ester (0, 5, 10, 15, 20 mg L<sup>-1</sup>) can accelerate fruit ripening in fig cv. 'Sabz'. Fruits were sprayed three times by foliar application at 15-days intervals for two consecutive years. The highest fruit ripening percentage occurred with the second time that ethephon (200 mg L<sup>-1</sup>) was sprayed. Application of 100 mg L<sup>-1</sup> ethephon increased the total soluble solids with the second and third foliar spraying. Ostiole cracking in dry fig fruits became most prevalent when applying 50 mg L<sup>-1</sup> ethephon by the third foliar spraying. The highest carotenoid content was caused by 100 mg L<sup>-1</sup> ethephon by the second foliar application. Furthermore, the highest sugar content occurred in response to the second foliar spraying of 50 mg L<sup>-1</sup> ethephon. The second 2,4-D isopropyl ester (10 mg L<sup>-1</sup>) foliar spraying significantly increased fruit diameter. Since the crop has asynchronous nature of ripening, harvesting continued for several weeks. Ethephon (200 mg L<sup>-1</sup>) accelerated fig harvesting and made it occur about 7 days sooner under rain-fed conditions. Therefore, accelerating fig fruits ripening can reduce the likelihood that fruits will be exposed to drought near the end of the season. Also, ethephon treatment can help prevent loss of fruit quality.

**Keywords:** Asynchronous ripening, *Ficus carica*, Hastening ripening, Foliar spraying, Ostiole cracking.

### INTRODUCTION

The fig (*Ficus carica* L.) is one of the earliest cultivated fruit trees in human history. It is native to Iran, Asia Minor and Syria (Flaishman *et al.*, 2008; Rahemi and Jafari, 2008). Figs are consumed as fresh and dry, although dry consumption plays an important role in export, especially when cultivated under rain-fed conditions. Rain-fed conditions are managed by the establishment of a micro-chachment around each tree, so that rainwater is made more available to the tree during the rainy season. Since trees are not irrigated during the growing season (from March to

September), they rely on the natural amount of moisture in the soil. In Iran, there are many rain-fed fig orchards that have historical background (Rahemi and Jafari, 2008). Nowadays, fig tree is mainly widespread in the Mediterranean region since it has high adaptation to different edaphoclimatic conditions (Hssaini *et al.*, 2020).

Fig growth and development in rain-fed conditions, like other fruit crops, is regulated by endogenous (carbohydrates, type and source of pollen grain and nitrogen) and exogenous factors (environmental conditions and pruning). Other factors such as planting density, irrigation and fertilization can induce

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growth and tree development. Using plant growth regulators is one way to stimulate early ripening by changing the internal physiological processes in plants. Different plant growth regulators can be used for achieving this purpose (Rademacher *et al.*, 2004; Gaaliche *et al.*, 2017; Lama *et al.*, 2019). Among them, ethephon can stimulate ethylene production and can accelerate the physiological process of fruit maturation (Owino *et al.*, 2006). Ethephon (2-chloroethylphosphonic acid), an ethylene releasing compound, is considered as non carcinogenic to humans by IARC (International Agency for Research on Cancer). It penetrates into the fruit and decomposes to ethylene, and has been shown to hasten ripening of several fruits including bananas, apples, tomatoes, mango, peaches and guava (Maduwanthi and Marapana, 2019). When ethylene is released from ethephon, endogenous ethylene is created, fruit sugar is increased and color change is hastened by fruit ripening (Zhang *et al.*, 2012; Hussain *et al.*, 2015). Synthetic auxins can be applied during the second stage of fruit development and could positively influence fruit growth (Stern *et al.*, 2007). In addition, the only form of 2,4-Dichlorophenoxyacetic acid (2,4-D) registered for use as a Plant Growth Regulator (PGR) on citrus in the USA is the isopropyl ester. In 1992, a task force was formed to pursue studies needed for the re-registration of selected forms of 2,4-D used in the USA in herbicide formulations. The isopropyl ester is not a part of this group and decomposes gradually in the plant after induction (Anthony and Coggins, 1999). Application of 2,4-Dichlorophenoxypropionic acid (2,4-DP), and 3,5,6-Trichloro-2-Pyridyloxyacetic Acid (3,5,6-TPA) at the beginning of the pit-hardening in 'Bing' cherry fruits stimulated cell enlargement in the mesocarp and resulted in a significant improvement of fruit size and total yield (Stern *et al.*, 2007). Also, 2,4-DP sprays have reportedly enhanced the skin coloration of 'Cripp's Pink' apple at commercial harvest (Stern *et al.*, 2010). Fruit size of 'Salustiana' sweet orange was enhanced by 2,4-D treatment during flowering

without affecting the yield (Rebolledo *et al.*, 2012). On the other hand, a remarkable information indicates that inhibitors of ripening could be auxins that resist ripening by the delay in the ripening processes in some fruits following treatments with either 2,4-D or indoleacetic acid (Tingwa and Young, 1975). The application of auxins at higher concentrations shows an adverse effect on fruit ripening which, in turn, prevents the ripening of date palm fruits (Tavakoli and Rahemi, 2014).

Fig tree (*Ficus carica* L.) is one of the most important crops grown in Iran. Most of the fig trees in the country are cultivated in rain-fed orchards of Estahban Region (Fars Province), providing 90% of dried fig production (Abdolahipour *et al.*, 2018). Fig syconium growth occurs in three different stages, described by a double sigmoid curve. The first stage (period I), during the first six weeks of growth, is characterized by rapid diameter increase and slightly lower increases in moisture, fresh and dry weight, and sugar content. The second growth stage (period II), during the successive four weeks, is characterized by a reduction in diameter growth, moisture content, and fresh and dry weight accumulation rates. The third stage of growth (period III), during the remaining four weeks prior to maturation, is characterized by a marked increase in diameter growth, rates of fresh and dry weight, and both moisture and sugar content (Crane *et al.*, 1970; Crane and Brown, 1950).

