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EFFECT OF CALCIUM AND GIBBERELLIC ACID SPRAYS ON YIELD, QUALITY AND ABSCISSION OF GRAPEFRUIT

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ABSTRACT

This study was carried out to evaluate the effect of Calcium nitrate ($Ca(NO_3)_2$) at 0.1% and Gibberellic acid (GA_3) at 25 ppm on grapefruit trees during the petal fall (PF) through the two successive seasons 1999/2000 and 2000/2001. It could be concluded that, ($Ca(NO_3)_2$) and GA_3 increased the fruit yield per tree. In addition to,the application of ($Ca(NO_3)_2$) or GA_3. separately or combined together significantly improved the physical and chemical fruit properties; e.g. fruit weight (g),fruit size (cm^3), peel thickness (cm), juice %, TSS %, acidity %, TSS/acidity ratio, total sugar (mg), vitamin C (mg). In addition to, Chlorophyll (A and B) content in leaf tissues. All treatments also significantly increased leaf mineral content. The anatomical results indicated that, treatments fundamentally affected the internal features of both the juncture of pedicel and fruitlet as well as the leaf structure. Combined treatments gave the best results in reducing abscission as the thickness of both the juncture zone and the pedicel as well as degree of both connections of vascular system and cell adhesion in union zone were the highest. Moreover, the anatomical leaf features were the best as the combined treatments were applied.

INTRODUCTION

Grapefruit (*Citrus paradisi* Macf.) belongs to the family Rutaceae (order: Sapindales) which consists of 150 genera and 900 species (Cronquist, 1981). Grapefruit is cultivated in Egypt on a commercial scale for exportation and as a source for hard currency. Grapefruit is not a popular fruit for Egyptians, although some people use the fresh fruits for medical purposes as the fruits contain vital constituents such as flavanones (naringin, narirutionosides (NR) limonin 17-B-D glucopyranoside (LG) and Vitamin C. These compounds were found to have potential human health benefits, including cancer cardiovas disease prevention. (Patil *et. al.*, 2000). For these reasons, this study was carried out for increasing yield and improving fruit quality (physical and chemical fruits properties) by using ($Ca(NO_3)_2$) or GA_3 separately or their combination.

Concerning the effect of Calcium, many investigators reported that it has a critical role in cell division, cell development, carbohydrate movement, neutralization of cell acids, cell wall deposition and the formation of pectate salts in middle lamella (Huber,1980). Moreover, Zhou *et. al.*(1998) found that chlorophyll reached its maximum concentration with Ca application and John and Bower (2000) found that Ca^{+2} application appears to decrease the effects of water stress.

Additionally, Smith (1964) found that vigorous trees occurred when leaf Ca was 2.5 percent or above, while values over 4 percent were associated with a sparse fruiting habit. Xie *et. al.*, (1992) reported that the Ca status of the fruit may be responsible for basipetal transport of IAA and may regulate the movement of IAA. Regarding the effect of GA_3, Farmahan (1999) reported that spraying with GA_3 during the on year at 30 and 23 days before anthesis reduced the production of fruits compared to the control treatment, which resulted in significantly higher average fruit weight and higher fruit yield per tree. GA_3 also increased fruit number and average fruit weight in both off years compared with controls in Kinnow mandarin trees .In addition, Davies *et. al.* (1999) found that sprayed fruits of orange trees with GA_3 at color break stage had statistically greater juice yield than those of non sprayed ones. Moreover, spraying with 10 ppm. GA_3 at the beginning of vegetative growth increased the number of fruits and fruit yield of naval orange (Koller *et. al.*, 2000).

Fruitlets abscission phenomenon is one of the most critical problems facing the growers of fruit trees , which cause a great loss of yield and in turn in mass of income (Hollman,1995). This phenomenon has been demonstrated as a complicated correlative process , which involves phytohormones and nutrition status, as well as environmental conditions (Guinn,1984).

Fruits abscise in different stages of development. The location of the abscission zone varies in different fruits. In citrus the separation layer is formed beneath the ovary where the vascular diverge from the receptacle into the carple.The degree of differentiation of the abscission zone varies in different fruits. In citrus, large quantities of starch accumulate in the abscission region and pectin disappears, with the consequent weakening of cell walls. The abscission zone in most fruits is often structurally weak in having reduced amounts of sclerified tissue and a vascular system that is condensed in the center instead of distributed near the periphery (Esau,1976).

This investigation was carried out on grapefruit trees to overcome the fruitlets drop phenomenon by using GA_3 and / or calcium treatments as spraying solutions , which could delay dropping of the fruitlets till reaching the maturity or reducing the percentage of fruit set abscission .

To give more details about the reasons of fruitlets abscission, anatomical study was carried out on abscission zone using different treatments to find out whether these treatments would stimulate or inhibit this phenomenon and to study the anatomical features of the abscission zone of both cases.

