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Effect of putrescine, GA_3, 2, 4-D, and calcium on delaying peel senescence and extending harvest season of navel orange

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Abstract

The present study was conducted in 2007/2008 and 2008/2009 seasons in order to extend harvest season and maintain fruit quality for better marketability of Washington navel oranges growing in clay soil by preharvest foliar sprays of GA_3, 2,4-D, putrescine and calcium either alone or in combinations. Fruits were harvested on two different harvest dates, the first was at the estimated commercial harvest date (middle December), and the second was late in the harvest season (during February). At both harvesting dates, all spray treatments delayed fruit softening, peel ageing and fruit color break and decreased creasing and fruit drop. Also, fruit TSS, sugars and vitamin C contents increased. The treatments had positive influence on extending harvest season without any deterioration in fruit characteristics. Spraying the different substances in combinations gave better results, especially with putrescine.

Key words: Putrescine, GA_3, 2, 4-D, calcium, peel senescence, Navel orange

Introduction

Oranges are one of the worldwide popular fruits mostly consumed fresh. Orange fruit faces, pre- or post-harvest, a number of rind disorders such as creasing, splitting, puffing, peel pitting and senescence. Delaying peel senescence prolongs the fruits life, improves fruit quality and extends its marketing season, which enhances the crop value and contributes to growers returns. Thus, any manipulation of the very final stage of fruit development in order to delay rind senescence improves fruit quality and extends its exporting season.

In Egypt, navel orange growers tend to expand harvest period by keeping the fruit for longer time on the trees to extend marketing season, for late export date. This practice leads to the appearance of the previous mentioned disorders, mainly on the rind, ending with fruit senescence and shorten its shelf life and marketability. Controlling of rind aging, mainly rind softening in navel oranges is important for the marketing of quality fresh orange and to prolong the life of the fruit with high quality characters as long as possible after harvest.

Growth regulators are one of several tools, when properly used, enable citrus growers to extend marketing period with no loss of fruit quality (Ismail, 1997). Gibberellic acid when applied pre-harvest retarded rind softening and fruit maturation (Ismail 1997; Coggins, 1981). Creasing, splitting, puffing and peel pitting can be reduced in intensity or minimized using gibberellic acid and synthetic auxins or a mixture of both (Agusti *et al*., 2002). According to Chapman (1983), exogenous applications of GA_3 at the citrus fruit color change stage maintained the peel quality of late-harvested fruit and reduced mesocarp cracking. In addition, pre-harvest sprays of 2,4-D alone or with GA_3 is proven effective technique for better peel quality in tree-stored fruit, and reducing late season fruit drop therefore, extending the harvest season, as well as retarding rind senescence and lowering fruit decay (Golddchmidt and Eilati, 1970; Ismail, 1997). Moreover, Tumminelli *et al*. (2005) noticed an increase in ethylene production in the albedo tissues of Satsuma mandarin with the ripening stage and it increased with peel aging. Zheng and Zhang (2004) reported a gradual decline in the concentrations of free polyamines in mandarin fruits after harvest that was parallel to peel senescence. Polyamines are group of natural compounds that are believed to have anti-senescence function by inhibition of the formation of enzymes essential to the synthesis of ethylene (Ke and Romani, 1988) thus, retard ripening and extend fruit shelf life. They also improve fruit quality by reducing mechanical damage and increasing fruit firmness (Valero *et al*., 1998a and b, Perez-Vicente *et al.*, 2002). In addition, many researches focused on extending citrus fruit life by pre- or post-harvest treatments with calcium (El-Hilali *et al.*, 2004; Valero *et al*., 1998b).

In view of the above findings, the present study was conducted to evaluate the effect of pre-harvest foliar sprays of GA_3, 2, 4-D, putrescine and calcium on internal and external quality of navel orange fruit and fruit abscission during late in the season, to determine whether it is possible to prolong the shelf life and delay the harvest of navel orange trees growing in clay soil without economical loss.