Dried figs should be fully ripe on the tree and partially dry there (Ferguson *et al.*, 1990). They fall to the ground, where they are collected by labors and are then placed under the sun to lose their moisture. In Estahban Region, in order to achieve fig drying process, the fruits are exposed to sunlight and dried for three days in a special place (Eshpang) until the desirable amount of moisture is reached (about 11-14%) (Sedaghat and Rahemi, 2018). Dried figs are harvested in late August until early September. During this period, a sudden rainfall can change the color and taste of dried figs in the area. Therefore, the use of

chemical compounds that induce early fig fruit ripening to prevent reducing the quality and taste is inevitable. The early fig fruits ripening can be accelerated by auxins and ethephon applications. The practice of stimulating growth to hasten fig fruit ripening has been the subject of several studies (Çelikel *et al.*, 1997; Lama *et al.*, 2019, 2020). Using ethephon on 'Mission' fig fruits at or after a critical stage of fruit development stimulated their ripening within 8 days (Crane *et al.*, 1970). Dramatic pigment changes occurred during this period as the fruit skins turned from green to bluish black (Puech *et al.*, 1976). Ethephon application accelerated fruit maturation period in two date fruit cultivars at kimri stage by improving the biochemical attributes (Hussain *et al.*, 2015). Moreover, the application of synthetic auxin (2,4-DP) influenced the ripening capacity of 'La France' pear (Kondo *et al.*, 2004). Ethephon and auxin treatments can hasten the ripening of dried fruits of fig cv. Sabz under rain-fed conditions, but the specifications of this hypothesis have not been studied systematically. In addition, the late occurrence of ripening, harvesting and drying in fig fruits could make fruits susceptible to fungal diseases, browning and sourness due to increased relative humidity or rainfall at the end of season. Therefore, early and compact ripening of rain-fed figs can serve as key roles in reducing these damages.

Therefore, the aim of this study was to assess the changes in physicochemical properties of dried fig fruits after ethephon and 2,4-D isopropyl ester applications to hasten fig fruit ripening. The idea of this research was carried out for the first time under rain-fed conditions.

## MATERIALS AND METHODS

This experiment was conducted during two consecutive years (2016 and 2017) by three times of foliar application of two plant growth regulators. It involved three blocks as a factorial experiment in the framework of a completely randomized block design.

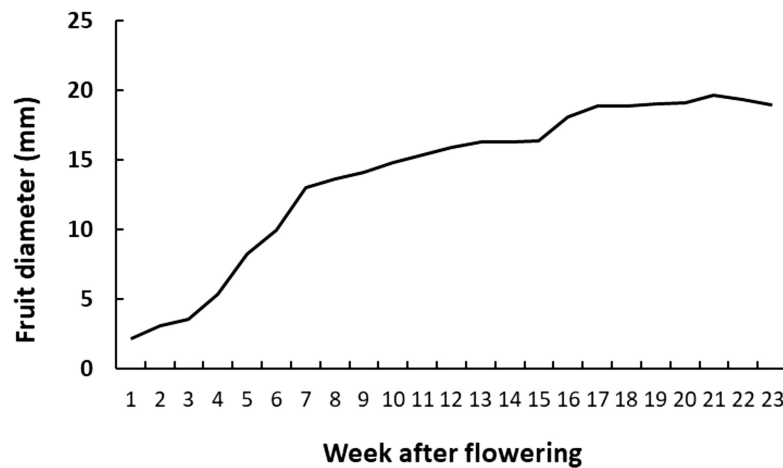
To enable the timely use of ethephon (2-chloroethyl phosphonic acid, MW= 144.49 g mol<sup>-1</sup>, Sigma CO) and 2,4-D isopropyl ester (2,4-dichlorophenoxy isopropyl ester, MW= 263.11 g mol<sup>-1</sup>, Sigma CO), a fruit growth-in-diameter curve was developed using fig 'Sabz' growing at the Fig Research Center of Estahban (longitude: 29° 6' 52", latitude: 54° 4' 27"), Iran. Data related to the meteorological conditions of the experimental site during both years are displayed in Table 1. Cross diameters of 15 fruits were recorded per branch on each tree. In total, 25 trees were used in this experiment and measurements were made weekly using a digital caliper (Figure 1). During the second half of period II (slow growth period), when fruit growth and diameter remained constant (13-15 mm), 100 fruits of 162 branches were numbered and the fruits and leaves were sprayed with 0, 50, 100, 150 and 200 mg L<sup>-1</sup> of ethephon. Then, 0, 5, 10, 15 and 20 mg L<sup>-1</sup> of 2,4-D isopropyl ester were applied three times as foliar application (at 15-days intervals) for two years (2016 and 2017). Three trees sprayed with deionized water (0 ppm) were served as a control. The dates of foliar sprays in the 1<sup>st</sup> year were July 11<sup>th</sup> (at the constant time of fruit diameter), July 26<sup>th</sup> (07 weeks after flowering) and August 08<sup>th</sup> (15 weeks after flowering). Whereas in 2017, they were June 28<sup>th</sup>, July 14<sup>th</sup> and July 29<sup>th</sup>. Finally, the branches were covered with white nets to prevent bird eating. Fruit samples were collected when ripening began. The fruits were harvested at 7-days intervals. Fruits that had attained their maximum diameter were harvested when they were considered fully ripe.

## Fruit Ripening Percentage

Fruits were harvested and the fruit ripening percentage was calculated weekly according to the ripening indices (color change, decrease of firmness and pedicle bent). Fruit ripening percentage was measured in relation to the total fruit.

**Table 1.** Meteorological data during the two seasons 2016 and 2017.

Year	Month	Minimum temperature (°C)	Maximum temperature (°C)	Mean temperature (°C)	Mean relative humidity (%)	Average rainfall (mm)
2016	Jan.	0.0	14.3	7.2	13	29
	Feb.	2.8	16.6	9.7	24	28
	Mar.	1.8	14.7	8.3	18	31
	Apr.	8.4	22.9	15.7	50	17.0
	May	11.4	29.1	20.3	38	10.7
	Jun.	17.8	34.3	26.1	24	0.0
	Jul.	21.3	35.6	28.5	24	0.8
	Aug.	19.1	34.3	26.7	24	0.0
	Sep.	16.6	32.3	24.5	31	0.4
	Oct.	11.3	29.1	20.2	32	0.0
	Nov.	7.3	21.3	14.3	52	71.6
	Dec.	0.5	15.5	8.0	51	5.9
2017	Jan.	0.3	13.6	7.0	63	78.0
	Feb.	-0.4	14.4	7.0	48	0.5
	Mar.	4.1	19.6	11.9	45	1.0
	Apr.	6.4	20.7	13.6	50	44.0
	May	12.6	29.5	21.1	33	1.4
	Jun.	14.6	34.2	24.4	20	0.0
	Jul.	20.6	37.1	28.9	21	0.0
	Aug.	20.8	35.5	28.2	25	1.6
	Sep.	17.0	33.9	25.5	24	0.0
	Oct.	11.4	29.2	20.3	31	0.0
	Nov.	4.9	25.0	15.0	34	0.0
	Dec.	2.4	17.5	10.0	53	14.2

**Figure 1.** Fruit growth and development curve of ‘Sabz’ fig fruit under rain-fed conditions (n= 3).

### Total Soluble Solids (TSS)

One gram of full ripening fig fruit was added to distilled water (9 mL) and was shaken overnight to reach a smooth extract. The TSS was measured using a hand refractometer (ATAGO- Japan).