MATERIALS AND METHODS

This study was carried out during two successive seasons (1999-2000 & 2000-2001) on 28 years old grapefruit trees (*Citrus paradisi* Macf. cv. Ruby red), nearly similar in vigour, budded on sour orange rootstock (*Citrus aurantium* L.) grown in Hort. Res. Inst., Agri. Res. Center,Giza. The trees were grown in a clay loam soil under flooding irrigation system and spaced at 5 X 5 meters. Twenty four trees particularly uniform in vigour and productivity were chosen for this study and treated as a complete randomized block experimental design (CRBD) with three replicates, each replicate consisted of one tree.

Treatments were as follows:

1- Control trees = left without foliar spray
2- $Ca(NO_3)_2$ sprayed only at 30% PF = a single foliar spray with calcium nitrate only at 30% of petal fall.
3- $Ca(NO_3)_2$ sprayed only at 75% PF = a single foliar spray with calcium nitrate only at75% of petal fall.
4- $Ca(NO_3)_2$ sprayed 6 times (every 30 days) = six times frequent foliar spray with calcium nitrate, starting from end of February to end of July, i.e. one month between each spray.
5- GA_3 sprayed only at 30% PF = a single foliar spray with gibberellic acid only at 30% of petal fall.
6- GA_3 sprayed only at75% PF = a single foliar spray with gibberellic acid only at 75% of petal fall.
7- GA_3 + $Ca(NO_3)_2$ (only at 30% PF) = a single foliar spray with mixture of calcium nitrate + gibberellic acid only at 30% of petal fall.
8- GA_3+ $Ca(NO_3)_2$ (only at 75% PF) = a single foliar spray with mixture of calcium nitrate + gibberellic acid only at 75% of petal fall.

In this study calcium nitrate ($Ca(NO_3)_2$); (15.5% N, 18.8% Ca) and gibberellic acid (GA_3) were used at 0.1% and 25ppm, respectively. Each tree was individually sprayed with 10 litres of solution which was sufficient for a thorough coverage of the canopy salient-film as a wetting agent at 0.3% was used as a wetting agent.

A sample of ten fruits was taken from each replicate to be tested for fruit weight (g), fruit size (cm^3), peel thickness (cm), juice %, total soluble solids (TSS) %, acidity %, total sugar (mg /100 cm^3 juice) and ascorbic acid (VC) as mg /100 cm^3 juice .All these analyses were evaluated according to A.O.A.C. methods (1977). Leaf chlorophyll (A & B) contents as mg /g of mature fresh leaf tissue were estimated according to Arnon (1945).

Yield as weight of fruits (kg) per tree was recorded at harvest. Leaf samples for both chlorophyll and leaf mineral contents were taken from mature leaves of each treatment and washed several times with tap water then with distilled water, oven dried at 60-70^0 C. The dried ground samples were digested with sulphuric acid and hydrogen peroxide according to Evnhuis and waard (1980) to determine N, P, K, Ca, Mg, Fe, Zn, and Mn, contents. N was determined by micro –Kjeldahl method as outlined by Pregl (1945), Phosphorus was colorimetrically determined according to Murphy and Riley (1962). K, Mg, and Ca were determined by using Flame Photometer as mentioned by Brown and Lilleland (1946). Fe, Zn, and Mn were determined by using Perkin Elemer Atomic Absorption Spectrophotometer. Data were statistically analysed according to Snedecor and Cochran (1972).

Anatomical studies:

For anatomical studies of the juncture of the fruitlet and the pedicel, and its relationship with the leaf anatomical features, 3 samples of fruitlets (including the pedicel), and leaves of each treatment were taken 30 days after fruit set in the second season (2000/2001).

Specimens were killed and fixed for at least 48 hours in F.A.A. (10 ml formalin, 5 ml glacial acetic acid, 50 ml ethanol 95% and 35 ml distilled water). Thereafter, samples were washed in 50% ethanol and dehydrated in a normal butanol series before being embedded in paraffin wax (m.p. 56-58°C). Longitudinal sections 20 µ thick were cut using rotary microtome, stained with crystal violet/erythrosin in leaves, or safranin/light green in fruitlets and mounted in Canada balsam (Sass,1967). Slides were microscopically examined and photomicrographed.

Microscopical counts and measurements (µ) were taken through specimens of the median leaf of the corresponding twings and conjunction zone between the fruitlet and the pedicel. Ten readings from 5 slides were taken for each treatment.

RESULTS AND DISCUSSION

I-Yield:

Results in Tables (1 and 2) indicate that spraying grapefruit trees with calcium nitrate ($Ca(NO_3)_2$) at 30% and 75% of petal fall (PF) or each 30 days significantly increased the yield than the control trees. Amongst the three $Ca(NO_3)_2$ treatments, the highest yield was obtained by spraying $Ca(NO_3)_2$ each 30 days; 175.5 and 243.5 Kg/tree for the two successive seasons, respectively.

