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EFFECT OF PLANT GROWTH REGULATORS ON FRUIT SPLINTING IN THOMPSON NAVEL ORANGE

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ABSTRACT

Fruit splitting is one of main problems extensively observed in citrus-growing area located in the northern parts of Iran. In order to investigate the effect of 2,4-D and GA_3 on incidence of fruit splitting in Thompson navel orange, an experiment, as a factorial arrangement in a randomized complete block design (RCBD) with three replications, was carried out in two consecutive years of 2016 and 2017. The main factors of this experiment included timing of spray at different growth stages (full bloom, petal fall, and June drop), 2, 4-D (0, 10, 20 mg L^{-1}), and GA_3 (0, 50, and 100 mg L^{-1}). In the end of experiment, the characteristics including fruit length, fruit diameter, rind thickness, cellulase and polygalacturonase activities, total soluble solid, percentage of split fruit, and fruit yield were measured. Results showed that using 2, 4-D and GA_3 not only decreased the rate of fruit splitting but also increased fruit yield as compared to controls. The highest fruit length, fruit diameter, rind thickness, total soluble solid and the lowest activities of cellulase and polygalacturonase were obtained using 100 mg L^{-1} GA_3 and 20 mg L^{-1} 2, 4 D during the full bloom. In general, the results revealed that using 2, 4-D and GA_3 during the full bloom increased fruit yield while reduced fruit splitting and maintained the qualitative characteristics of Thompson navel orange.

Key words: cellulase, polygalacturonase, 2, 4-D, GA_3, citrus

INTRODUCTION

Iran annually produces 2717000 ton Thompson navel orange consisting 0.04 of worldwide orange production [FAO 2016]. Some factors such as hot climate, water deficiency, soil texture, light deficiency, pests and diseases, and fruit splitting damage citrus production in the world as much as 60% [Hoffman 2009]. In citrus, fruit splitting is a physiological disorder emerging in navel orange and hybrid tangerines and leading to extend microcracks located at the blossom end of fruits. In immature fruits, the cracks surrounding wound extend and lead to fruit fall off trees at this stage of growth. The fruit cracking occurs in thin-rind cultivars during pre-harvest mainly due to an internal pressure of pulp to thin rinds, and such rinds is not able to accommodate the exerted pressure to them and consequently start to split. The severity of fruit splitting in different years and locations is not the same, indicating a remarkable change in climate paving the way for emerging this phenomenon [Maritnez-Fuentes and Mesejo 2002]. Moreover, the rate of splitting among the navel fruits is higher than that in other fruits because the navel is a chimeric tissue and the resistance of different parts of this type of tissue toward an internal pressure of pulp is not alike. It has been

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reported that in a navel cultivar, the bigger the navel of a fruit, the higher the risk of its splitting [Rabe and Rensburg 1996]. The appearance of fruit is a factor affecting fruit splitting. According to some research, it has been found that an increase in ratio of fruit length to fruit diameter leads to emerging fruit splitting [Rabe and Rensburg 1996].

Plant growth regulators (PGRs) such as auxin and gibberellin can be served as an appropriate tool to reduce fruit splitting [Garcia-Luis et al. 1994]. Spray 40 mg L^{-1} of 2, 4-D in Nova tangerine declined fruit splitting as much as 20% while increased its yield and fruit size [Greenberg et al. 2006]. In comparison to controls, application of 2, 4-D at 20, 30, and 40 mg L^{-1} on pomegranate at full bloom as well as in 45 and 90 d after fruit set significantly increased fruit length, width, volume, and weight as well as aril percentage, number of fruit, and yield of fruit [Reddy and Prasad 2012]. Also, foliar spray of 2% $Ca(No_3)_2$ along with 2,4-D as well as GA3 at 20 mg L^{-1} through strengthening fruit rind, reduced fruit splitting, in a way that it reinforced the fruit rind rather than increasing rind thickness in citrus [Almela et al. 1994]. The success of these treatments was believed to be strongly dependent on their concentration and time of utilizing [Mupambi 2010]. In our research, the effect of 2, 4-D and GA_3 at different concentrations and spraying times on fruit splitting, in terms of some qualitative characteristics, fruit yield, and activities of cellulase and polygalacturonase (PG) enzymes were investigated.