Materials and methods

Plant material and treatment: Thirty-five years old Washington navel orange trees (*Citrus sinensis*, L.), budded on sour orange rootstock planted at 4.5x4.5 m apart in a private citrus orchard at El-Tarh region, EL- Behera Governorate were selected for the study. The soil was clay, well-drained with water table about 110 cm and pH 8. Trees were subjected to the standard cultural practices in the orchard. In January of both seasons, calcium superphosphate (15.5% P_2O_5) was added at the rate of 250 kg per feddan. Ammonium nitrate (33% N) was applied at the rate of 250 kg in March, 250 kg in May and 200 kg in August of both seasons per feddan. In August of both seasons, 100 kg per feddan potassium sulfate (48% K_2O_5) was added. Trees were irrigated with Nile water every 15-20 days.

One hundred and ten trees were selected as uniform as possible and were subjected to two foliar sprays. The first was at the beginning of fruit color change (about month and half before normal harvest date), and the second after 25 day from the first spraying date. Trees were sprayed with, GA_3 (10 ppm), 2, 4-D (10 ppm), putrescine (PUT) (5 mmol) and chelated calcium (1500 ppm) either alone or in combinations. Treatments were arranged in a complete randomized design with five replicates (each replicate consisting of two trees) and the trees were treated with eleven foliage treatments (2 x 5 x 11 = 110 trees), water only, GA_3, 2,4-D, putrescine, calcium, GA_3 + 2,4-D, GA_3 + putrescine, GA_3 + calcium, 2,4-D + putrescine, 2,4-D + calcium, and putrescine + calcium. The surfactant Nourfilm (produced by Alam Chemca) at the rate of 40 cm/100 L water was added to all sprayed chemicals in order to obtain best results. Trees were harvested at two different dates, the first at the estimated commercial harvest date (middle September), and the second late in the harvest season (during February).

Determination of fruit physical characteristics: The percentage of pre-harvest fruit drop, rind ageing, softening, creasing and fruits unfit for export and peel thickness (mm) and fruit color were recorded at each harvest date (middle September and February). Peel softening and fruits unfit for export were measured depending on the scale of export specifications. Rind ageing was estimated as the percent of fruit with peel pitting. Fruit color was estimated by giving five degree of color stage as follows; 1= 100 % green, 2= 25% green, 3= 50% orange, 4= 75% orange and 5=100% orange.

Determination of fruit chemical characteristics: A sample of ten fruits were taken from each replicate at each harvest date in both growing seasons in order to estimate electrolyte leakage (EL), fruit carotenoids, total soluble solids (TSS), acidity, TSS/acidity ratio, sugars, and vitamin C (VC) contents.

Acidity (%) and VC (mg/100 mL juice) was determined by titration according to AOAC (1980). Electrolyte leakage (EL) as ppm was estimated in fruit peel by Conductivity/TDS Meter. Carotenoids (mg/100 g peel fresh weight) were measured according to the method of Moran and Porath (1980). Sugar content (%) was determined according to the method of Malik and Singh (1980).

Statistical analysis: Data obtained were subjected to analysis of variance (ANOVA) to detect treatment effect. Mean separation were performed and compared using least significant difference (LSD) at $P \leq 0.05$ level. The data were analyzed using Statistical Analysis System (SAS) version 6.03.

Results and discussion

Fruit physical characteristics: The effect of the different treatments on fruit physical characteristics at the first and second harvest dates are presented in Tables 1 and 2. The data of the first harvest date showed that all treatments (except spraying PUT alone, 2,4-D alone in both seasons, and PUT + 2,4-D in the first season) increased fruit peel thickness when compared with the control in both seasons. Spraying GA_3 either alone or + PUT, 2, 4-D or Ca had significanty higher effect in increasing peel thickness than 2,4-D + Ca in the first season only. In addition, fruit color break was delayed significantly by spraying PUT, GA_3, PUT + 2, 4-D, PUT + GA_3 and GA_3 + Ca in both seasons. A significant decrease in the rind ageing percent was recorded in both seasons by all foliar sprays (except 2, 4-D alone in the first season). The percent of fruit softening, fruit creasing, fruit drop and fruits unfit for export were decreased by all foliar sprays in both seasons as compared with the control. All foliar sprays had higher effect in decreasing fruit softening than spraying 2,4-D alone in both seasons. Also, spraying PUT alone resulted in the lowest creasing percent in the first season when compared with 2, 4-D alone, 2, 4-D + GA_3, 2, 4-D + Ca and GA_3 + Ca.