Concerning GA_3 treatments, it could be noticed that the yield was significantly increased by spraying GA_3 at both 30 and 75 % PF .The highest values were obtained by foliar application of GA_3 at 75% PF compared with the other treatment being 199.3 and 260.3 kg/tree for both seasons, respectively.

Maximum yield occurred when trees sprayed with GA_3+$Ca(NO_3)_2$ at 30 % PF being 232.2 and 283.9 kg/tree in both seasons, respectively, as compared with the other treatments. These results are in line with Fidalski *et. al.* (2000) on Valencia orange; Ouyang Guochun (1998) on *Satsuma mandarin* who found that good yields were obtained by Ca application. In the same respect. Dai (1995) found that spraying calcium had positive effects on flower bud formation and increment in yield due to increment in fruit set. Moreover several workers (Ibrahim *et. al.*, 1994; El-Wazzan *et. al.*, 1993 and Tafazoli *et. al.*,1989) reported that spraying GA_3 at different concentrations during flowering increased the yield of navel orange and Valencia orange. These increments also could be attributed to the stimulated of GA_3 sprays on cell elongation of fruit tissue (Brion and Hemming, 1958). In the same respect, Wang and Yu (2000) found that the fruit set increased by spraying GA_3 after flowering on *C. medica*.

Table(1): Effect of Calcium nitrate ($Ca(NO_3)_2$), gibberellic acid (GA_3) and their mixture sprays on yield and fruit quality of grapefruit cultivar Ruby red during the first season (1999/2000).

Treatments	Yield (kg/tree)	Fruit weight (gm)	Fruit size (cm3)	Peel thickness (cm)	Juice (%)	T.S.S. (%)	Acidity (%)	T.S.S/ acidity ratio	Total sugar (mg/100 cm3 juice)	V.C. (mg/100 cm3 juice)	Chl A (mg/g F.W.)	Chl B (mg/g F.W.)
1- Control	131.2	293.5	303.2	0.43	43.83	11.20	1.96	5.71	41.89	16.67	1.407	0.339
2- Calcium nitrate at 30% PF	165.7	308.3	317.7	0.47	44.11	10.10	2.01	5.04	54.18	20.40	1.804	0.570
3- Calcium nitrate at 75%PF	163.0	309.0	320.1	0.48	43.83	11.10	1.90	5.86	46.06	22.47	2.060	0.772
4- Calcium nitrate each 30 days	175.5	313.3	326.1	0.56	46.68	11.13	1.75	5.78	47.08	23.37	2.240	0.814
5- GA_3 at 30%PF	185.8	308.7	320.8	0.46	46.10	10.13	2.03	4.98	58.54	24.77	1.662	0.510
6- GA_3 at 75%PF	199.3	318.2	332.7	0.48	46.37	10.33	2.15	4.82	48.64	24.00	1.857	0.726
7- GA_3 +Calcium nitrate at 30%PF	232.2	334.7	360.7	0.46	46.68	10.07	1.82	5.52	50.73	23.40	2.158	0.771
8- GA_3 +Calcium nitrate at 75%PF	210.4	347.2	380.9	0.55	48.75	11.83	2.13	5.56	62.40	25.40	2.589	0.902
L.S.D. at 5%	9.9	NS	5.9	0.04	0.31	0.36	0.06	0.24	3.34	0.26	0.130	0.077

PF=Petal fall T.S.S.= Total soluble solids V.C.=Ascorbic acid Chl A =Chlorophyll A Chl B=Chlorophyll B

Table(2):Effect of Calcium nitrate (Ca(NO3)2), gibberellic acid (GA3) and their mixture sprays on yield and fruit quality of grapefruit cultivar Ruby red during the second season (2000/2001).

Treatments	Yield (kg/tree)	Fruit weight (gm)	Fruit size (cm3)	Peel thickness (cm)	Juice (%)	T.S.S. (%)	Acidity (%)	T.S.S/ acidity ratio	Total sugar (mg/100 cm3 juice)	V.C. (mg/100 cm3 juice)	Chl A (mg/g F.W.)	Chl B (mg/g F.W.)
1- Control	180.7	298.7	303.0	0.51	39.32	12.17	1.62	7.49	45.41	18.32	1.055	0.510
2- Calcium nitrate at 30% PF	231.5	310.3	322.2	0.52	40.30	11.00	1.78	6.18	43.36	22.55	1.308	0.572
3- Calcium nitrate at 75%PF	224.4	312.5	325.4	0.56	48.50	11.33	1.61	7.05	40.77	23.42	1.522	0.424
4- Calcium nitrate each 30 days	243.5	320.3	332.1	0.65	45.41	11.83	1.57	7.52	54.02	24.43	1.637	0.535
5- GA3 at 30%PF	255.2	321.5	332.6	0.46	43.72	10.17	1.77	5.75	49.93	24.31	1.449	0.638
6- GA3 at 75%PF	260.3	329.7	335.1	0.56	42.34	11.83	1.86	6.36	47.71	24.15	1.526	0.690
7- GA3 +Calcium nitrate at 30%PF	283.9	349.2	366.5	0.59	49.37	11.17	1.61	6.93	50.39	24.25	1.654	0.184
8- GA3 +Calcium nitrate at 75%PF	272.2	370.2	385.1	0.61	49.41	12.33	1.88	6.55	54.53	25.35	1.869	0.623
L.S.D. at 5%	6.0	7.2	2.6	0.03	0.55	0.47	0.09	0.38	0.93	0.31	0.060	0.037