### Ostiole-End Cracking

The number of full ripening fruits affected by cracking for each treatment at the last harvesting time was counted and the data were expressed as the percentage of fruit ostiole-end cracking.

### Fruit Diameter

At full ripening stage, average fruit diameter (mm) was measured on a sample of 10 fruits using a digital caliper.

### Chlorophyll and Carotenoid Determination

Chlorophyll a, chlorophyll b, total chlorophyll and carotenoid content of fruit were determined according to the formula described by Arnon (1949) when Dimethylsulfoxide (DMSO) is used as the extraction solvent for pigments. Seven mL of dimethyl sulfoxide was added to 0.1 g fresh leaves. Following incubation at 65°C for 30 minutes, the extract volume reached 10 mL by adding dimethyl sulfoxide. The Optical Density (OD) of the extractions was recorded against DMSO as a blank at 663, 645 and 470 nm by the spectrophotometer (Epoch, Bio Tek<sup>®</sup>, United States). After calculation by the mentioned formula, final content of pigments was expressed as mg per g fresh weight of fruit (Hiscox and Israelstam, 1979).

### Total Soluble Carbohydrates

Soluble sugars were analyzed according to Rangana (2007) method with some modifications. A volume of 10 mL 80%

ethanol was added to 0.1 g of tissue powder and mixed with vigorous shaking. After centrifugation at 5,000 rpm for 10 minutes, the supernatant was separated. This procedure was repeated two times and the supernatants was combined. One mL of supernatant was placed into test tubes and 5 mL of 5% phenol solution was added and 5 mL of sulfuric acid was added to each tube and vortexes for 30 s. The tubes were immediately transferred into an ice bath and cooled down to room temperature. Absorbance of the samples was recorded at 630 nm using spectrophotometer. Total sugar concentrations of the samples were calculated using calibration curve drawn for glucose standard solutions.

### Starch Content

Five milliliter of cold water plus 6.5 mL perchloric acid (52%) were added to residues left from sugar analysis and mixed for 15 min. About 20 mL water were then added. The samples were centrifuged at 5,800 rpm for 10 minutes and the supernatant was removed. The same procedure was repeated for residue and the resulting supernatants were combined and left for 30 minutes at 0°C. Then, the volumes of supernatants were adjusted to 100 mL with distilled water. About 2.5 mL of supernatant was added into the test tubes and 10 mL of cold 2% anthrone solution was added to them. The samples were then heated at 100°C for 7.5 minutes. The tubes were immediately transferred to an ice bath and then kept at room temperature. The absorbance of the samples was recorded at 630 nm with a spectrophotometer. Starch concentration was determined using the calibration curve drawn for glucose standard solutions and multiplied by 0.92 (McCready *et al.*, 1950).

### Data Analysis

Data collected during the experiment were subjected to one-way Analysis Of Variance (ANOVA) using SAS software (Version 9.4;



Institute Inc., Cary, NC). The mean values were compared using LSD Test in order to determine the significant differences at the probability level of 0.05.

## RESULTS

### Fruit Ripening Percentage

Data were presented as mean values of both 2016 and 2017, because of the similarity between the results of the two years of study, i.e., since no significant differences were observed between the years. The second ethephon (200 mg L<sup>-1</sup>) foliar spraying exhibited the highest fruit ripening percentage at the first week of harvesting. By contrast, no fruit ripening was recorded by the control and 2,4-D isopropyl ester treatments (Table 2). In the third week of harvesting, the highest fruit ripening percentage was caused by 200 mg L<sup>-1</sup> ethephon treatment at the second time of foliar spray, while there was no significant difference with some treatments (Table 2). Ethephon application at 200 mg L<sup>-1</sup> caused the highest fruit ripening percentage at the second time of foliar application in fig fruits under rain-fed conditions. However, there were no significant differences between the effects of 200 mg L<sup>-1</sup> at the third time of foliar application and 100 mg L<sup>-1</sup> at the second and third time of applications. Application of 10 mg L<sup>-1</sup> isopropyl ester caused the lowest fruit ripening percentage at the first time of foliar spray (Table 2).

Significant differences ( $P < 0.05$ ) were observed between plant growth regulators and fruit ripening percentage in rain-fed figs in both years of the experiment (Figure 2). The highest fruit ripening percentage (71.09%) was recorded with 200 mg L<sup>-1</sup> ethephon application, while the lowest one (51.48%) was caused by 20 mg L<sup>-1</sup> 2,4-D isopropyl ester (Figure 2).

As shown in Figure 3, the highest fruit ripening percentage (68.16%) was recorded in 2016 at the second time of foliar application; whereas the lowest one

(43.39%) was registered in 2017 at the first time of foliar spray.

### Total Soluble Solids and Fruit Ostiole-End Cracking

Data were presented as mean values of both 2016 and 2017, because of the similarity between the results of the two years of study, i.e., since no significant differences were observed between the years. The Total Soluble Solids (TSS) was increased with 100 mg L<sup>-1</sup> ethephon application at the second and third times of foliar spraying (27.46 and 27.24° Brix, respectively) (Table 3). The lowest TSS was observed in the control at the third time of foliar application. However, the differences among the first and second times of foliar application in the control and 2,4-D isopropyl ester treatments at the first time of application were not statistically significant. The fruit ostiole-end cracking in dried figs reached the highest level with 50 mg L<sup>-1</sup> ethephon application at the third time of foliar spray. This was brought to a minimum by 15 mg L<sup>-1</sup> 2,4-D isopropyl ester at the third time of foliar application (Table 3).