MATERIALS AND METHODS

This research was carried out in Ghaemshar region located in Mazandran province, Iran. Some seven-year-old trees of Thompson navel orange (*Citrus sinensis* L.) were used and distanced 5 × 5 m, grafted onto bitter oranges (*Citrus aurantium,* L. Osbeck). The average annual temperature and yearly rainfall of this region consisted of 16.7°C and 724.9 mm, respectively. The experiment was set up as a factorial in a randomized complete block design with three replications. The treatments included 2, 4-D (0, 10, and 20 mg L^{-1}) and GA_3 (0, 50, 100 mg L^{-1}) applied at three times of plan growth (full bloom, petal fall, and June drop). The PGRs were sprayed on leaves of trees under investigation; meanwhile distilled water was used instead of PGRs treatment in controls. In the end of experiment, the following characteristics were measured:

Yield. In order to determine the yield of fruit, all fruits (split and non-split ones) belonging two trees in each replication were initially picked up in the end of harvest season (in first half of December), and weighted after their counting. Then, split fruits were counted, and after weighting, the percentage of fruit splitting was calculated as follows [Abd-El-Rahman et al. 2012]:

$$\text{Percentage of split fruit} = \text{Number of split fruits} \times 100 / \text{Total fruits}$$

Fruit length and diameter. According to Akhlaghi et al. [2012], 50 fruits for each replication were used to measure fruits length and diameter using caliper. In order to determine the length of fruit, the distance between peduncle and naval of fruit was taken into account.

Rind thickness. First, 50 fruits were randomly chosen at each replication and spliced meticulously with a sharp knife. Then, rind thickness was determined using caliper, and their average was reported [Akhlaghi et al. 2012].

Cellulase and PG activities. In order to measure the activities of cellulase and PG, 50 fruits were picked up from each tree and peeled off. Then, the peels were washed with distilled water and dried with a freezing dryer and grounded under liquid nitrogen. In order to measure the activity of mentioned enzymes, a method introduced by Ghose [1987] and Adney et al. [1996] was used for measuring cellulase activity; likewise, a method proposed by Faize et al. [2003] also used for measuring polygalacturonase. TSS (Brix) was measured using a handy refractometer (Atago model) under room temperature, and the resultant was expressed as percentage. In this regard, the refractometer was initially calibrated with distilled water; then, two drops of provided juice was put in the lenses of refractometer to determine its TSS [Ayala-Zavala et al. 2007].

The statistical analysis was carried out using SAS. The comparison of means was performed based on multiple Duncan test ($p < 0.05$) and the graphs were plotted using Excel software.

RESULTS

Fruit length. The data presented in Table 1 showed that main effects of year (Y), 2, 4-D and GA_3 and timing (T) and interaction effect of 2, 4-D × GA_3 × T on fruit length were found significant. Moreover, the results of comparison means of Y showed that the average of fruit length at first year (Y1) (80.24 mm) was higher than that in second year (Y2) (75.23 mm) (Tab. 2). Throughout our experiment, using 2, 4-D and GA_3 showed to have better effect on fruit length as compared to control. The highest fruit length (average 95.14 mm) was gained by employing 100 mg L^{-1} GA_3 at full bloom that increased 37.81 mm in comparison to control. In other words, the lowest fruit length (59.16 mm) was obtained by control at full bloom (Fig. 1).

Fruit diameter. The results of our research revealed that the main effects of Y, 2, 4-D and GA_3 and T along with the interaction effect of 2, 4-D × GA_3 on fruit diameter were significant at $p < 0.01$ (Tab. 1). The fruit diameter in Y1 was approximately 4.98% larger than that in Y2. The average of fruit diameter in Y1 and Y2 was 82.63 and 78.51 mm, respectively (Tab. 2). As the concentration of 2, 4-D and GA_3 was enhanced, the fruit diameter significantly increased, in a way that the highest fruit diameter was gained at 100 mg L^{-1} of GA_3 and 20 mg L^{-1} of 2, 4-D (Fig. 2).

Rind thickness. The results of ANOVA showed that the main effects of all treatments (Y, 2, 4-D, GA_3, and T) as well as the interaction effect of Y×2, 4-D×GA_3 on rind thickness were significant (Tab. 1). Likewise, the highest rind thickness (average 9.15 mm)

Table 1. Analysis of variance (ANOVA) for year, 2.4-D, GA_3, time and their interactions effects on evaluated dependent variables in Thomson navel orange