PF=Petal fall T.S.S.= Total soluble solids V.C.=Ascorbic acid Chl A =Chlorophyll A Chl B=Chlorophyll B

II-Fruit quality:

The effect of $Ca(NO_3)_2$ singly on fruit quality:

1- Physical properties:

Generally, $Ca(NO_3)_2$ treatments when sprayed on grapefruit trees improved fruit quality (physical properties) as compared with control in both seasons. $Ca(NO_3)_2$ each 30 days gave the highest values in both seasons compared with the other $Ca(NO_3)_2$ treatments, it gave (313.3, 326.1, 0.56 and 46.68) in the first season and (320.3, 332.1, 0.65 and 45.41) in the second season for fruit weight, fruit size, peel thickness and juice percentage, respectively. These results are logic since Ca++ plays an important role in the auxin induction inside plant cells. Also auxin caused cell elongation seems to require Ca^{++} (Jones, 1990). In addition, Shishova and Lindberg (1999) who found that Calcium provides cell wall rigidity by cross – linking the pectic chains of the middle lamella, and Treeby *et. al.* (2000) who cleared that, Ca sprays reduce creasing approximately 30%, attributed to the increase of thickness, and also Fidalsk *et. al.* (2000) who reported that, the fruit weight was related with both Ca^{++} content of leaves and soil solution which gave the highest orchards productivity.

2- Chemical properties:

Chemical properties as affected with $Ca(NO_3)_2$ each 30 days gave the highest values in both seasons compared with the other $Ca(NO_3)_2$ treatments, it gave 11.13 %, 1.75%, 5.78 %, 47.08, 23.37 and (2.240 & 0.814) in the first season and (11.83%, 1.57 %, 7.52 %, 54.02, 24.43 and (1.637 & 0.535) in the second season for TSS, Acidity, TSS/acidity, total sugar, V.C and chlorophyll (A & B), respectively. These results are in accordance with those obtained by Kim *et. al.* (2000) who found that, spraying Ca^{++} on Satsuma *mandarin* in increased fruit quality such as total sugar and TSS. Moreover, Zhou *et. al.* (1998) and Thukral *et. al.* (1994) on *Citrus limonum* and Lavon *et. al.* (1995) on rough lemon reported that, leaf chlorophyll content increased with Ca spraying.

The effect of GA_3 singly or plus $Ca(NO_3)_2$on fruit quality:

1- Physical properties:

Data in Tables (1&2) indicate that spraying GA_3 singly or in combination with $Ca(NO_3)_2$ improved fruit quality (physical properties) compared with control in both seasons. GA_3+$Ca(NO_3)_2$ at 75% PF gave the highest values in both seasons (347.2, 380.9, 0.55 and 48.75) in the first season and (370.2, 385.1, 0.61 and 49.41) in the second season for fruit weight, fruit size, peel thickness and juice percentage, respectively.These findings are in harmony with those obtained by Mazumdar and Bhatt (1977) who reported that, spraying GA_3 increased fruit size on sweet orange and lima, also the similar results was reported by Tafazoli *et. al.* (1989) on Valencia orange trees.

2- Chemical properties:

Data presented in Table (1&2) show that, GA_3 singly or plus $Ca(NO_3)_2$ improved chemical properties, as GA_3 + $Ca(NO_3)_2$ at 75% PF gave the highest values with TSS, acidity, TSS/acidity, total sugar, V.C and chlorophyll (A&B) content (11.83, 2.13, 5.56, 62.40, 25.40 and (2.589 & 0.902) in the first season, respectively, while in the second season were 12.33, 1.88, 6.55, 54.53, 25.35 and (1.869 & 0.623), respectively. These results are in line with the findings of El-Wazzan, *et. al.* (1993) on Navel orange that the ascorbic acid content increased slightly with spraying GA_3. Moreover, Casagrande *et. al.* (1999) showed that, citric acid percentage (acidity%) increased with increasing GA_3 concentration on tangerine. While Davies, *et. al.* (1999) reported that, GA_3 sprays on Hammlin orange gave peels with higher (peel puncture), peel colour, and Juice yield. On the other hand, Matthew and Frederick (2000) reported that, citric acid was significantly reduced (about 0.5% - 0.8 % compared with control) when Hamlins and Valencia orange trees were sprayed with GA_3.

