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# Calyx biochemical changes and possibility of reducing thomson orange June drop by nutrition elements and growth regulators

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**Abstract**

Summer fruit drop (June drop) is one of the main reasons for low yield in some citrus varieties in northern Iran. Recognition of physiological changes in fruit abscission zone (calyx) and application of suitable treatments would reduce or control abscission. Hence, the changes of auxin, cellulose, and polygalacturonase in calyx of Thomson navel fruitlets were measured and their relations with abscission and mineral nutrition of fruitlets as well as the effect of different treatments on them at June drop were evaluated. A randomized complete block design (RCBD) experiment was performed with 8 treatments and 4 replicates. Treatments were as follows: 1. control; 2. urea (1%); 3. urea + 2,4-D (15 mg $l^{-1}$); 4.urea + $GA_3$ (15 mg $l^{-1}$); 5.urea + sucrose (1%); 6.urea + $ZnSO_4$ (0.5%); 7. urea + $ZnSO_4$ + 2,4-D; and 8. urea + ZnSO4 + $GA_3$. Results showed that abscission had a significant negative correlation with auxin and a significant positive correlation with cellulase and polygalacturonase of the calyx. The control group had the lowest auxin and the highest cellulase in calyx. Maximum effect (Approximately 24% reduction in abscission) was obtained after foliar application of urea + sucrose (treatments 5). The use of urea alone or in combination with 2, 4-D and $GA_3$ had no effect on abscission; however, using it in combination with sucrose was the most efficient treatment in this experiment. Furthermore, the combination of urea and Zn provided an effective treatment. A majority of micronutrients (Mg, Fe, Mn, Zn, Cu and B) had a positive correlation with auxin and a negative correlation with enzymes. Therefore, nutritional management and providing an appropriate condition to maximize photosynthesis can balance physiological metabolism and reduce fruitlets abscission.

**Keywords:** Abscission, Auxin, Citrus, Enzyme activity, Nutrition.

**Abbreviations: ABA,** Abscisic Acid; **ACC,** 1-Aminocyclopropane-1-Carboxylic Acid; **$GA_3$,** Gibberellic Acid; **PG,** Polygalacturonase; **MCP,** Methylcyclopropene; **TLC,** Thin Layer Chromatography; **2,4-D,** 2,4-Dichloro Phenoxy Acetic Acid.

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## Introduction

Annual citrus production in Iran is more than 4.34 million tons, representing the highest production of horticultural crops in the country (NN., 2015). Abscission during different growth stages is one important reason to yield reduction and high losses in citrus. In addition to flower drop in citrus, there are other natural fruit drops like initial drop and June drop caused by multiple reasons including both physical and pathological ones. Sudden changes in temperature or humidity, poor nutrition management, hormonal imbalance, improper soil moisture and so on are the physiological causes and the fungal infections pathologically results in fruit drop (Racsko *et al*., 2007).

Summer physiological abscission (June drop) include abscission of fruitlets with 0.5-2 cm diameter (Iglesias *et al.*, 2007). This is a physiological process that is likely to compete among fruitlets for carbohydrates, water, hormones and other metabolites and would be intensified by stresses caused by high temperature and water deficit (Huchche, 2001).

Many researchers have proved that phytohormones can regulate fruit abscission. Racsko *et al*. (2006) concluded that the loss of hormonal balance (including auxins, cytokinins, gibberellins and abscisic acid) causes fruit drop. Some synthetic auxins delay the formation of abscission layer by increasing internal auxin (Huchche, 2001).

Onguso *et al.* (2004) reported that auxins spraying prevented the senescence of fruits presumably by maintaining the cell turgidity at abscission zone. This prevents the synthesis of hydrolytic enzymes such as cellulose, which hydrolyze the cell walls. Polygalacturonase (PG) usually act in correlation with cellulose, to degrade two main components of the cell wall (namely cellulose and pectin) (Ibrahim *et al*., 2011). To control fruit abscission in early growth stages, foliar application of 15 mg $l^{-1}$ gibberellic acid ($GA_3$) with 1000 mg $l^{-1}$ benomyle and urea (1%) was effective (Huchche *et al*., 1993). GA application in partenocarpic citrus varieties reduces June drop (Huchche, 1993; Pozo, 2001). Optimum use of some nutrient elements like nitrogen can also delay or reduce abscission (El-Otmani *et al*., 2000). There are also some evidences indicating that nitrogen is required for auxin synthesis. Urea foliar application eliminated the need for some growth factors like polyamines in nitrogen metabolism (Huchche, 2001). Application of urea at full bloom and summer physiological drop improved the yield due to an increase in flower number and fruit size (El-Otmani *et al*., 2000).