Dependent variable	Fruit length	Fruit diameter	Rind thickness	Total yield	Cellulase	Polygala-cturonase	Total soluble solid	Percentage of split fruit
Year (Y)	**	**	**	ns	**	**	*	**
2,4-D	**	**	**	ns	**	**	*	**
GA_3	**	**	**	*	**	**	**	**
Y×GA_3	ns	ns	**	ns	**	**	ns	**
2,4-D ×GA_3	**	**	**	ns	**	**	ns	**
Y× GA_3 × 2,4-D	ns	ns	ns	**	**	**	**	**
Time (T)	**	**	**	*	**	**	**	**
Y×T	ns	ns	ns	ns	ns	**	ns	**
2,4-D ×T	ns	ns	**	ns	**	**	ns	ns
2,4-D×Y×T	ns	ns	ns	**	ns	**	ns	ns
T × GA_3	**	ns		ns	**	*	ns	ns
GA3×T×Y	ns	ns	ns	ns	ns	*	ns	ns
2,4-D×T GA_3	**	ns	**	ns	**	**	ns	ns
2,4-D×GA_3×T×Y	ns	ns	ns	ns	ns	**	ns	ns
C.V	2.52	2.72	2.41	4.13	2.40	4.89	5.71	12.70

** and * represent significance at the 0.01 and 0.05 levels, respectively, and ns represents non-significance at $p < 0.05$

Table 2. The effect of year on some characteristics of Thompson navel orange

Year	Fruit length (mm)	Fruit diameter (mm)
2016	80.24a	82.63a
2017	75.23a	78.51b

Values followed by different letters were significant difference according to Duncan's Multiple Range Test at $p < 0.05$

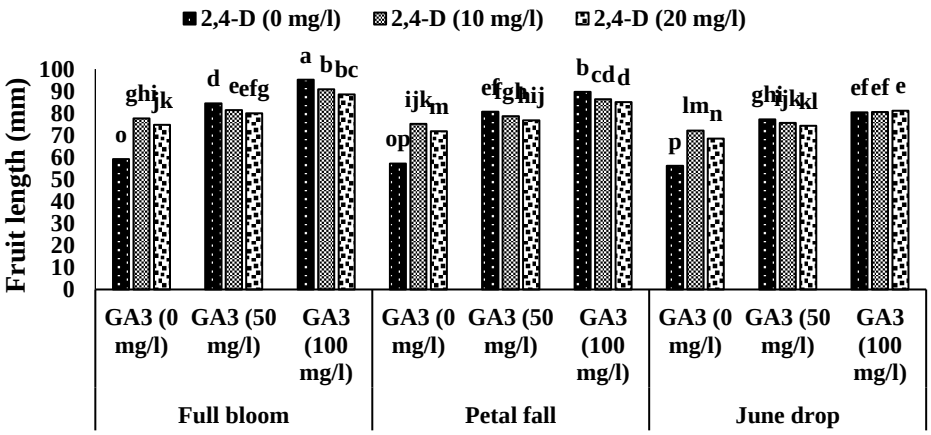

Fig. 1. The interaction effect of 2, 4-D×GA3×Time of spray on fruit length of Thompson navel orange

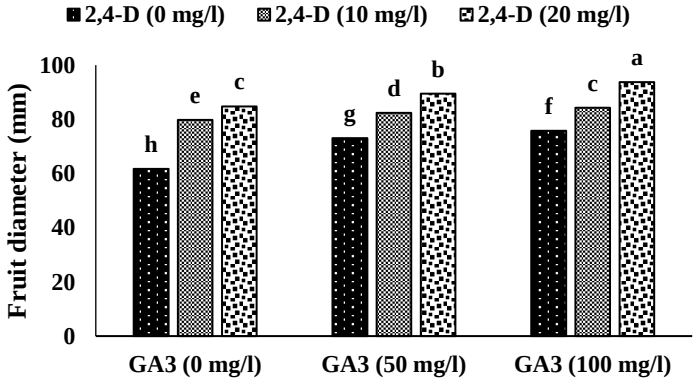

Fig. 2. The interaction effect of 2, 4-D×GA3 on fruit diameter of Thompson navel orange