III-The leaf mineral content:

1-The effect of $Ca(NO_3)_2$ singly:

Data presented in Tables (3&4) indicated that the different experimented treatments have increased the values of leaf mineral content as compared with the untreated trees in both seasons. Also when the trees sprayed with solution of $Ca(NO_3)_2$ each 30 days, the leaf calcium content increased than the control in both seasons. Moreover, this treatment recorded the highest values (5.45% and 3.10 %) in the first season and (5.28 % and 3.21 %) in the second season for both treatments ($Ca(NO_3)_2$ each 30 days and control), respectively. These results are in accordance with those reported by Li *et. al.* (1999) on navel orange that as the concentration of nutrient in the solution rises, the contents of the nutrient in the plant increase accordingly.

2-The effect of GA_3 singly or plus $Ca(NO_3)_2$:

The effect of GA_3 plus $Ca(NO_3)_2$ treatments gave values higher than control for all elements except nitrogen had no significant in the first season while it was significant in the second season, but GA_3 plus $Ca(NO_3)_2$ at 75% PF gave the higher values than GA_3 plus $Ca(NO_3)_2$ at 30% PF, being (0.159, 0.83, 5.32, 0.34, 69.34, 36.00 and 32.67) in the first season and (0.145, 0.75, 4.79, 0.42, 94.14, 62.66 and 43.37) in the second season for P, K, Ca, Mg, Fe, Zn and Mn, respectively. Generally, these results are in harmony with the findings of Ibrahim *et. al.* (1994) and Shawki *et. al.* (1978) on navel orange.

In conclusion, it is evident from these data that spraying grapefruit cv. Ruby red trees with calcium nitrate each 30 days or GA_3 plus $Ca(NO_3)_2$ at 75% PF followed by GA_3 plus $Ca(NO_3)_2$ at 30% PF gave the highest values for the yield, fruit quality, leaf mineral content and Chlorophyll (A&B) content compared with the control.

IV- Anatomical studies:

The anatomical study was designed to find out the effect of spraying different doses of GA_3 and calcium nitrate, separately or in combination, on both quantity and quality of fruit yield. Thus, the anatomical features of the juncture of the fruitlet and the pedicel as well as the fruitlet pedicel were used as criteria, measuring the changes which may have occurred in the abscission zone as a result of treatments.

Table(3): Effect of foliar application of calcium nitrate ($Ca(NO_3)_2$), gibberellic acid (GA_3) and their combination sprays on Leaf mineral content of grapefruit cultivar Ruby red during the first season (1999/2000).

Treatments	N%	P%	K%	Ca%	Mg%	Fe (ppm)	Zn (ppm)	Mn (ppm)
1- Control	2.30	0.122	0.45	3.10	0.26	61.34	26.33	26.67
2- Calcium nitrate at 30% PF	2.37	0.135	0.62	3.40	0.26	75.34	28.33	28.67
3- Calcium nitrate at 75%PF	2.56	0.136	0.66	3.47	0.33	83.34	29.33	26.67
4- Calcium nitrate each 30 days	2.67	0.147	0.87	5.45	0.38	145.34	50.00	31.67
5- GA_3 at 30%PF	2.46	0.132	0.74	5.10	0.27	66.66	29.67	26.67
6- GA_3 at 75%PF	2.41	0.145	0.66	4.35	0.29	64.66	28.34	28.00
7- GA_3 +Calcium nitrate at 30%PF	2.51	0.142	0.67	5.26	0.32	54.66	30.00	31.33
8- GA_3 +Calcium nitrate at 75%PF	2.60	0.159	0.83	5.32	0.34	69.34	36.00	32.67
L.S.D. at 5%	N.S	0.017	0.06	0.19	0.02	2.85	4.40	2.21

PF= Petal fall

Table(4): Effect of foliar application of calcium nitrate ($Ca(NO_3)_2$), gibberellic acid (GA_3) and their combination sprays on Leaf mineral content of grapefruit cultivar Ruby red during the second season (2000/2001).

Treatments	N%	P%	K%	Ca%	Mg%	Fe (ppm)	Zn (ppm)	Mn (ppm)
1- Control	2.30	0.124	0.67	3.21	0.28	55.40	26.51	26.01
2- Calcium nitrate at 30% PF	2.60	0.144	0.64	4.34	0.32	81.40	28.80	26.17
3- Calcium nitrate at 75%PF	2.65	0.135	0.69	4.54	0.40	90.86	29.58	31.75
4- Calcium nitrate each 30 days	2.72	0.147	0.86	5.28	0.56	106.54	72.83	45.78
5- GA_3 at 30%PF	2.26	0.144	0.73	3.74	0.28	87.86	40.67	31.50
6- GA_3 at 75%PF	2.35	0.134	0.76	4.63	0.31	81.22	44.78	31.43
7- GA_3 +Calcium nitrate at 30%PF	2.50	0.136	0.73	4.30	0.32	75.00	45.33	35.29
8- GA_3 +Calcium nitrate at 75%PF	2.60	0.145	0.75	4.79	0.42	94.14	62.66	43.37
L.S.D. at 5 %	0.09	0.004	0.06	0.29	0.04	2.85	2.90	2.01

PF= Petal fall

The fruit quality found in the previous results could be related to the changes which may have occurred in leaf structure due to the treatments.