At the second harvest date (Table 2), peel thickness increased significantly in the first season by spraying GA_3 alone, Ca alone, PUT + Ca and 2,4-D + Ca with no significant differences among them. However, in the second season foliar sprays of Ca alone, PUT + 2, 4-D, PUT + GA_3, 2, 4-D + GA_3 and Ca + GA_3 resulted in higher increase in fruit thickness in comparison with the control, with the highest increase obtained by GA_3 + Ca. On the other hand, fruit color was not significantly affected by any of the sprayed substances as compared with the control in both seasons. All treatments (except PUT + GA_3 and 2, 4-D + Ca) decreased rind aging in the first season, whereas, no significant effect was obtained in the second season as compared with the control. The percents of rind softening and creasing were decreased by all foliar sprays in comparison with the control. In general, spraying the substances in combinations resulted in the highest decrease in rind softening and creasing. In both seasons, the percent of late in the season fruit drop and fruit unfit for export was significantly decreased by all treatments when compared with the control.

The data of the present study showed that in general all sprayed growth regulators had positive influences in decreasing fruit external characteristics disorder and delaying fruit senescence at both harvesting dates. Senescence is the final stage of fruit growth and development. It is a process mainly characterized by disintegration of organelle structures, intensive loss of chlorophyll and proteins, membrane leakage and breakdown of cell wall components leading to loss of tissue structure (Paliyath and Droillard 1992, Buchanan-Wollaston 1997), which all contribute to the weakening of peel structure and leads to the senescence of the fruit. Delaying peel and rind senescence improves fruit quality and prolongs the life of citrus fruit and extends its value (Ismail, 1997). Numerous studies have suggested the promotive role of ethylene on the process of fruit ripening and senescence (Abeles *et al.*, 1992; Zarembinsiki and Theologis, 1994; Lelievre *et al.*, 1997; Dilley, 1977). In non-climacteric fruits, such as citrus, ethylene is not required for the coordination of ripening of the fruit (Giovannoni, 2001; Lelierve *et al.*, 1997; Yang and Hoffman,1984), however, it plays an important role in the senescence process. Considerable progress has been made during the past decade in understanding the possible relationship between ethylene and fruit ripening, and association between the sprayed substances specially the polyamines (for example putrescine in our study). There is evidence of an interrelationship between

Table 1. Effect of PUT, GA_3, 2, 4-D and Ca, foliar sprays on fruit physical characteristics at the first harvest date during 2007/2008 and 2008/ 2009