Additionally, it is reported that carbohydrate content may be a biochemical signal involved in controlling abscission. The fruit set in citrus is highly dependent upon carbohydrate availability (Iglesias *et al*., 2003, 2006). Researches have shown that sucrose concentration in leaves reduced during June drop, causing competition among growing fruitlets for carbohydrate. Reduction of sucrose and other carbohydrates in peel of fruitlets during June drop could indicate the association between free sugar level and fruitlets drop. Like leaf removal which greatly reduced the transfer of sucrose to fruitlets, increased ethylene synthesis and ACC accumulation by fruitlets, the deficiency of free sugar could stimulate ethylene synthesis (Ruiz *et al*., 2001). Mehouachi *et al*. (2009) reported a relationship between the reduction of early abscission rates and higher concentrations of carbohydrates and GAs induced by girdling in developing fruitlets. These findings revealed that girdling certainly increased the GA concentration and strongly suggested that its effect on early fruitlet abscission delay is likely to be mediated by both GA and carbohydrates. It is reported that sucrose foliar application has an adjusted alternate bearing cycle in Satsuma mandarin trees. Also, this treatment increased the average diameter and weight of fruits (Akhlaghi Amiri *et al*., 2006).

Abscission of young fruits has a positive correlation with cellulose activity in abscission zone (YangLiang *et al*., 1997; Burns, 1998). It is also claimed that benzyl adenine and GA prevented the abscission of young fruits by delaying the cellulose activity (Yang Liang *et al*., 1997). Also, researches in citrus gardens located at the eastern part of Mazandaran province in northern Iran showed that the Zn deficiency is crystal clear because of calcareous soil and imbalanced consumption of chemical fertilizers. This factor limits the regional production and probably increases the citrus abscission in this area (Asadi Kangarshahi *et al*., 2002). Accordingly, this experiment was performed to investigate the effects of auxin, cellulose, and PG on the abscission zone of Thomson navel orange and their relationship with fruitlet abscission and mineral nutrition at June drop. Single and combination treatment of urea, zinc, 2, 4-D, sucrose and $GA_3$ were used to investigate their effects on the occurrence of abscission in fruitlet stage of Thomson orange.

## Materials and Methods

The experiment setting was located at 36 degrees north latitude and 52 degrees east longitude. The local meteorological information obtained during the experiment was as follows: Annual precipitation: 570.6 mm; Absolute minimum temperature: -2.6°C; Absolute maximum temperature: 37.9°C; Average minimum temperature: 13.37°C; Average maximum temperature: 22.5°C; Average daily temperature: 17.9°C; Absolute minimum humidity: 10%; Absolute maximum humidity: 100%; Average minimum humidity: 56%; Average maximum humidity: 92%; Average daily humidity: 73%; and Average height above sea level: 29 m.

The experiment used a randomized complete block design with eight treatments and four replicates (one tree for each replication) on 14-year Thomson navel orange (*Citrus sinensis* L.) trees on sour orange rootstock in Sari, Iran. All agricultural practices like drip irrigation and pest control were similar for all trees based on the tree requirement.

The treatments (1. control, 2. Urea (1%), 3. urea+2,4-D (15 mg $l^{-1}$), 4. urea+ $GA_3$ (15 mg $l^{-1}$), 5. urea+sucrose (1%), 6. urea+$ZnSO_4$ (0.5%), 7. urea+$ZnSO_4$+2,4-D and 8. urea+ $ZnSO_4$+$GA_3$) were sprayed in late May, 2006, 20 days after full bloom (after early abscission of flowers and fruitlets and before starting summer fruit drop (June drop)) when the diameter of fruitlets was about 10 mm.