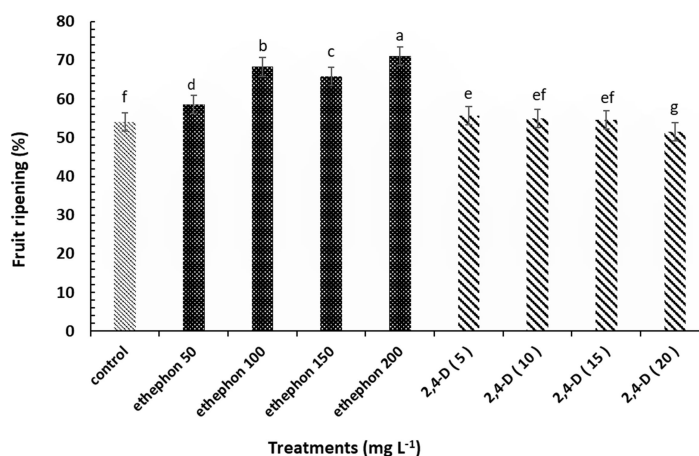
### Chlorophyll *a*, Chlorophyll *b*, Total Chlorophyll and Carotenoid Content

Data were expressed as mean values of 2016 and 2017 because similar results were obtained for the two years of the experiment (no significant difference between years). The highest chlorophyll *a* and *b* were recorded with 20 mg L<sup>-1</sup> 2,4-D isopropyl ester at the second time of foliar spray (Table 4). The lowest content of chlorophyll *a* was registered with 100 mg L<sup>-1</sup> ethephon by the first and second foliar application and, separately, with 200 mg L<sup>-1</sup> ethephon by the second instance of foliar application. Also, this latter treatment caused the lowest content of chlorophyll *b*, but there were no significant differences between the effects of 50 mg L<sup>-1</sup> ethephon at all three instances of

**Table 2.** Effects of plant growth regulators and time of foliar spray on fruit ripening percentage in fig fruits under rain-fed conditions (Data are means of two years 2016 and 2017).<sup>a</sup>

Treatments (mg L <sup>-1</sup> )	Time of foliar spray <sup>b</sup>	First week of fruit harvesting (September 01-04)	Second week of fruit harvesting (Mid-September)	Third week of fruit harvesting (September 27-30)
Control	1	0 <sup>c</sup>	2.12 <sup>i</sup>	4.45 <sup>ki</sup>
	2	0 <sup>c</sup>	4.38 <sup>e-h</sup>	52.08 <sup>i</sup>
	3	0 <sup>c</sup>	5.95 <sup>b-e</sup>	67.53 <sup>d</sup>
Ethephon 50	1	0.33 <sup>de</sup>	1.86 <sup>i</sup>	43.50 <sup>k</sup>
	2	0.52 <sup>cd</sup>	4.38 <sup>e-h</sup>	73.62 <sup>bc</sup>
	3	0.16 <sup>de</sup>	6.61 <sup>a-d</sup>	58.44 <sup>s</sup>
Ethephon 100	1	0.08 <sup>de</sup>	2.52 <sup>h-i</sup>	42.60 <sup>kl</sup>
	2	1.24 <sup>bc</sup>	6.28 <sup>a-d</sup>	80.64 <sup>a</sup>
	3	0.33 <sup>de</sup>	5.43 <sup>c-f</sup>	80.80 <sup>a</sup>
Ethephon 150	1	0.18 <sup>de</sup>	3.34 <sup>i</sup>	48.63 <sup>j</sup>
	2	1.51 <sup>b</sup>	7.13 <sup>a-c</sup>	75.53 <sup>d</sup>
	3	0.38 <sup>de</sup>	5.67 <sup>b-e</sup>	73.21 <sup>b</sup>
Ethephon 200	1	0.27 <sup>de</sup>	3.17 <sup>g-i</sup>	51.57 <sup>j</sup>
	2	3.82 <sup>a</sup>	8.21 <sup>a</sup>	80.86 <sup>a</sup>
	3	0.76 <sup>cd</sup>	5.37 <sup>c-f</sup>	81.42 <sup>a</sup>
2,4-D 5	1	0 <sup>e</sup>	3.65 <sup>i-i</sup>	41.40 <sup>l</sup>
	2	0 <sup>e</sup>	4.62 <sup>d-g</sup>	60.74 <sup>f</sup>
	3	0 <sup>e</sup>	5.43 <sup>c-f</sup>	64.85 <sup>e</sup>
2,4-D 10	1	0 <sup>e</sup>	2.25 <sup>i</sup>	37.74 <sup>m</sup>
	2	0 <sup>e</sup>	4.70 <sup>d-g</sup>	66.52 <sup>de</sup>
	3	0 <sup>e</sup>	7.52 <sup>ab</sup>	60.64 <sup>f</sup>
2,4-D 15	1	0 <sup>e</sup>	2.66 <sup>h-i</sup>	42.01 <sup>ki</sup>
	2	0 <sup>e</sup>	4.75 <sup>d-g</sup>	65.49 <sup>de</sup>
	3	0 <sup>e</sup>	5.93 <sup>b-e</sup>	56.19 <sup>h</sup>
2,4-D 20	1	0 <sup>e</sup>	2.00 <sup>i</sup>	40.58 <sup>l</sup>
	2	0 <sup>e</sup>	6.52 <sup>a-d</sup>	57.14 <sup>gh</sup>
	3	0 <sup>e</sup>	6.85 <sup>a-c</sup>	56.73 <sup>gh</sup>

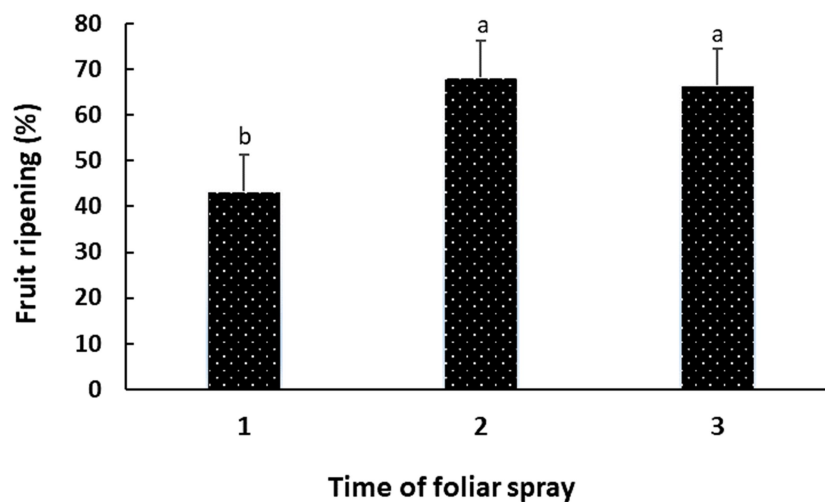
<sup>a</sup> Values in the same column with different superscript letters represent significant differences at  $P < 0.05$  according to LSD test, ( $n = 3$ ). <sup>b</sup> Times of foliar spray: 1 (at the constant time of fruit diameter), 2 (7 weeks after flowering) and 3 (15 weeks after flowering).

**Figure 2.** Effects of plant growth regulators and on fruit ripening percentage in 'Sabz' fig. Values in the same column with different letters represent significant differences at  $P < 0.05$  according to LSD test ( $n = 3$ ) and error bars represent Standard Errors.