Treatment	Peel thickness (mm)	Color	Rind ageing (%)	Softening (%)	Creasing (%)	Fruit drop (%)	Fruit unfit for export (%)
			Season 2007/2008				
water	28.8c	4.86a	2.72a	0.98a	4.94a	8.47a	18.11a
PUT	30.7bc	4.34b	1.16c	0.32c	1.05d	4.98b	9.86bc
2,4-D	30.6bc	4.68a	2.13a	0.54b	2.86bc	3.34c	11.23bc
GA_3	34.6a	4.42b	1.20c	0.26cd	1.75cd	2.38c	7.79c
Ca	32.3ab	4.69a	2.05b	0.13d	2.06cd	3.50bc	8.97bc
PUT +2,4-D	30.4bc	4.36b	1.30c	0.24cd	2.03cd	3.26c	7.96c
PUT + GA_3	34.3a	4.43b	1.14c	0.15d	1.98cd	2.04c	6.76c
PUT + Ca	32.8ab	4.40b	1.18c	0.12d	2.08cd	2.13c	7.97c
2,4-D +GA_3	34.6a	4.87a	1.26c	0.21cd	2.46c	2.23c	8.43c
2,4-D + Ca	31.6b	4.67a	1.30c	0.23cd	3.05bc	3.56bc	9.76bc
GA_3 + Ca	34.4a	4.37b	1.31c	0.19cd	2.32c	2.89c	8.43c
			Season 2008/2009				
water	30.6b	5.00a	1.72a	2.78a	7.98a	10.65a	22.57a
PUT	32.6b	4.68ab	0.42c	0.19e	2.65bc	6.67bc	11.86bc
2,4-D	32.4b	4.89ab	1.13b	1.34b	2.64bc	2.98cd	9.36c
GA_3	34.4a	4.44b	0.50c	0.78c	1.86bc	5.40bc	10.05bc
Ca	33.8a	4.82a	0.98bc	0.53de	2.26bc	2.03d	6.87c
PUT +2,4-D	33.8a	4.58b	0.60bc	0.64c	3.09bc	3.98c	7.64c
PUT + GA_3	34.4a	4.46b	0.44c	0.85c	2.03bc	1.02d	6.87c
PUT + Ca	34.8a	4.45b	0.78bc	0.42de	2.43bc	2.94cd	7.98c
2,4-D +GA_3	33.9a	4.76ab	0.56c	0.51de	3.14bc	1.00d	6.87c
2,4-D + Ca	33.8a	4.96a	1.02b	0.53de	3.09bc	2.86cd	6.76c
GA_3 + Ca	34.6a	4.64b	0.71bc	0.49de	1.69c	1.46d	5.87c

Values within a column with same letter are not significantly different (P<0.05).

Table 2. Effect of PUT, GA_3, 2, 4-D and Ca foliar sprays on fruit physical characteristics at the second harvest date

Treatment	Peel thickness (mm)	Color	Rind ageing (%)	Softening (%)	Creasing (%)	Fruit drop (%)	Fruit unfit for export (%)
			Season 2007/2008				
water	30.4b	5.00	8.66a	31.54a	28.62a	18.05a	57.36a
PUT	32.7ab	4.94	5.26b	18.46b	14.65b	10.42b	30.46cd
2,4-D	31.8b	5.00	4.21c	15.86c	15.34b	5.37c	25.67de
GA_3	34.2a	4.86	5.26b	14.85c	15.76b	6.39c	23.54de
Ca	35.3a	5.00	3.45c	19.64b	17.76b	5.45c	21.68e
PUT +2,4-D	32.6ab	4.96	4.78bc	6.28e	9.76cd	7.68c	27.54d
PUT + GA_3	33.3ab	4.83	6.16ab	8.64de	10.78cd	10.60b	28.87d
PUT + Ca	34.8a	4.94	5.54b	7.87e	9.87cd	9.03bc	26.32de
2,4-D +GA_3	32.7ab	4.98	2.28c	7.75e	8.87d	4.57c	23.46de
2,4-D + Ca	35.4a	5.00	10.00a	6.87e	10.76cd	8.64c	36.87b
GA_3 + Ca	33.2ab	5.00	4.23c	6.07e	9.87cd	4.46c	24.67de
			Season 2008/2009				
water	31.8d	5.00	6.89	34.78a	37.64a	20.53a	54.35a
PUT	33.8cd	5.00	4.86	15.64c	20.32c	16.18b	34.28bc
2,4-D	33.6cd	5.00	6.57	17.08bc	21.14b	9.57b	28.26c
GA_3	34.6cd	4.87	4.60	12.87d	15.43d	11.00b	24.34ce
Ca	34.8c	4.98	4.26	17.64b	20.16c	10.63b	30.34c
PUT +2,4-D	35.4bc	4.98	3.98	11.98d	16.49d	6.63c	23.48e
PUT + GA_3	34.8c	4.86	3.74	10.86d	10.63f	8.85bc	20.34e
PUT + Ca	33.6cd	5.00	6.21	9.76d	9.08f	10.54b	32.66bc
2,4-D +GA_3	34.8c	5.00	4.68	11.87d	13.32e	6.74c	24.68ce
2,4-D + Ca	34.4cd	4.96	6.64	14.73c	13.74e	4.58c	28.48c
GA_3 + Ca	43.8a	5.00	7.42	10.04d	16.06d	7.67c	35.86b