After spraying until the ending of June drop, dropped fruitlets in each tree were counted every ten days. Sampling was simultaneously carried out at the time of maximum drop in all trees, and 50 fruits (about 10 g) were randomly picked and their calyxes along with a part of peduncle were separated.

Mineral elements (N, P, K, Mg, Fe, Mn, Zn, Cu and B) of fruitlets were measured (Emami, 1996). Then, Calyxes were washed with distilled water, dried in a freeze dryer and powdered with liquid nitrogen. Calyx samples were used for measuring IAA, polygalacturonase (EC. 3.2.1.15) and cellulase (EC. 3.2.1.4).

To measure auxin, the modified method of Unyayar *et al*. (1996) was used (Ergun *et al*., 2002). Accordingly, first, 0.5 g of the calyx sample was extracted by auxin buffer. Then purification steps were performed and separation was conducted by thin layer chromatography (TLC). Auxin concentration in each sample was measured by densitometer at 222 nm wavelength (Ergun *et al*., 2002).

To measure PG, the modified method of Gross (1982) was used (Faize *et al*., 2003). To do so, 0.2 g from each sample was extracted and then, incubated. Absorption was determined by a spectrophotometer with 274 nm and enzyme concentration was calculated by sample absorption and a standard curve (Faize *et al*., 2003).

Moreover, cellulase was measured by the modified method of Ghose (1987). First, 0.1 g of each sample was extracted. Whatman filter paper No. 1 was used as a substrate. After several steps of incubation and freezing, absorption was determined by a spectrophotometer with 540 nm and enzyme concentration was calculated by sample absorption and a standard curve (Adney and Baker, 1996).

Fruit number in each tree was calculated based on the yield and fruit average weight. Abscission percentage was determined based on fruit number at harvest and number of dropped fruitlets.

## Results

Analysis of variance indicated that percentage of abscission at June drop of Thomson navel orange had a significant difference at 1% level. Except the treatment 2, all the treatments reduced abscission compared to the control, which was significant at 5% level (Table 1) in the treatments 5, 6 and 8. Maximum abscission reduction was observed in the treatment 5 with about 24%, as compared to the control.

Analysis of variance of IAA did not show any significant difference. The treatments 5 and 6 had the highest auxin content among the other treatments; however, this difference was not statistically significant (Table 1).

Analysis of variance for PG showed a 5% difference. The treatments 2 and 5 had the highest and lowest PG, respectively; except the treatment 5, the other treatments did not have any significant difference with the control treatment (Table 1).

Also, analysis of variance indicated that cellulase had a significant difference at 1% level. Cellulase content was the highest in the control treatment. Except in the treatment 2, cellulase in the other treatments was reduced significantly, as compared to the control (Table 1).

Analysis of variance of mineral elements of fruitlets did not show any significant difference. Mean comparison of mineral nutrition in the different treatments is shown in Table 2. As can be observed, only magnesium, iron and copper had significant differences among the different treatments. The control treatment had the lowest magnesium, iron, manganese, and zinc; however, magnesium level in the control was equal to that in the treatment 2 (urea), which had significant reduction compared to all the other treatments, and iron level in the control was equal to that in the treatment 8. The treatment 6 (urea + $ZnSO_4$) had the highest magnesium and iron among the other treatments, which was statistically significant compared to the control. The treatment 5 (urea + sucrose) had the highest copper and boron among the other treatments, but it was not statistically significant (Table 2).

The Pearson correlation coefficient and linear regression among percentages of abscission of Thomson navel fruitlets, PG, cellulase and auxin content in calyx and fruitlets mineral elements in the different treatments at June drop are shown in Table 3. As is indicated, abscission and auxin had a significant negative correlation at 1% level. Also, abscission had a significant positive correlation with cellulase at 1% and with PG at 5% level (Table 3). PG and auxin had a negative significant correlation at 1% level. Cellulase had a negative correlation with auxin and a positive correlation with PG at 5% level. Also, the correlation between cellulase with magnesium and manganese was negative and significant at 5% level. Overall, Table 3 indicated that abscission, PG, and cellulase had a positive correlation with N, P and K, whereas they had a negative correlation with Mg, Fe, Mn, Zn, Cu and B of Thomson navel fruitlet at June drop. In contrast, auxin had a negative correlation with N and K, while it had a positive correlation with Mg, Fe, Mn, Zn, Cu and B of fruitlets. All significant correlations had also significant regressions (Table 3).