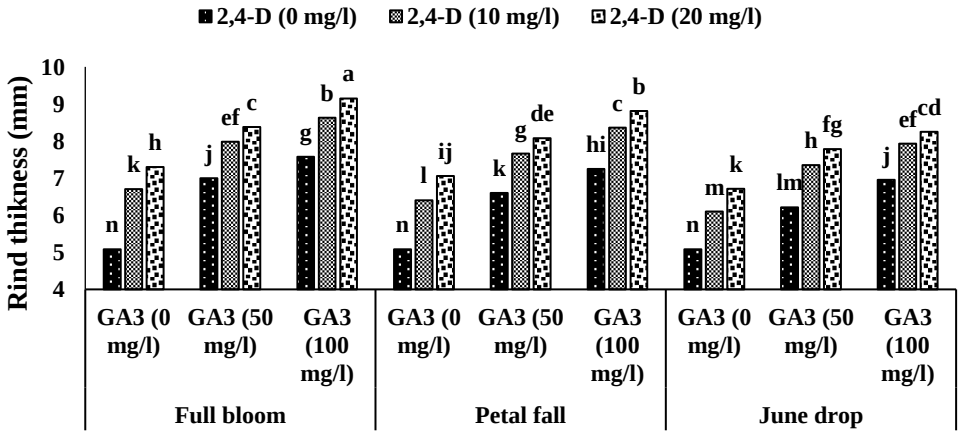

Fig. 3. The interaction effect of 2, 4-D×GA3×timing of spray on rind thickness of Thompson navel orange

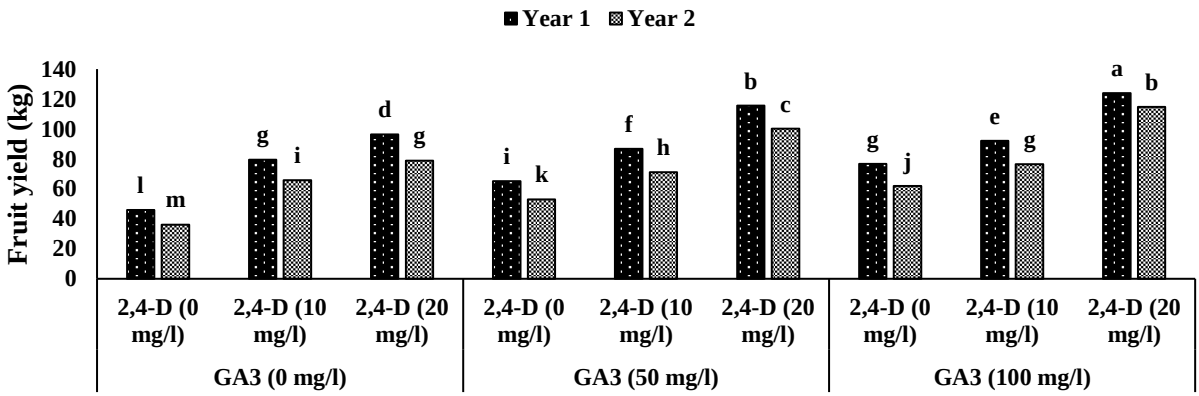

Fig. 4. The interaction effect of 2, 4-D×GA3 on fruit yield of Thompson navel orange

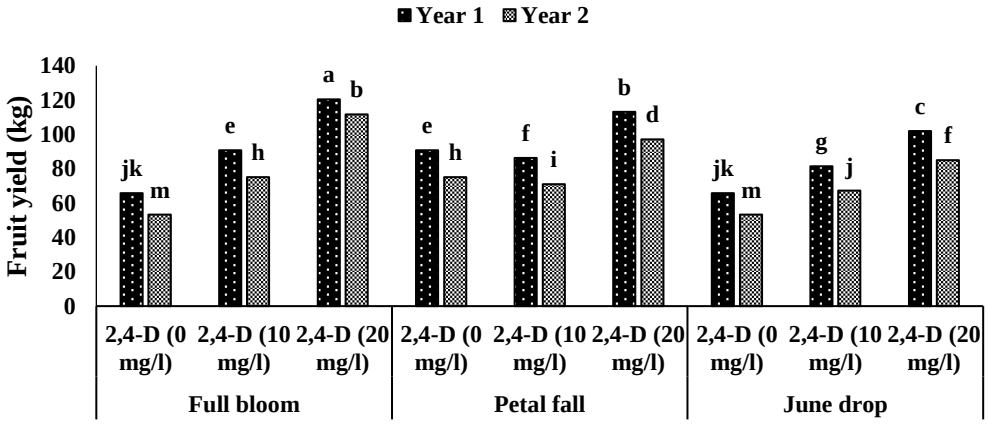

Fig. 5. The interaction effect of 2, 4-D ×timing of spray on fruit yield of Thompson navel orange