Values within a column with same letter are not significantly different (P<0.05).

ethylene and polyamines during fruit ripening and senescence (Pandey *et al*., 2000). They play an inhibitory role on ethylene production through inhibition of ACC synthesase and ACC oxidase (Apelbaum *et al*., 1981; Lee *et al*., 1997), thus delaying ethylene emission. Polyamines have been reported to reduce softening, delay senescence and reduce decay in several fruits (Saftner and Baldi, 1990; Kramer *et al*., 1991). Other beneficial effects of polyamines application on fruit are: retarding color changes and increasing fruit firmness (Valero *et al*., 1999; Valero *et al*., 2002). Putrescine application leads to changes in cell wall stability (Messiaen *et al*., 1997) by inhibition of the action of polygalacturonase and pectin methyl esterase involved in softening, and also cross-link pectic substances in the cell wall, producing rigidification and increasing fruit firmness (Martinez-Romero *et al*., 2002; Perez-Vicente *et al*., 2002). This might explain the increase in firmness by putrescine application obtained for navel orange in our study. Similar results were reported for lemon by Valero *et al*. (1998b). Moreover, GA_3 sprays in the present study increased peel thickness and peel firmness and decreased fruit ageing, creasing, color change and the number of unfit export fruits. Similar results were obtained for Hamlin, Valencia, navels and blood oranges and mandarins (Coggins, 1973; Greenberg *et al*., 1992; Davies *et al*., 1997; Davies *et al*., 1999; Pozo *et al*., 2000). GA_3 is known to delay and retard chlorophyll degradation in citrus (El-Otmani and Coggins, 1991; Agusti *et al*., 1981). Moreover, its role is not limited only to the regulation of rind color, but also in delaying the more general process of peel ageing (Baez-Sanudo *et al*., 1992). This might be due to the association of GA_3 with the reduction of fruit peel growth as has been reported for mandarins (Pozo *et al*., 2000).

Also, we recorded improvement in fruit physical characters by calcium sprays specially an increase in peel firmness, peel thickness and decrease in rind ageing and creasing. Sayed *et al*. (2004) working on grapefruit and El-Hilali *et al*. (2004) working on mandarin obtained similar results. Storey *et al*. (2005) reported fruit rind disorders as a result of calcium deficiency. Calcium role in the physiological disorder related to ripening, fruit quality and shelf life is well established (Chaplin and Scott, 1980; Wimwright and Burbage, 1989). Calcium is involved in cell wall membrane metabolism and it contributes to the maintenance of configuration of specific enzymes (Jones and Lunt, 1967). Addition of calcium improves rigidity of cell walls and obstructs enzymes such as polygalacturonase from reaching their active sites (John, 1987), thereby retarding tissue softening and delaying ripening. Repeated sprays of Ca solutions increased the proportion of unaffected navel orange fruit with albedo breakdown (Treeby and Storey, 2002).

The positive influence on decreasing late in the season fruit drop by the sprayed substances in our study is obvious. It is well established that plant growth regulators are involved in controlling abscission. Ethylene accelerates mature citrus fruit abscission (Sexton and Roberts, 1982), and as previously mentioned that putrescine inhibits ethylene production, this might explain its effect on decreasing fruit drop. In addition, 2, 4-D is widely used in citrus in order to reduce the incidence of mature fruit drop and its primary action is to delay the development of the abscission layer (Coggins, 1973). GA_3 and Ca sprays influence might be due to the increase in the thickness of both juncture zone and the pedical as well as increasing the connections of vascular system and cell adhesion in union zone as reported for grapefruit by Sayed *et al*. (2004).