A- Anatomical features of the juncture of the fruitlet and the pedicel:

Anatomical counts and measurements at the junction of fruitlet and pedicel for treated grapefruit trees are presented in Table (5). It is obvious that, trees regularly sprayed at 30 days intervals by 0.1% calcium nitrate showed a remarkable increase in thickness of both union zone and pedicel by 154.6 and 10.6%, respectively, as compared with their respective control. However, these plants showed well brought-up degree of vascular connection and enough amounts of cells adhesion in the union zone comparing with the untreated plants (Figure , 1 A and B) . The same trend was observed by GA_3 treatments , since 25 ppm GA_3 sprayed at 75% PF increased the thickness of both union zone and pedicel by 159.1 and 23.7%, respectively, as compared with their respective control. This increase in thickness of both pedicel and union zone is relatively equivalent to that occurred as a result of calcium nitrate treatments. More consistency was observed in union zone either for vascular connection or amount of cells adhesion due to GA_3 treatments, comparing with that of control plants (Figure,1 A and C).

Regarding the effect of the combined treatment of GA_3 and calcium nitrate at 75% PF, data presented in Table (5) and Figure, 1(A and D) proved highly positive synergistic effect on the anatomical features of abscission zone at the junction of fruitlet and pedicel when compared with the untreated plants, since the increase in thickening measurements of the juncture of the fruitlet and the pedicel reached 263.6%. However, this increase was accompanied with an increase of 39.5% in pedicel thickness. Moreover, relative to the control, these treatments resulted in increasing the fruitlet pedicel junction (i.e; degree of connection in the vascular system especially xylem as well as degree of cells adhesion in attachment zone),consequently prevented fruitlet abscission.

Anatomical observations for the treatments under study revealed that abscission zone at the junction of fruitlet and pedicel occurred with intense vascular system development as a result to spraying grapefruit trees with GA_3 and / or Ca solutions relative to the control.

Table (5): Measurements (μ) of the abscission zone at the juncture of the fruitlet and the pedicel of grapefruit trees untreated and treated with calcium nitrate and/or GA_3 .

Measurements (μ) / Treatments	Thick. of union zone	Thick. of pedicel	Degree of connection of vascular system	Degree of cell adhesion in union zone
Control	770	1380	+	+
$Ca(NO_3)_2$ each 30 days	1960	1526	++	++
GA_3 at 75% PF	1995	1707	+++	+++
$Ca(NO_3)_2+GA_3$ at 75% PF	2800	1925	++++	++++