**Table 3.** Effects of plant growth regulators and time of foliar spray on TSS and ostiole cracking in fig fruits under rain-fed conditions (Data are means of two years 2016 and 2017).<sup>a</sup>

Treatments (mg L <sup>-1</sup> )	Time of foliar spray <sup>b</sup>	TSS (°Brix)	Ostiole cracking (%)
Control	1	18.85 <sup>hi</sup>	3.91 <sup>c-f</sup>
	2	18.90 <sup>hi</sup>	2.16 <sup>d-f</sup>
	3	18.35 <sup>i</sup>	4.00 <sup>b-f</sup>
Ethephon 50	1	22.14 <sup>d</sup>	6.16 <sup>a-d</sup>
	2	22.57 <sup>b</sup>	4.00 <sup>b-f</sup>
	3	25.34 <sup>b</sup>	10.00 <sup>a</sup>
Ethephon 100	1	23.92 <sup>c</sup>	7.58 <sup>a-c</sup>
	2	27.46 <sup>a</sup>	6.41 <sup>a-d</sup>
	3	27.24 <sup>a</sup>	7.00 <sup>a-c</sup>
Ethephon 150	1	23.63 <sup>c</sup>	4.58 <sup>b-f</sup>
	2	24.35 <sup>c</sup>	5.42 <sup>b-f</sup>
	3	24.05 <sup>c</sup>	6.00 <sup>a-c</sup>
Ethephon 200	1	23.71 <sup>c</sup>	4.91 <sup>b-f</sup>
	2	24.26 <sup>c</sup>	1.83 <sup>ef</sup>
	3	24.20 <sup>c</sup>	5.50 <sup>b-f</sup>
2,4-D 5	1	18.92 <sup>hi</sup>	3.75 <sup>c-f</sup>
	2	21.38 <sup>ef</sup>	3.66 <sup>c-f</sup>
	3	21.19 <sup>e-g</sup>	3.75 <sup>c-f</sup>
2,4-D 10	1	19.34 <sup>h</sup>	6.00 <sup>a-c</sup>
	2	21.74 <sup>de</sup>	4.25 <sup>b-f</sup>
	3	20.53 <sup>g</sup>	8.25 <sup>ab</sup>
2,4-D 15	1	19.47 <sup>h</sup>	5.58 <sup>b-e</sup>
	2	21.34 <sup>e-g</sup>	7.41 <sup>a-c</sup>
	3	21.00 <sup>e-g</sup>	1.25 <sup>f</sup>
2,4-D 20	1	19.55 <sup>h</sup>	3.41 <sup>c-f</sup>
	2	21.61 <sup>d-f</sup>	4.00 <sup>b-f</sup>
	3	20.96 <sup>fg</sup>	5.66 <sup>b-e</sup>

<sup>a</sup> Values in the same column with different superscript letters represent significant differences at  $P < 0.05$  according to LSD test, ( $n = 3$ ). <sup>b</sup> Times of foliar spray: 1 (at the constant time of fruit diameter), 2 (7 weeks after flowering) and 3 (15 weeks after flowering).



**Figure 3.** Effects of the application number of foliar sprays on fruit ripening percentage in ‘Sabz’ fig. Times of foliar spray: 1 (at the constant time of fruit diameter), 2 (7 weeks after flowering) and 3 (15 weeks after flowering). Values in the same column with different letters represent significant differences at  $P < 0.05$  according to LSD test ( $n = 3$ ) and error bars represent Standard Errors.



**Table 4.** Effects of plant growth regulators and time of foliar spray on chlorophyll and carotenoids content in fig fruits under rain-fed conditions (Data are means of two years 2016 and 2017).<sup>a</sup>

Treatments (mg L <sup>-1</sup> )	Time of foliar spray <sup>b</sup>	Chlorophyll a (mg g <sup>-1</sup> Fw)	Chlorophyll b (mg g <sup>-1</sup> Fw)	Carotenoid (mg g <sup>-1</sup> Fw)
Control	1	1.14 <sup>h</sup>	0.269 <sup>hi</sup>	0.102 <sup>se-i</sup>
	2	1.17 <sup>g</sup>	0.290 <sup>h</sup>	0.112 <sup>fe-j</sup>
	3	1.13 <sup>h</sup>	0.319 <sup>g</sup>	0.088 <sup>i-k</sup>
Ethephon 50	1	0.84 <sup>l-m</sup>	0.182 <sup>o</sup>	0.089 <sup>ij</sup>
	2	0.88 <sup>o-i</sup>	0.202 <sup>m-o</sup>	0.116 <sup>fg</sup>
	3	0.94 <sup>i</sup>	0.186 <sup>o</sup>	0.158 <sup>ab</sup>
Ethephon 100	1	0.75 <sup>o</sup>	0.183 <sup>o</sup>	0.089 <sup>ij</sup>
	2	0.78 <sup>o</sup>	0.187 <sup>no</sup>	0.127 <sup>df</sup>
	3	0.87 <sup>k-m</sup>	0.228 <sup>kl</sup>	0.165 <sup>a</sup>
Ethephon 150	1	0.42 <sup>n</sup>	0.238 <sup>jk</sup>	0.148 <sup>bc</sup>
	2	0.42 <sup>n</sup>	0.211 <sup>l-n</sup>	0.157 <sup>ab</sup>
	3	0.91 <sup>ij</sup>	0.220 <sup>k-m</sup>	0.126 <sup>ef</sup>
Ethephon 200	1	0.84 <sup>n</sup>	0.226 <sup>k-m</sup>	0.123 <sup>f</sup>
	2	0.77 <sup>o</sup>	0.178 <sup>o</sup>	0.141 <sup>cd</sup>
	3	0.90 <sup>jk</sup>	0.256 <sup>j</sup>	0.138 <sup>ce</sup>
2,4-D 5	1	1.28 <sup>e</sup>	0.384 <sup>d</sup>	0.125 <sup>ef</sup>
	2	1.17 <sup>g</sup>	0.386 <sup>d</sup>	0.061 <sup>mn</sup>
	3	1.31 <sup>de</sup>	0.370 <sup>de</sup>	0.060 <sup>mn</sup>
2,4-D 10	1	1.23 <sup>f</sup>	0.347 <sup>ef</sup>	0.079 <sup>i-i</sup>
	2	1.32 <sup>d</sup>	0.418 <sup>c</sup>	0.71 <sup>l-n</sup>
	3	1.29 <sup>de</sup>	0.340 <sup>fg</sup>	0.062 <sup>mn</sup>
2,4-D 15	1	1.37 <sup>bc</sup>	0.327 <sup>fg</sup>	0.71 <sup>l-n</sup>
	2	1.37 <sup>bc</sup>	0.441 <sup>bc</sup>	0.73 <sup>k-m</sup>
	3	1.41 <sup>ab</sup>	0.465 <sup>ab</sup>	0.66 <sup>l-n</sup>
2,4-D 20	1	1.33 <sup>d</sup>	0.344 <sup>f</sup>	0.097 <sup>hi</sup>
	2	1.41 <sup>a</sup>	0.468 <sup>a</sup>	0.062 <sup>mn</sup>
	3	1.36 <sup>c</sup>	0.426 <sup>c</sup>	0.058 <sup>n</sup>

<sup>a</sup> Values in the same column with different superscript letters represent significant differences at  $P < 0.05$  according to LSD test, ( $n = 3$ ).