Fruit chemical characteristics: The data of the first and second harvesting dates are presented in Tables 3 and 4. Data of the first harvest date showed that all foliar sprays (except 2, 4-D alone and PUT + 2, 4-D in the first season) increased fruit TSS content during both seasons when compared with the unsprayed control trees. Fruit acidity was significantly decreased by all treatments (except for GA_3, Ca, PUT + GA_3 and GA_3 + Ca) in the first season. However, in the second season, acidity content was not significantly affected by the treatments (except for 2, 4-D alone which resulted in decreasing fruit acidity). TSS/Acidity ratio was significantly higher than the control by spraying PUT, 2,4-D, PUT + Ca, 2, 4-D + GA_3 and 2, 4-D + Ca in the first season. In the second season, foliar sprays of GA_3 alone, 2, 4-D + PUT and 2, 4-D + Ca significantly increased TSS/acidity ratio. Vitamin C content was significantly increased in both seasons by spraying GA_3 alone, Ca alone, PUT + GA_3 and GA_3 + Ca with no significant difference obtained among them. The electrolyte leakage (EL) was lower than the control by spraying PUT alone and GA_3 + Ca in the first season, whereas, in the second season all foliar sprays significantly decreased the EL as compared with the control. The highest decrease was obtained by PUT + GA_3 and PUT + Ca sprays.

Fruit reducing sugars content was increased by PUT + Ca, 2,4-D + GA_3 and 2,4-D + Ca, whereas, it decreased due to spray of 2,4-D alone, GA_3 alone, PUT + 2,4-D, PUT + GA_3 and GA_3 + Ca as compared with the control. Only foliar sprays of GA_3 alone and 2,4-D + Ca increased reducing sugars content in the second season, whereas, it was decreased by spraying 2,4-D + GA_3, PUT + GA_3 + PUT and GA_3 + Ca. In the first season, fruit non reducing sugars content increased by all sprays (except PUT + GA_3, PUT + Ca and GA_3 + Ca). In the second season, foliar sprays of 2,4-D alone, Ca alone, 2,4-D + GA_3 and 2,4-D + Ca gave higher non reducing sugars content than the control with no significant difference among them. In both seasons, total sugars content was significantly higher than the control by spraying GA_3 alone, Ca alone and 2,4-D + Ca. A significant decrease in fruit carotenoids content was observed as a result of spraying GA_3 + Ca (in both seasons), PUT + GA_3 (in the first season) and (PUT or GA_3 alone) in the second season.

Data on the effect of the sprayed substances at the second harvest (Table 4) indicated that all foliar sprays (except 2, 4-D alone) increased fruit TSS content in both seasons as compared with the control. In addition, spraying of GA_3 alone, PUT + GA_3, PUT + CA and 2,4-D + Ca gave higher TSS content than spraying PUT alone in the first season. A significant increase in fruit acidity was obtained by spraying GA_3 alone, Ca alone, PUT + GA_3 and GA_3 + Ca in the first season. Whereas, in the second season fruit acidity was not significantly affected by any of the treatments. The ratio of TSS to acidity increased significantly by spraying 2, 4-D alone, 2,4-D + Ca and PUT + Ca in the first season, whereas, in the second season all foliar sprays increased TSS/Acidity ratio when compared with the control. Vitamin C content increased in all treatments (except PUT + 2, 4-D in both seasons and 2,4-D + GA_3 in the first season) in both seasons. However, VC content was decreased by spraying 2, 4-D alone in the second season

Table 3. Effect of putrescine (PUT), GA_3, 2, 4-D and Ca foliar sprays on fruit chemical characteristics at the first harvest date