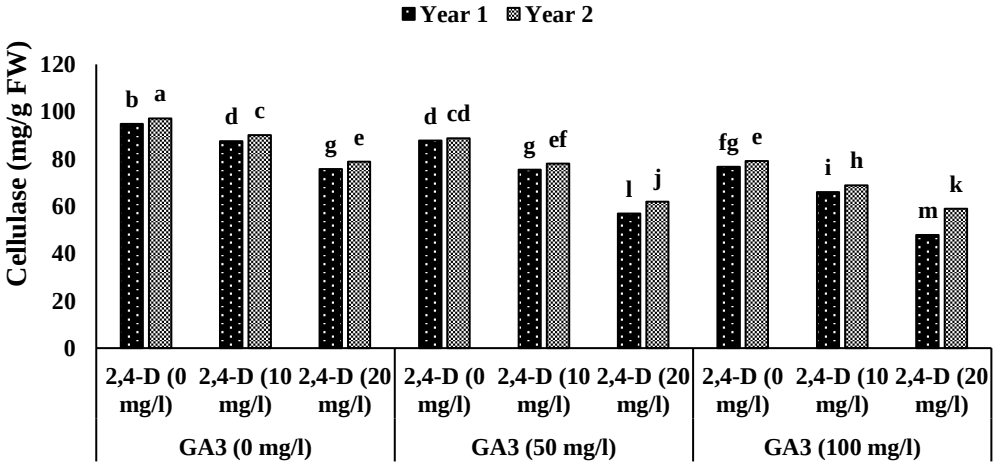

Fig. 6. The interaction effect of 2, 4-D×GA3 on cellulase activity of Thompson navel orange

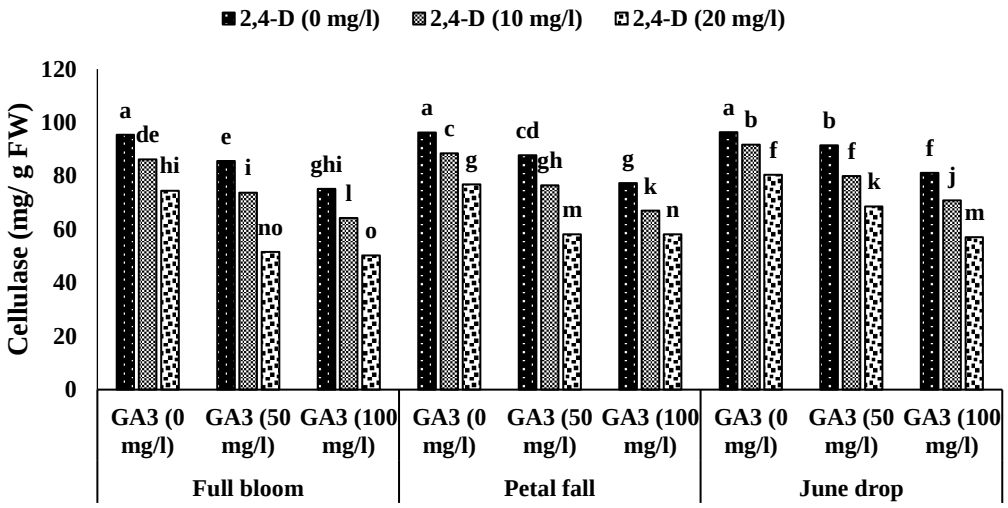

Fig. 7. The interaction effect of 2, 4-D×GA3×timing of spray on cellulase activity of Thompson navel orange