<sup>b</sup> Times of foliar spray: 1 (at the constant time of fruit diameter), 2 (7 weeks after flowering) and 3 (15 weeks after flowering).

the foliar application and 100 mg L<sup>-1</sup> ethephon at the second time of foliar application. The maximum carotenoid content occurred with the second time that ethephon (100 mg L<sup>-1</sup>) was sprayed, whereas the lowest one was observed with the third 2,4-D isopropyl ester (20 mg L<sup>-1</sup>) foliar spraying (Table 4).

#### Total Soluble Carbohydrate Content, Starch Content and Fruit Diameter

The highest sugar content (92.77 mg g<sup>-1</sup> dw) was caused by 50 mg L<sup>-1</sup> ethephon at the second time of foliar spraying, while the lowest one (65.91 mg g<sup>-1</sup> dw) was recorded in the control by the first instance of the foliar application (Table 5). Furthermore,

application of 15 mg L<sup>-1</sup> 2,4-D isopropyl ester caused the highest starch content in 2016 at the second time of foliar spray, but there were no significant differences between 10 and 15 mg L<sup>-1</sup> 2,4-D isopropyl ester at the second time of foliar application and 20 mg L<sup>-1</sup> 2,4-D isopropyl ester at the first time of foliar spraying in rain-fed figs (Table 5). The highest starch content occurred in response to 15 mg L<sup>-1</sup> 2,4-D isopropyl ester at the first and second times of foliar spraying, with values of 7.89 and 7.63 mg g<sup>-1</sup> dw in 2017, respectively.

As shown in Table 5, application of 10 mg L<sup>-1</sup> 2,4-D isopropyl ester resulted in greatest fruit diameter in 2016 and 2017 (25.38 and 24.03 mm, respectively) with the second time of foliar spraying, whereas no

**Table 5.** Effects of plant growth regulators and time of foliar spray on total carbohydrate, starch and diameter in fig fruits under rain-fed conditions during 2016 and 2017.<sup>a</sup>

Treatments (mg L <sup>-1</sup> )	Time of foliar spray <sup>b</sup>	Total carbohydrates (mg g <sup>-1</sup> dw)		Starch (mg g <sup>-1</sup> dw)		Diameter (mm)	
		2016	2017	2016	2017	2016	2017
Control	1	65.91 <sup>n</sup>	64.92 <sup>l</sup>	7.57 <sup>bc</sup>	7.33 <sup>cd</sup>	19.45 <sup>op</sup>	19.57 <sup>k</sup>
	2	72.19 <sup>m</sup>	70.39 <sup>jk</sup>	7.16 <sup>d</sup>	6.98 <sup>e</sup>	19.54 <sup>op</sup>	18.91 <sup>n</sup>
	3	71.24 <sup>m</sup>	69.47 <sup>k</sup>	7.51 <sup>c</sup>	7.58 <sup>bc</sup>	19.16 <sup>p</sup>	19.03 <sup>mn</sup>
Ethephon 50	1	78.66 <sup>hi</sup>	75.05 <sup>g</sup>	5.93 <sup>k</sup>	5.82 <sup>k</sup>	24.43 <sup>b</sup>	23.39 <sup>bc</sup>
	2	92.77 <sup>a</sup>	90.49 <sup>a</sup>	6.19 <sup>i-k</sup>	6.03 <sup>i-k</sup>	24.82 <sup>b</sup>	23.52 <sup>b</sup>
	3	82.39 <sup>e</sup>	80.04 <sup>c</sup>	6.13 <sup>jk</sup>	6.02 <sup>i-k</sup>	21.34 <sup>hi</sup>	21.13 <sup>g</sup>
Ethephon 100	1	86.94 <sup>c</sup>	85.49 <sup>c</sup>	6.38 <sup>g-j</sup>	6.22 <sup>g-i</sup>	22.72 <sup>ef</sup>	22.35 <sup>ef</sup>
	2	90.77 <sup>b</sup>	88.17 <sup>b</sup>	6.09 <sup>i-k</sup>	5.89 <sup>jk</sup>	22.83 <sup>de</sup>	22.14 <sup>f</sup>
	3	85.32 <sup>cd</sup>	83.03 <sup>d</sup>	6.25 <sup>h-j</sup>	6.12 <sup>ij</sup>	19.92 <sup>mn</sup>	19.10 <sup>l-n</sup>
Ethephon 150	1	86.13 <sup>cd</sup>	83.32 <sup>d</sup>	6.52 <sup>gh</sup>	6.27 <sup>g-i</sup>	21.42 <sup>h</sup>	20.69 <sup>h</sup>
	2	84.43 <sup>d</sup>	81.99 <sup>d</sup>	6.45 <sup>g-i</sup>	6.42 <sup>f-h</sup>	21.29 <sup>h-j</sup>	21.05 <sup>gh</sup>
	3	82.14 <sup>ef</sup>	80.01 <sup>e</sup>	6.19 <sup>i-k</sup>	6.13 <sup>hi</sup>	20.74 <sup>j-l</sup>	20.10 <sup>ij</sup>
Ethephon 200	1	80.52 <sup>fg</sup>	78.14 <sup>f</sup>	6.13 <sup>jk</sup>	6.12 <sup>ij</sup>	20.71 <sup>kl</sup>	20.03 <sup>ij</sup>
	2	86.65 <sup>c</sup>	83.10 <sup>d</sup>	6.39 <sup>g-j</sup>	6.27 <sup>g-i</sup>	20.90 <sup>h-l</sup>	20.10 <sup>ij</sup>
	3	79.51 <sup>gh</sup>	75.17 <sup>g</sup>	6.15 <sup>jk</sup>	6.00 <sup>ik</sup>	20.83 <sup>i-l</sup>	20.10 <sup>ij</sup>
2,4-D 5	1	76.63 <sup>hi</sup>	73.03 <sup>hi</sup>	7.21 <sup>d</sup>	7.09 <sup>de</sup>	22.16 <sup>g</sup>	22.01 <sup>f</sup>
	2	82.16 <sup>ef</sup>	80.02 <sup>e</sup>	7.51 <sup>c</sup>	7.57 <sup>bc</sup>	22.26 <sup>fg</sup>	22.08 <sup>f</sup>
	3	78.01 <sup>h-j</sup>	74.01 <sup>gh</sup>	7.13 <sup>d</sup>	6.97 <sup>e</sup>	21.20 <sup>h-k</sup>	20.97 <sup>gh</sup>
2,4-D 10	1	75.11 <sup>l</sup>	72.04 <sup>i-j</sup>	7.51 <sup>c</sup>	7.46 <sup>bc</sup>	23.41 <sup>c</sup>	23.03 <sup>cd</sup>
	2	86.12 <sup>cd</sup>	83.12 <sup>d</sup>	7.65 <sup>a-c</sup>	7.58 <sup>bc</sup>	25.38 <sup>a</sup>	24.03 <sup>a</sup>
	3	76.03 <sup>ki</sup>	74.04 <sup>gh</sup>	7.04 <sup>de</sup>	7.00 <sup>c</sup>	21.14 <sup>h-k</sup>	19.71 <sup>jk</sup>
2,4-D 15	1	78.73 <sup>hi</sup>	75.08 <sup>g</sup>	7.83 <sup>ab</sup>	7.89 <sup>a</sup>	20.36 <sup>lm</sup>	20.15 <sup>i</sup>
	2	81.38 <sup>ef</sup>	79.23 <sup>ef</sup>	7.87 <sup>a</sup>	7.63 <sup>ab</sup>	23.38 <sup>cd</sup>	22.77 <sup>de</sup>
	3	77.26 <sup>i-k</sup>	73.03 <sup>hi</sup>	6.83 <sup>ef</sup>	6.66 <sup>f</sup>	20.14 <sup>mn</sup>	19.50 <sup>ki</sup>
2,4-D 20	1	76.76 <sup>l</sup>	74.72 <sup>gh</sup>	7.54 <sup>b</sup>	7.50 <sup>bc</sup>	20.76 <sup>j-l</sup>	20.19 <sup>i</sup>
	2	85.46 <sup>cd</sup>	83.09 <sup>d</sup>	7.68 <sup>a-c</sup>	7.72 <sup>ab</sup>	24.56 <sup>b</sup>	23.79 <sup>ab</sup>
	3	77.04 <sup>ik</sup>	74.32 <sup>gh</sup>	6.68 <sup>fg</sup>	6.49 <sup>fg</sup>	19.62 <sup>np</sup>	19.42 <sup>k-m</sup>