Treatment	TSS (%)	Acidity (%)	TSS/ Acidity	VC[1] (mg/100 mL)	EL[2] (ppm)	Sugars (%)			Carotenoids (mg/100g)
						Reducing	Non-reducing	Total	
2007/2008									
Water	10.44c	1.22a	8.56b	58c	294a	3.67cd	4.27b	7.94b	7.65a
PUT	11.04b	1.04c	10.62a	62bc	256b	3.61d	5.20a	8.81b	6.79ab
2,4-D	10.64c	0.97c	10.97a	57c	268ab	3.33g	5.43a	8.76b	8.03a
GA_3	11.08b	1.24a	8.94b	71a	280ab	3.45f	5.75a	9.20a	6.78ab
Ca	11.44a	1.16ab	9.86ab	67ab	276ab	3.64d	5.48a	9.12a	7.17ab
PUT +2,4-D	10.47c	1.12b	9.35b	58c	271ab	2.94h	5.63a	8.57b	7.02ab
PUT + GA_3	11.18ab	1.18a	9.47b	65b	261ab	3.21g	4.94b	8.15b	5.67b
PUT + Ca	11.43a	1.08b	10.58a	64b	269ab	3.84bc	4.46b	8.30b	6.23ab
2,4-D +GA_3	11.58a	1.00c	11.58a	60c	274ab	4.94a	5.24a	10.18a	7.09ab
2,4-D + Ca	11.0b6	1.01c	10.95a	57c	269ab	3.98b	5.46a	9.44a	6.98ab
GA_3 + Ca	11.42a	1.26a	9.06b	67ab	249b	3.46f	4.48b	9.94a	5.76b
2008/2009									
Water	10.28d	1.09a	9.43b	52c	312a	3.86bc	4.41c	8.27b	6.59ab
PUT	11.42c	1. 00a	11.42ab	59b	280c	3.78cd	4. 96bc	8.74a	4.43c
2,4-D	11.08c	0.96b	11.54ab	50c	294b	3.82cd	5.84a	9.66a	7.00a
GA_3	11.89b	1.02ab	11.66a	65a	278cd	4.32a	4.98bc	9.30a	4.98bc
Ca	11.84b	1.10a	10.76ab	63a	284bc	3.82cd	5.22ab	9.04ab	6.12ab
PUT +2,4-D	12.36a	1.02ab	12.12a	56bc	290bc	4.00b	4.68bc	8.68b	7.13a
PUT + GA_3	12.48a	1.14a	10.95ab	68a	263de	3.42de	4.86bc	8.28b	5.89b
PUT + Ca	11.64b	1.06ab	10.98ab	56bc	248e	3.77cd	4.67bc	8.44b	6.02b
2,4-D +GA_3	11.82b	1.03ab	11.48ab	59b	266d	3.46de	5.22ab	8.68b	7.08a
2,4-D + Ca	12.48a	0.98a	12.73a	52c	269cd	4.23a	5.47ab	9.70a	6.12ab
GA_3 + Ca	12.86a	1.14a	11.28ab	64a	268cd	3.36e	4.86b	8.22b	4.98bc

[1] Vitamin C, [2] Electrolyte leakage. Values within a column with same letter are not significantly different (P<0.05).

Table 4. Effect of putrescine (PUT), GA_3, 2, 4-D and Ca foliar sprays on fruit chemical characteristics at the second harvest date