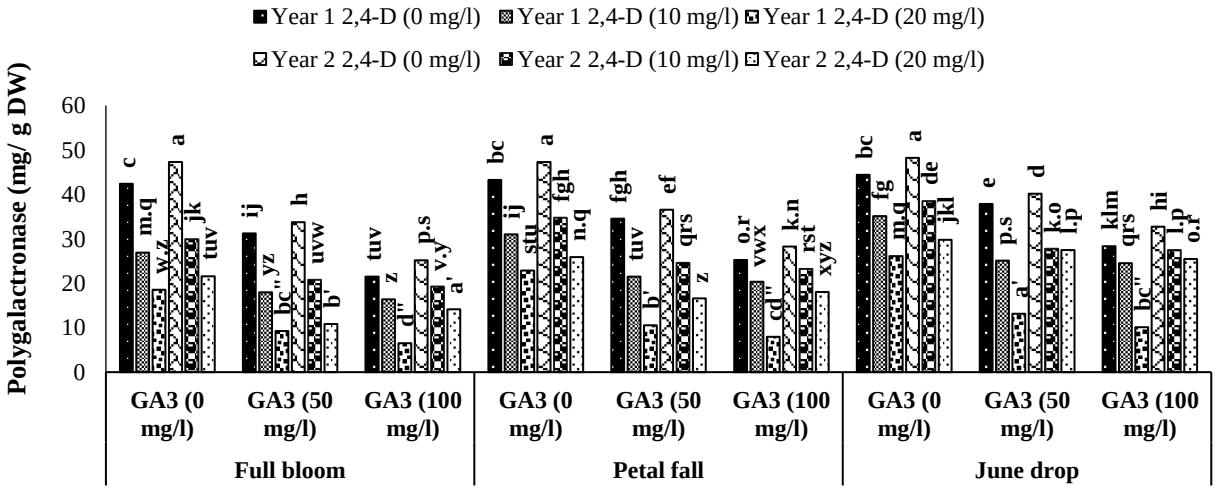

Fig. 8. The interaction effect of 2, 4-D×GA3×timing of spray on PG activity of Thompson navel orange

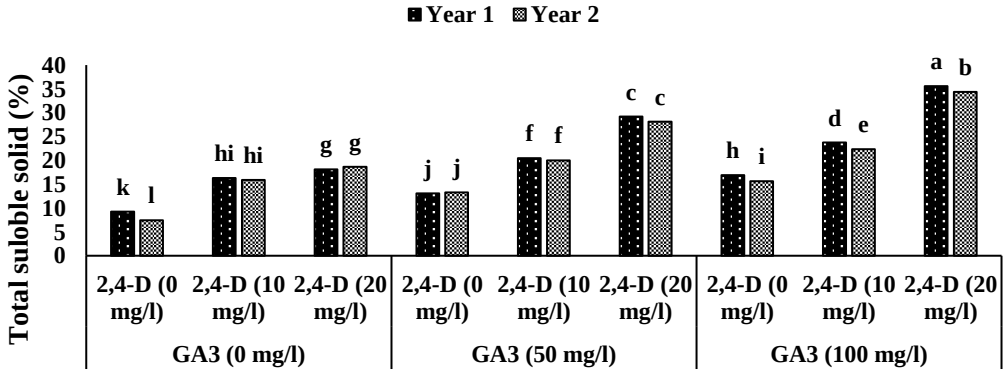

Fig. 9. The interaction effect of 2, 4-D×GA3 on TSS of Thompson navel orange

was observed by employing the highest concentrations of the both PGRs (i.e. 2, 4-D and GA_3 at 20 and 100 mg L^{-1}, respectively) at full bloom that gained 44.4% more rind thickness in comparison to control. The lowest average of rind thickness (average 5.08 mm) was attained by control (Fig. 3).

Fruit yield. According to Table 1, the main effects of GA_3 and T along with the interaction effect of Y× 2, 4-D×T were significant on fruit yield. The highest fruit yield (average 128.89 kg) was gained by applying the both PGRs at their highest concentrations in Y1 that gained higher fruit yield (64.4%) as compared to control. The lowest average of fruit yield was shown by control in Y2 (average 36.15) (Fig. 4). However, the highest fruit yield was found by employing 2, 4-D at 20 mg L^{-1} at full bloom that obtained an increase of 45.3% in contrast to control (Fig. 5).

Cellulase activity. The activity of cellulase in rind was affected by different treatments. The results showed that application of 2, 4-D and GA_3 significantly decreased activity of cellulase as compared to control (Fig. 6). However, the highest activity of cellulase (average 96.23 mg/fresh weight) was shown by control while the lowest (average 50.27 mg/fresh weight) was observed through employing both GA_3 and 2, 4-D at full bloom (Fig. 7).

PG activity. The results of our research revealed that PG was significantly influenced by main effects of Y, 2, 4-D, GA_3, and T as well as their interaction effects (Tab. 1). In both years (Y1 and Y2), application GA_3 and 2, 4-D significantly reduced activity of PG. The lowest PG activity was observed at full bloom during Y1 by employing GA_3 (100 mg L^{-1}) and 2, 4-D (20 mg L^{-1}) (Fig. 8).

TSS. The results of our research revealed that the main effects of Y, 2, 4-D, GA_3, and T as well as interaction effect of Y× 2, 4-D × GA_3 on TSS were significant at $p < 0.0$. (Tab. 1). By comparing the means of spray timing, it was observed that the highest TSS (average 22.10%) and lowest (average 17.46%) were recorded at full bloom and June drop, respectively (Tab. 3). In both years of experiment, using 2, 4-D and GA_3 significantly increased the rate of TSS.