**Table 1. Percentage of abscission, auxin (IAA), PG and cellulase content in the abscission zone of Thomson navel orange fruitlets in the different treatments at June drop**

| Treatments | Abscission (%) | Auxin ($\mu g\ g^{-1}$) | Polygalacturonase ($U\ g^{-1}$) | Cellulase ($U\ g^{-1}$) |
|---|---|---|---|---|
| 1. Control | 44.89 a | 35.19 a | 27.84 ab | 101.50 a |
| 2. Urea | 45.04 a | 38.25 a | 28.97 a | 97.90 ab |
| 3. Urea + 2,4-D | 43.48 ab | 44.31 a | 26.38 ab | 85.06 cd |
| 4. Urea + $GA_3$ | 43.15 ab | 48.81 a | 22.32 bc | 85.23 cd |
| 5. Urea + sucrose | 34.26 c | 82.86 a | 19.54 c | 78.86 d |
| 6. Urea + $ZnSO_4$ | 38.40 bc | 63.51 a | 24.10 abc | 80.75 d |
| 7. Urea + $ZnSO_4$ + 2,4-D | 44.15 ab | 37.26 a | 27.95 ab | 91.29 bc |
| 8. Urea + $ZnSO_4$ + $GA_3$ | 38.29 bc | 42.15 a | 26.17 ab | 84.35 cd |

In each column, means followed by the same letter are not significantly different using DNMRT at 5% level.

**Table 2. Effect of different treatments on mineral elements concentration in Thomson navel fruitlet at June drop**

| Treatments | N ns | K ns | P ns (%) | Mg* | Fe* | Mn ns | Zn ns (mg/kg) | Cu* | B ns |
|---|---|---|---|---|---|---|---|---|---|
| 1. Control | 1.895 | 1.370 | 0.1650 | 0.1275c | 25.00b | 5.675 | 12.50 | 14.65a | 88.25 |
| 2. Urea | 1.920 | 1.452 | 0.1600 | 0.1275c | 29.88ab | 6.225 | 16.00 | 14.10a | 87.88 |
| 3. Urea + 2,4-D | 1.870 | 1.308 | 0.1600 | 0.1500ab | 26.38ab | 7.100 | 13.55 | 14.10a | 95.13 |
| 4. Urea + $GA_3$ | 1.890 | 1.378 | 0.1525 | 0.1500ab | 30.70ab | 8.150 | 14.10 | 14.65a | 89.68 |
| 5. Urea + sucrose | 1.888 | 1.308 | 0.1600 | 0.1400ab | 29.38ab | 6.775 | 15.75 | 15.47a | 100.8 |
| 6. Urea + $ZnSO_4$ | 1.860 | 1.313 | 0.1600 | 0.1600a | 34.78a | 8.325 | 14.13 | 8.38bc | 79.47 |
| 7. Urea + $ZnSO_4$ + 2,4-D | 1.940 | 1.183 | 0.1575 | 0.1550ab | 32.05ab | 6.500 | 14.67 | 3.91c | 80.05 |
| 8. Urea + $ZnSO_4$ + $GA_3$ | 1.857 | 1.370 | 0.1525 | 0.1550ab | 25.00b | 7.325 | 13.05 | 10.50ab | 93.13 |

In each column, means followed by the same letter are not significantly different using DNMRT at 5% level.
ns: no significant difference
*: significant difference at 5% level

**Table 3. The correlation coefficient and regression between fruitlet abscission, PG, cellulase and mineral elements of Thomson navel fruitlet at June drop in the different treatments (R: correlation; $R^2$: regression)**