Treatment	TSS (%)	Acidity (%)	TSS/ Acidity	VC[1] (mg/100 mL)	EL[2] (ppm)	Sugars (%)			Carotenoids (mg/100g)
						Reducing	Non-reducing	Total	
2007/2008									
Water	10.69c	0. 94b	11.37ab	56fg	304a	3.26d	4.39b	7.65d	8.34a
PUT	11.62cd	1.08ab	10.76bc	66c	262b	3.76bc	4.67ab	8.43c	6.54b
2,4-D	10.93f	0.86b	12.71a	59e	248b	3.60cd	4.80ab	8.40c	8.78a
GA_3	12.03b	1.18a	10.19c	68b	278a	3.74c	5.04ab	8.78bc	5.68c
Ca	11.82bc	1.16a	10.19c	65c	284a	3.78bc	5.14ab	8.92bc	6.12bc
PUT +2,4-D	11.45d	0.96b	12.31ab	54g	268b	3.68cd	5.26a	8.94bc	7.12b
PUT + GA_3	12.28a	1.12a	10.96bc	68b	253b	3.76bc	4.78ab	8.54c	5.65c
PUT + Ca	12.18a	0.96b	12.69a	73a	277ab	3.94bc	4.86ab	8.80bc	6.12bc
2,4-D +GA_3	11.24e	0.92b	12.21ab	58ef	287a	4.66a	4.96ab	9.62a	6.56b
2,4-D + Ca	12.06ab	0.96b	12.56a	59ef	289a	4.68a	5.25a	9.93a	7.48ab
GA_3 + Ca	12.28a	1.14a	10.77bc	64d	289a	4.46a	4.98ab	9.44a	4.89c
2008/2009									
Water	11.14c	1.02	10.92d	52f	282a	3.67c	4.21c	7.88f	8.97a
PUT	12.08ab	0.93	12. 99bc	59c	260c	3.88c	4. 68b	8.56e	7.65b
2,4-D	12.66a	0.98	12.92bc	49a	294a	3.89c	4.86ab	8.75de	8.65a
GA_3	11.86b	0.89	13.33b	67a	257c	4.02b	4.78ab	8.80d	6.13bc
Ca	12.28a	0.84	13.64	64b	273b	4.45b	4.88ab	9.33bc	6.43bc
PUT +2,4-D	12.14ab	0.90	13.49b	50fg	248c	4.47b	5.00ab	9.47b	6.64bc
PUT + GA_3	11.96b	0.94	12.72bc	60c	260c	4.36b	4.94ab	9.30bc	6.76bc
PUT + Ca	12.42ab	0.86	14.44a	64b	265b	4.28b	4.87ab	9.15c	6.87bc
2,4-D +GA_3	11.82b	0.80	14.77a	55e	270b	4.66a	5.08ab	9.74a	7.87ab
2,4-D + Ca	12.16ab	1.00	12.16c	56d	261c	4.75a	5.17a	9.92a	8.01ab
GA_3 + Ca	12.20ab	0.89	13.71ab	65a	274b	4.66a	5.06ab	9.72a	5.98c

[1] Vitamin C, [2] Electrolyte leakage. Values within a column with same letter are not significantly different (P<0.05).

only. Electrolyte leakage was significantly decreased in the first season by spraying PUT alone, 2, 4-D alone, PUT + 2, 4-D and PUT + GA_3. Whereas, in the second season it decreased in all treatments (except 2,4-D alone) as compared with the control. In both seasons, fruit reducing sugars content was significantly increased by all sprays (except 2, 4-D alone in both seasons, PUT + 2,4-D in the first season and PUT alone in the second season). In addition only foliar sprays of PUT + 2,4-D and 2,4-D + Ca resulted in higher non reducing sugars content than the control in the first season, whereas, all foliar sprays significantly increased non reducing sugars content in the second season. Total sugars content increased significantly by all treatments in both seasons. Moreover, spraying 2, 4-D + GA_3, 2,4-D + Ca and GA_3 + Ca resulted the highest total sugars content when compared with all other treatments in both seasons. The data of both seasons indicated that all foliar sprays (except 2,4-D alone and 2,4-D + Ca in both season and 2,4-D + GA_3 in the second season) decreased fruit carotenoids content as compared with the control.

The improved fruit outer characteristics obtained for navel orange fruits in our study by the sprayed substances reflected better fruit internal characters at both harvest dates. Juice TSS and sugars contents were increased. It is reported that at the late stages of citrus fruit development, soluble solids accumulate in the juice sacs (Coggins, 1981). Similar increases in TSS, VC and sugar contents were reported in Clementine mandarin (El-Otmani *et al*., 2004); Fortune mandarin (EL-Hilali *et al*., 2004) and Blood Red orange (Saleem *et al*., 2008). Ethylene induced fruit coloration and increased carotenoids content in Navel oranges (Rodrigo and Zacarias, 2007), so it might be concluded that putrescine and GA_3 sprays inhibited ethylene production and then decreased carotenoids content and lowered fruit coloration.

In conclusion, the improvement, we recorded in the external and internal fruit characteristics even when harvest date was delayed, indicates that GA_3, PUT, Ca and 2, 4-D sprays might enable on-tree storage and late harvest by the benefit of their combined effect on delaying development of the abscission layer, keeping the phloem and xylem connections in better condition, delaying fruit coloration, thus delaying fruit senescence. This might allow navel orange growers to have longer harvest season with only modest losses from fruit drop and without risks of fruit quality loss and thus extend export season in Egypt.

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