Table 3. The effect of timing of spray on some studied characteristics of Thompson navel orange

Growth stages	Fruit diameter (mm)	Total soluble solid (%)
Full bloom	83.93a	22.10a
Petal fall	80.51b	19.97b
June drop	77.25c	17.64c

Values followed by different letters were significant difference according to Duncan's Multiple Range Test at $p < 0.05$

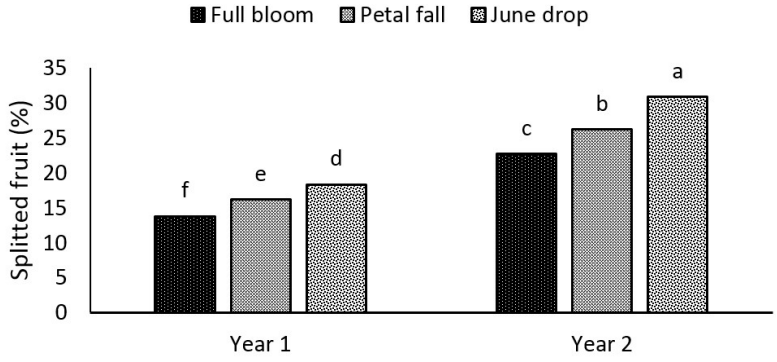

Fig. 10. The interaction effect of Y × timing of spray on fruit splitting of Thompson navel orange

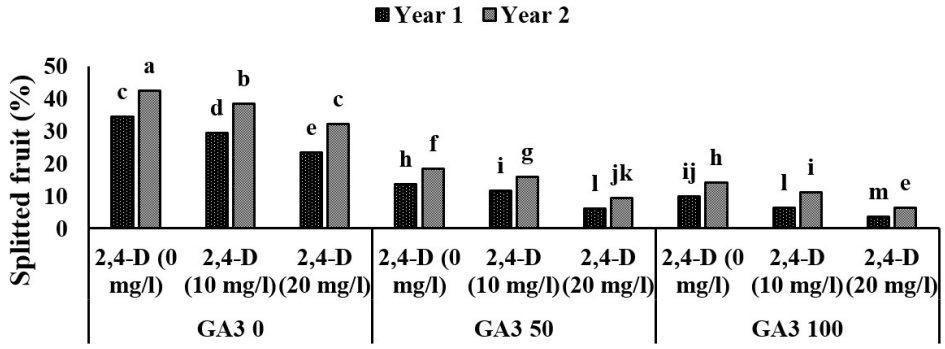

Fig. 11. The interaction effect of 2, 4-D×GA3 on fruit spray of Thompson navel orange

Moreover, the highest TSS in Y1 (average 35.57%) was obtained by employing GA_3 (100 mg L^{-1}) and 2, 4-D (20 mg L^{-1}) in comparison to control (an increase of 74.02%) (Fig. 9).

The data presented in Tab. 1 showed that the percentage of fruit splitting (PFS) was affected by different treatments. In both years, the lowest PFS was observed by applying treatments at full bloom (Fig. 10). In other words, the results showed that application 2, 4-D and GA_3 significantly decreased the rate of PFS in comparison to control. However, the lowest PFS was gained during Y1 by applying 2, 4-S (20 mg L^{-1}) and GA_3 (100 mg L^{-1}) (Fig. 11).

DISCUSSION

In our research, the effect of the PGRs on fruit splitting of Thompson navel orange was investigated. The results showed that application 2, 4-D and GA_3 increased fruit length and diameter. Our findings are in agreement with others in terms of PGRs effects on fruit length of apple, pear, olive, and banana [Watanabe et al. 2008, Yehia and Hassan 2005, Ramezani and Shekafandeh 2009, Sanna Ebeed et al. 2008]. Spraying GA_3 on trees of 'Valencia' orange enhanced fruit weight, diameter, and rind thickness [Sayed et al. 2004]. It seems that different gibberellins induce fruit length through synthesizing proteins in favor of regulating enzyme production, implementing a change in membrane permeability of cell wall, producing new microfibril, and absorbing more water [Guardiola et al. 1997]. However, Moghadas and Rahemi [2002] showed that 2, 4-D increased fruit length and diameter of tangerine native to Jahrom. There are some reports over enhancement of fruit size of orange by 2, 4-D [Almeida et al. 2004]. Also, it has been found that auxin is able to increase fruit diameter of orange as much as 8–13%; and this is contributed to formation of phloem and secondary xylem affecting the size of fruit [Anthony and Coggins 1999]. The effects of auxin on plants include enlarging and lengthening plant cells and their differentiation as well as restarting the activity of vascular bundles and intensifying cell division and producing flower. Role of 2, 4-D on lengthening cells paves the way for developing cells and enhancing their capability to absorb more water, thereby growing fruits earlier and consequently enlarging their size [Agusti et al. 2002].