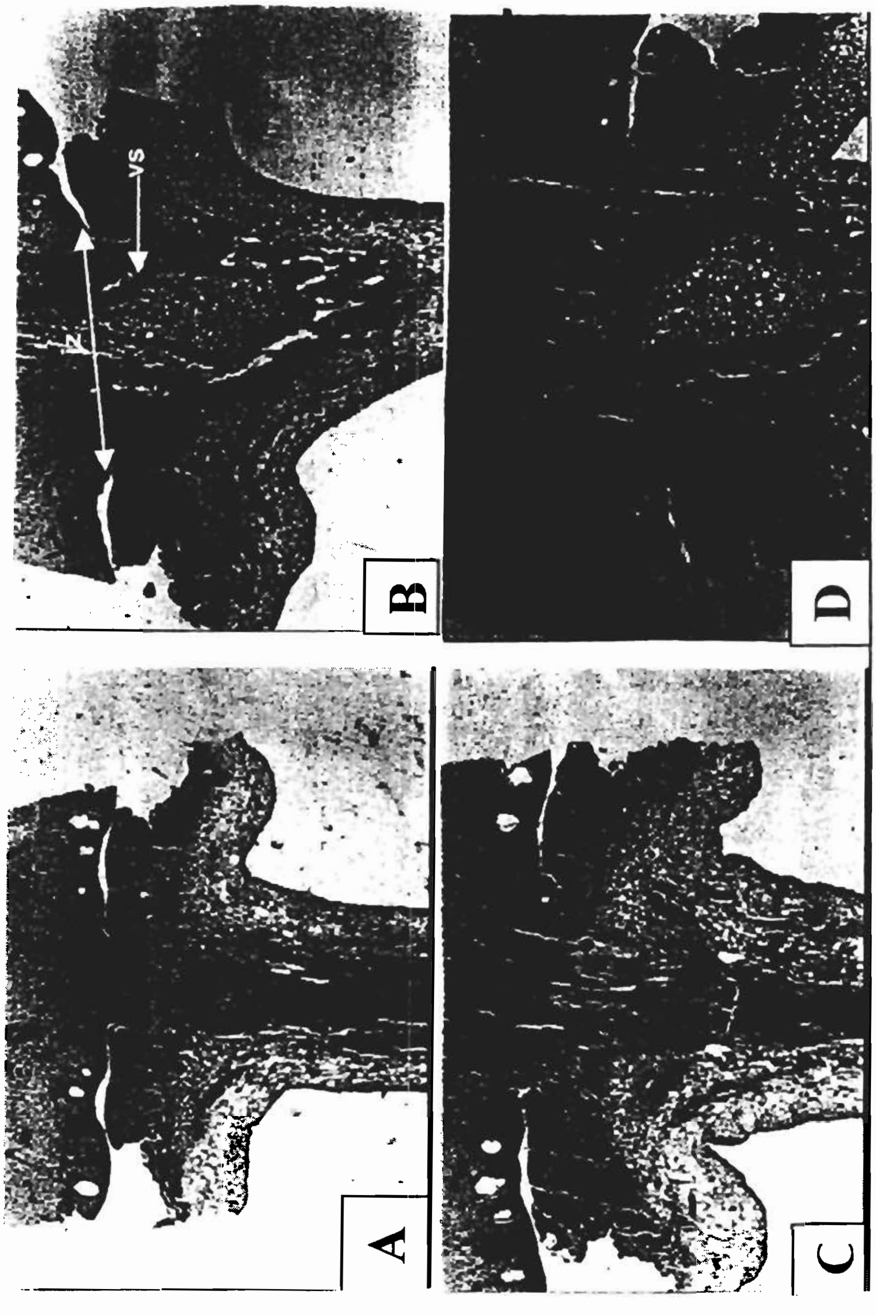

Fig.1(A-D): Longitudinal sections through the fruitlet-pedicel juncture 30 days after fruit set of grapefruit as affected by spraying with calcium nitrate and/or GA_3.
A-Untreated plant
B-Plant treated with calcium nitrate each 30 days
C-Plant treated with GA_3 at 75% PF
D-Plant treated with calcium nitrate +GA_3 at 75% PF
Details: jz, junction zone; vs, vascular system. (X 60)

The rapid development of the vascular system within a relatively short period could be attributed to that GA_3 moves in all plant tissues (including xylem and phloem) and promotes cambial activity, producing thick xylem and phloem layers. Addition of GA_3 in the basal medium induced cell divisions as well as promoting xylogenesis in *Citrus limonum* Risso tissue cultures (Aysha Khan,1996). Moreover, increase in GA_3 level is associated with the decline of the endogenous inhibitors (such as abscisic acid) . GA_3 also promotes auxin production,hence producing considerable amounts of auxins by citrus fruitlets at early stages of development will induce cambial activity and vascular proliferation (Goldschmidt, 1976).Thus,the cambial contacts between the fruitlet and pedicel as well as the vascular connection will grow most rapidly and export more assimilates and water into the fruits through the pedicel (Sachs,1991). Auxin also increases the secondary thickening of the pedicel which might be the reason for the bigger size of fruits. In addition to the direct effect of auxin on fruit growth (Bustan *et.al.*,1995).

An unusual upsurge in the flow rate of assimilates and water in the vascular system may act as a signal for the expansion of the vascular tissues. On the other hand, it is possible that the increase in fruit growth rate enhances the production and basipetal export of more growth substances by the fruit. This in turn, may enhance cambial activity and differentiation of additional phloem and xylem (Aloni,1987).As soon as the new vascular elements become active, the capacity of the system increases and more substrate is available to the fruit, hence increasing fruit growth rate.

Furthermore, calcium is considered one of the main constituents of cell wall-middlelamella. Thus, spraying trees with GA_3 and / or Ca caused higher transport capacity which might be responsible for retarding the fruitlet abscission processes , and consequently improving fruit set.

B- Anatomical features of the leaf:

Data in Table (6) represent certain microscopical measurements of grape fruit leaf blade as treated by different doses of gibberellic acid or calcium nitrate and their combined treatment. Photomicrographs that illustrate the effects of such treatments are given in Figure , 2 (A,B,C and D).

It is confirmed that calcium nitrate treatments of 0.1%, sprayed regularly each 30 days increased thickness of both leaf midvein and lamina of grapefruit leaves by 33.3 and 6.7% over the thickness of the untreated control for both traits, respectively. However, comparing with the control, the increase in lamina thickness was accompanied by an appreciable increment in thickness of both leaf palisade and spongy tissues, being 3.3% and 12.0% for the two tissues, respectively. Calcium nitrate treatments also affected the dimensions of the midvein bundles, since 39.1 and 73.3% increments were observed in length and width of midvein bundle, respectively, compared to the control. Nevertheless, the increase in bundle dimensions was positively coupled by an increase in thickness of xylem by 81.8 % and phloem by 20.0%. Moreover, xylem vessels showed wider vessels being 18.2% more than the control(Figure ,2 A and B).