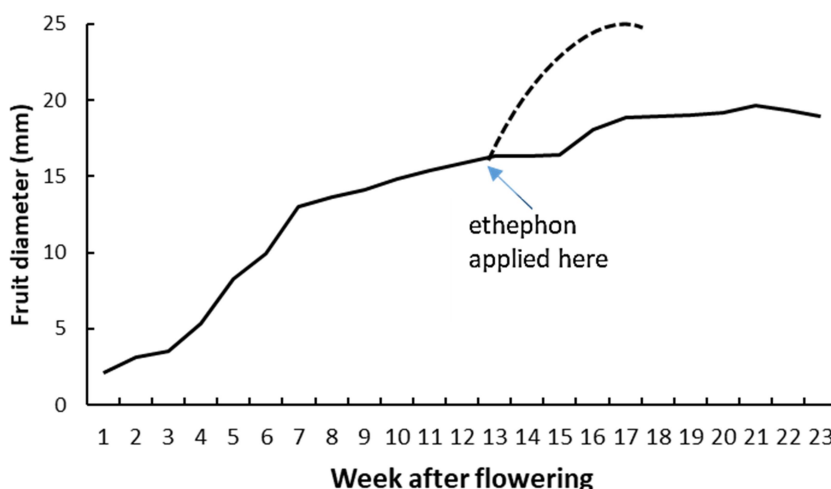
<sup>a</sup> Values in the same column with different superscript letters represent significant differences at  $P < 0.05$  according LSD test, ( $n = 3$ ). <sup>b</sup> Times of foliar spray: 1 (at the constant time of fruit diameter), 2 (7 weeks after flowering) and 3 (3 weeks after flowering).

significant effect was observed by applying 20 mg L<sup>-1</sup> 2,4-D isopropyl ester at the second time of foliar application ( $P < 0.05$ ). Rain-fed figs treated with ethephon at the later period of depressed growth rate (period II) accelerated the fruit growth rate as compared with the control (Figure 4).

## DISCUSSION

The present study showed that 200 mg L<sup>-1</sup> ethephon could accelerate fig fruits ripening by 7 days compared to the control and auxin treatments. The fruit ripening percentage

was also increased by ethephon treatment. Owino *et al.* (2006) reported that treating fruits with Ethrel<sup>®</sup> induced 'Masui Dauphine' fig fruits ripen about 7 days sooner, while the auxin-treated fruits ripened within 10 days after treatment and control samples ripened after 14 days, indicating good accordance with our findings. Since the fig is a climacteric fruit and the application of ethylene in the latter part of stage II induces growth, there is an accelerated rise in respiration, whereby endogenous ethylene production is stimulated and ripening is promoted (Mitra, 1997). Ethephon on rain-fed fig fruits can be



**Figure 4.** Fruit diameter of 'Sabz' fig sprayed with 200 mg L<sup>-1</sup> of ethephon as compared to the control fruits. The dotted line is the time of ethephon application. It is time that the growth rate of fruit is slow.

degraded within the plant and released as ethylene, which, in turn, hastens fruit ripening (Cui *et al.*, 2021). It seemed that foliar application of ethephon at the second half of fruit growth stage II enhanced the system II of ethylene regulation and facilitated the fruit ripening process, compared with auxin-treated and non-treated fruits. Ethephon treatment in late phase II has been reported to stimulate fig fruit growth and shorten the ripening period with no negative effect on fruit quality, resulting in 4- to 11-day earlier ripening than the untreated figs (Çelikel *et al.*, 1997). In addition, to enhance ripening, exogenous ABA and ethephon treatments also improved the fig fruit's color via early accumulation of cyanidin 3-O-glucoside and cyanidin 3-O-rutinoside. These two cyanidins have been reported to be the major anthocyanins in ripened fig fruit, responsible for the skin color intensity (Dueñas *et al.*, 2008).