The results of our research showed that application 2, 4-D and GA_3, apart from enhancing fruit qualitative characteristics, significantly increased yield as compared to control. The results of an experienced carried out by Ferrante et al. [2009] over enhancing quality and yield of persimmon fruit, application GA_3 at full bloom or fruit set increased vegetative growth characteristics (namely, stem length and diameter, total chlorophyll, and number of leaf per a stem) and bearing characteristics (number of fruit, yield, and quality of fruit). In this regard, it has been reported that citrus are more sensitive to PGRs such as 2, 4-D, in a way that if its dose goes beyond 20 mg L^{-1}, it leads to an increase in the size of fruit or rate of fruit production [Guardiola and Garcia-Luis 1997]. The pathogenic fungi use

PG enzymes to enter into plant tissues. In contrast, some plants possess polygalacturonase-inhibiting protein (PGIP) as a glycoprotein to delay or prevent the penetrance of their tissues by fungal hyphae and their colonization in their cells [Hossainzadeh et al. 2007]. Pectinase enzymes are of first enzymes secreted by fungi pathogens to penetrate plant cells. The importance of these enzymes in fungi pathogens has been substantiated by pectinase suppressors in cell wall of some plants [Favaron 2001]. Among pectinase enzymes, PG play a significant role in degrading plants' cell wall. In our research, application 2, 4-D and GA_3 decreased cellulase and PG activities in fruit cells.

Prior to occurring fruit splitting, selecting an appropriate time of spraying PGRs is crucial to control fruit splitting, increase rind thickness and rind mechanical resistance to splitting, and reduce rind cracking [Khehra and Bal 2014]. Application a synthesized auxin called 2, 4-D decreased the number of fruit split through increasing rind thickness and roughness in thin- or smooth-rind orange cultivars [Garcia-Luis et al. 2001]. Spray GA_3 at different growth stages of pomegranate fruit reduced fruit splitting and inversely increased fruit weight, volume, total acidity, TSS, juice content. By investigating the hormones found in fruit tissues of pomegranate at pre-harvest stage, it was observed that the split fruits possessed higher ABA and lower GA_3 and IBA in comparison to no-split fruits [Yilmaz and Ozguven 2005]. Our findings showed that application exogenous gibberellins and auxins may reduce fruit splitting. In this regard, it has been reported that using 2, 4-D (20 mg L^{-1}) at full bloom significantly decreased fruit splitting in citrus, and this was contributed to the thickness of the rind located in the end of style in treated fruits [Guardiola and Garcia-Luis 1997]. In present research, 2, 4-D and GA_3 significantly increased rind thickness, thereby reducing fruit splitting.

In citrus, fruit splitting is affected by status of rind structure, and using PGRs is suitable for reducing fruit splitting through affecting cell size and flavedo thickness [Hoffmann 2009]. Spraying 2, 4-D and NAA can reduce rind disorders such as splitting which is in agreement with our findings [Javidsafdari 2015]. In this respect, it has been exhibited that using GA_3, after fruit set stage, was the most efficient and applicable approach to prohibit fruit splitting in pomegranate [Eoliaiitarshiz and Emamian 2011]. GA_3 efficiently affects cell wall integrity or its elasticity, and by enhancing its elasticity it reduces fruit splitting [Khalil and Aly 2013]. Moreover, GA_3, through affecting permeability and/or cuticle elasticity of rind, influence indirectly fruit splitting [Sekse 2005]. Therefore, it seems that GA_3 reduces fruit splitting through increasing the flexibility of fruit skin [Yildirim and Koyuncu 2013].

CONCLUSION

The results of this research revealed that application 2, 4-D and GA_3 significantly reduces fruit splitting in Thomson navel orange. The highest efficiency was obtained when the both PGRs were simultaneously employed at full bloom stage. A reduction in fruit splitting may be due to an increase in rind thickness and reduction in activity of cellulase and PG. Moreover, application 2, 4-D and GA_3 improve the qualitative characteristics and fruit yield of Thompson navel orange and this justifies their practical application in citrus orchards.

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