| | Abscission | | PG | | Cellulase | | Auxin | |
|---|---|---|---|---|---|---|---|---|
| | R | R2 | R | R2 | R | R2 | R | R2 |
| Abscission | 1 | | | | | | | |
| PG | 0.768* | 0.586* | 1 | | | | | |
| Cellulase | 0.801** | 0.638* | 0.784* | 0.615* | 1 | | | |
| Auxin | -0.868** | 0.752** | -0.894** | 0.799** | -0.765* | 0.585 * | 1 | |
| N | 0.536 | 0.290 | 0.389 | 0.152 | 0.579 | 0.335 | -0.352 | 0.124 |
| K | 0.142 | 0.018 | 0.097 | 0.009 | 0.309 | 0.095 | -0.146 | 0.021 |
| P | 0.198 | 0.033 | 0.245 | 0.060 | 0.457 | 0.209 | 0.028 | 0.001 |
| Mg | -0.358 | 0.114 | -0.257 | 0.066 | -0.710* | 0.504* | 0.186 | 0.035 |
| Fe | -0.148 | 0.017 | -0.248 | 0.062 | -0.320 | 0.102 | 0.366 | 0.131 |
| Mn | -0.410 | 0.154 | -0.529 | 0.280 | -0.763* | 0.582* | 0.394 | 0.155 |
| Zn | -0.206 | 0.042 | -0.244 | 0.059 | -0.157 | 0.021 | 0.435 | 0.189 |
| Cu | -0.031 | 0.003 | -0.323 | 0.104 | 0.072 | 0.005 | 0.216 | 0.047 |
| B | -0.409 | 0.183 | -0.481 | 0.232 | -0.305 | 0.093 | 0.400 | 0.160 |

*: Significant difference at 5% level
**: Significant difference at 1% level

## Discussion

In this experiment, decrease of auxin resulted in an increase in the content of PG and cellulose. On the other hand, enzymes had a positive and strong relation with the percentage of abscission, which corresponds to results of other studies (Monselise and Goren, 1978; Yang Liang *et al*., 1997; Burns, 1998; Ibrahim *et al*., 2011). The use of urea alone, or in combination with 2,4-D and $GA_3$ did not have any effect on abscission, but in combination with sucrose was the best treatment in this experiment. Also, combining urea and Zn was an effective treatment. The treatment 5, in which sucrose was used in the combination, had the maximum auxin content, but the lowest content of cellulase and PG. It can be concluded that decreased enzyme activity caused reduction of abscission in this treatment. Reduction of fruitlet competition for carbohydrate can be mentioned as the main reason for the response of the treatment 5, which corresponds with the study by Gomez- Cadenas *et al.* (2000) who reported photo assimilate competition induced abscission. The positive effect of urea and sucrose combination on the reduction of June drop percentage in Thomson navel orange was also reported by other researchers (El-Otmani *et al*., 2000; Iglesias *et al*., 2003, 2006; Ruiz *et al*., 2001). Mehouachi *et al.* (1995) found that sucrose reserve is the main factor for regulation of fruit abscission during June drop. Iglesias *et al.* (2003) observed that sucrose reduced fruitlet abscission and increased carbohydrate availability to growing citrus fruitlets. Contrary to expectations, the treatments 3 and 7 that had 2,4-D in their combination did not show any significant effect on auxin content, and PG in them was similar to that in the control. Only, cellulase was significantly decreased when compared to the control. Adding $GA_3$ in the treatments 4 and 8 had similar effects on auxin, enzymes, and abscission in comparison with the treatments 3 and 7. In the treatments 7 and 8, the positive effects of urea and $ZnSO_4$ on the occurrence of abscission were decreased.

Results showed that auxin and micronutrients were positively correlated, whereas the correlation of abscission and enzymes with micronutrients was negative. Also, the relation of Mg and Mn with cellulase amount was significant. In the treatments 1 and 2, abscission was higher than that in the other treatments, whereas Mg of fruitlets was significantly lower than that in the other treatments. Also, Mn of these two treatments was lower than that in the others. In the treatments 6 and 8, in which $ZnSO_4$ was used in the combination, abscission reduction was significant, which corresponded to the results of the study by Asadi *et al.* (2002) conducted in Mazandaran, Iran, which reported Zn as a limiting factor for production in this province. Racsko *et al.* (2007) mentioned that poor nutrition management and hormonal imbalance are some physiological causes of fruit drop.

Generally, treatments that delay auxin reduction in abscission zone reduce synthesis or activity of hydrolytic enzymes and delay destroying of the cell wall and finally, can prevent abscission. Racsko *et al.* (2006) reported that auxin inhibits abscission by repression of the synthesis of hydrolytic enzymes, which is consistent with Burn's researches about citrus abscission in 1998.

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