The effect of ethephon application on fruit ripening was also reported in other crops such as *Ziziphus mauritiana* L., which occurred about 2 weeks earlier than the control group (Bal *et al.*, 1996), and 13 to 22

days earlier in persimmon (Kim *et al.*, 2004). Foliar application of ethephon at 4 mg/L reduced the fruit maturation period between kimri to rutab stages to 14 days (Hussain *et al.*, 2015). Our results showed that application of 2,4-D isopropyl ester at the first time of fruit harvesting had no effect on fruit ripening. It may be that auxin translocated slowly within the plant and took a long time to transmit and stimulate the induction of ethylene. Fig fruits had higher levels of total soluble carbohydrates and TSS, while having a lower amount of starch with the foliar application of ethephon in both years of the experiment. This finding is in accordance with previous reports on date fruits (Hussain *et al.*, 2015). It was reported that the rise of sugar contents depends on the conversion of starch on hydrolysis or that the enhanced ripening (as caused by the ethylene) resulted in an increase in TSS in ethephon-treated fruits. Moreover, foliar application of ethephon could induce the synthesis of ethylene and promote ripening, without any significant impact on fruit quality. In general, fruits that were treated with ethephon in the latter part of stage II (when the fruit diameter remained constant)



resulted in the accumulation of sugars as compared to fruits where the ethephon was not applied. This effect of ethephon on sugar accumulation was also reported in other fruit crops such as mango (Kulkarni *et al.*, 2004), date fruit (Hussain *et al.*, 2015) and *Ziziphus mauritiana* (Bal *et al.*, 1996).

The current study revealed that treating fig fruits with auxin and ethephon led to the larger fruit diameter, although the effect of 10 mg L<sup>-1</sup> 2,4-D isopropyl ester at the second time of foliar application was noticeable, which is in line with previous research on fig (Puech *et al.*, 1976; Cui *et al.*, 2021). It seemed that the increase in fruit diameter with foliar application of auxin could be a result of faster cell division and more elongation in rain-fed fig fruits. Auxin enhanced some enzyme activities responsible for loosening the cell wall and for producing plant cell materials. On the other hand, turgor pressure usually plays a key role in cell elongation. Ethylene facilitates the conversion of starch into sugars and increases the metabolism of cell walls when modification of pectin materials loosen the cell wall, thereby enabling the cell turgor pressure to contribute more to growth (Chaves and Mello-Farias, 2006). Also, it was reported that ethylene has a strong translucent capacity in figs and results in the movement of nutrients towards the fruit in the latter parts of stage II and causes the occurrence of cell expansion (Maxie and Crane, 1967). Therefore, an increase in fig fruit diameter could be associated with ethephon application under rain-fed conditions. According to the curve of growth in fig fruit diameter, since fruits were not irrigated at the period of depressed growth under rain-fed conditions, this stage showed a longer period (6-8 weeks) than in 'Calimyrna' fig under irrigated condition (3-4 weeks) (Crane *et al.*, 1970).

Chlorophyll and carotenoid contents in fig fruits treated with ethephon showed significant differences as compared with the control and auxin treatments. This confirms a previous study that claimed that ethephon can initiate fig ripening and also pigment

catabolism in response to ethylene. (Puech *et al.*, 1976). Although changes in the pigments of fig fruits follow a mode of action by ethylene, the exact mechanisms are not well known. This mechanism can be expressed and referred to other fruits such as citrus, apricot and kiwi fruits. It seemed that carotenoid formation and the loss of green color in dry figs could correlate with the loss of chlorophyll and with a lowered activity of Chlase enzyme. Moreover, ethylene can increase chlorophyll degradation in the peel of fruits by the *de nova* synthesis of Chlase enzyme (Azuma *et al.*, 1999).

## CONCLUSIONS

This study provides new information on the role of ethephon in fig-fruit ripening under rain-fed condition. The application of ethephon (200 mg L<sup>-1</sup>) accelerated ripening in fig fruits occurring about 7 days earlier under rain-fed conditions. In addition, ethephon application at 100 mg L<sup>-1</sup> was associated with increased total soluble solids and resulted in higher carotenoid content. Application of ethylene did not affect the fruit quality and caused the fruit with better ostiole- end cracking, which was one of the most important criterion in dried fig trade. In general, expediting the ripening of figs can reduce the chances of figs facing drought in the latter stages of the season. Presently, ethephon is involved in the federal IR-4 program [Minor crop pest Management Program Interregional Research Project # 4 (IR-\$)] and residue studies are ongoing as a protocol for prospect registration.

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### تأثیر تنظیم کننده های رشد اتفون و ۴-۲ دی ایزوپروپیل استر بر رسیدگی و کیفیت میوه انجیر در شرایط دیم

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#### چکیده

رشد و نمو میوه انجیر دیم با انجیر کشت شده در شرایط آبی متفاوت می-باشد و به کاربرد تنظیم کننده های رشد پاسخ متفاوتی می دهد. این مطالعه نشان می دهد که چگونه کاربرد اتفون (۰، ۱۰۰، ۵۰، ۱۵۰، ۲۰۰ میلی گرم در لیتر) و ایزوپروپیل استر (۰، ۴، ۱۰، ۱۵، ۲۰ میلی گرم در لیتر) می توانند رسیدن میوه را در انجیر رقم سبز تسریع -بخشد. میوه ها در دو سال متوالی، سه مرتبه به فاصله هر ۱۵ روز یکبار محلول پاشی شدند. بیشترین درصد رسیدن میوه در نوبت دوم محلول پاشی اتفون (۲۰۰ میلی گرم در لیتر) بدست آمد. استفاده از ۱۰۰ میلی گرم در لیتر اتفون باعث افزایش مواد جامد محلول در زمان محلول پاشی دوم و سوم شد. بیشترین شکاف خوردگی در میوه-های خشک انجیر با استفاده از ۵۰ میلی گرم در لیتر اتفون در محلول پاشی سوم بدست آمد. بیشترین مقدار کاروتنوئید در تیمار ۱۰۰ میلی گرم در لیتر اتفون در محلول پاشی دوم بدست آمد. همچنین بیشترین میزان قند در دومین زمان محلول پاشی با اتفون ۵۰ میلی گرم در لیتر ایجاد شد. زمان دوم محلول پاشی با ایزوپروپیل استر (۲، ۴، ۱۰) D- میلی گرم در لیتر) قطر میوه را به طور قابل توجهی افزایش داد. از آنجایی که محصول انجیر دارای ماهیت ناهمزمانی در رسیدن است، برداشت برای چند هفته ادامه یافت. اتفون با غلظت ۲۰۰ میلی گرم در لیتر برداشت انجیر را زودتر کرد و باعث شد که در شرایط دیم حدود ۷ روز زودتر برداشت انجام شود. بنابراین، تسریع در رسیدن میوه های انجیر می-تواند احتمال اینکه میوه-ها در اواخر فصل در معرض خشکی قرار گیرند را کاهش دهد. همچنین، تیمار با اتفون می-تواند به جلوگیری از کاهش کیفیت میوه کمک نماید.