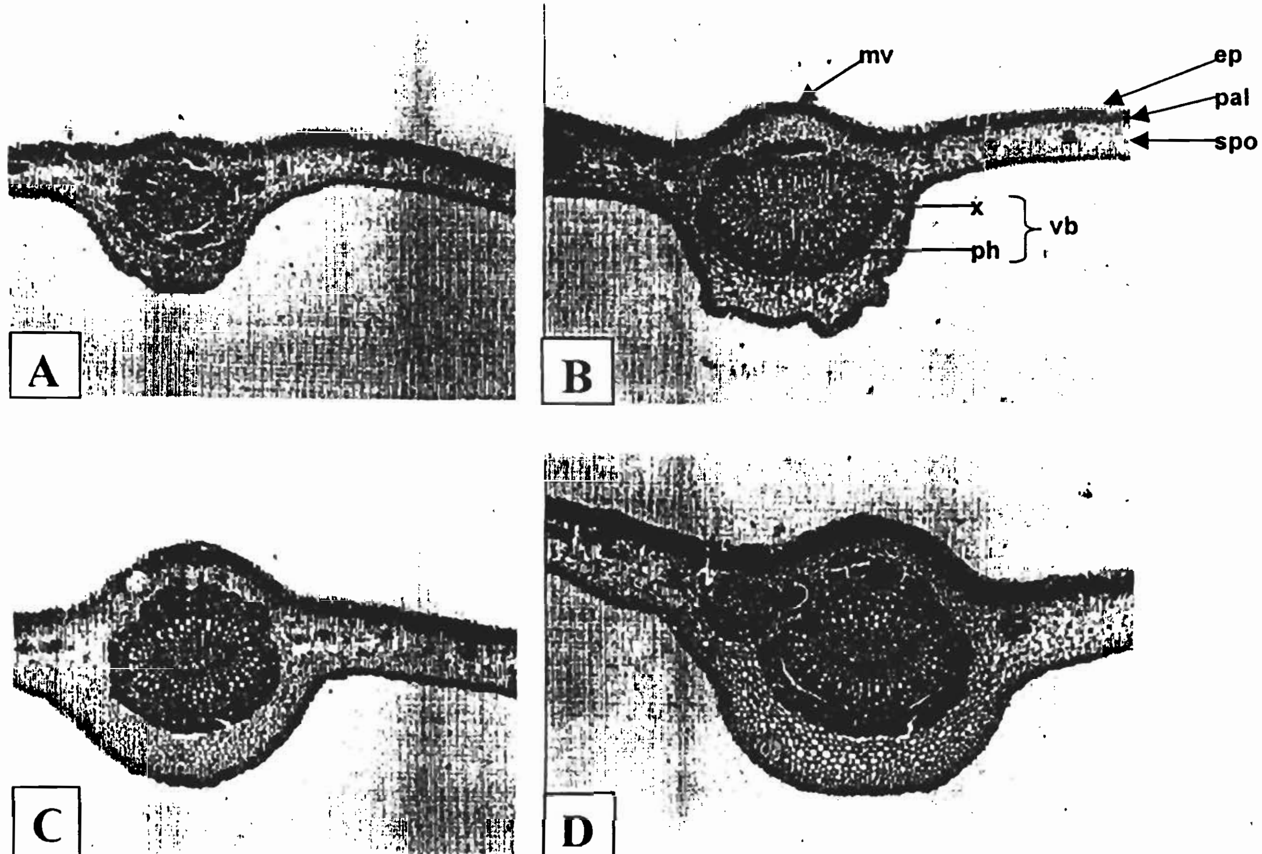

Fig.2(A-D):Transverse sections through the median portion of leaf blade on the corresponding twigs of grapefruit as affected by spraying with calcium nitrate and/or GA_3.
A-Untreated plant
B-Plant treated with calcium nitrate each 30 days
C-Plant treated with GA_3 at 75% PF
D-Plant treated with calcium nitrate + GA_3 at 75% PF
Details:ep,epidermis;mv,midvein;pal,palisad tissue;ph,phloem ;spo,spongy tissue;vb,vascular bundle; x, xylem. (X 40)

Regarding gibberellic acid treatments, data presented in Table (6) and Figure 2 (A and C) indicate that spraying 25 ppm GA_3 at 75% PF resulted in increasing thickness of both leaf midvein and lamina by 45.8% and 40.0% , respectively, more than the control. However, the increase in lamina thickness was mainly due to the increase in thickness of both palisade and spongy tissues, which amounted to 10.0% and 50.0% over the control, respectively. Moreover, dimensions of the main vascular bundle increased by 65.2% in length and 60.0% in width over the control. Though, the increase in vascular bundle dimensions is principally attributable to the increment in thickness of xylem tissue by 118.2% and phloem tissue by 28.0% over the control. Moreover, vessel diameter increased by 27.3% comparing with the control.

Regarding the effect of combined treatment of 0.1% calcium nitrate and 25 ppm GA_3 sprayed at 75% petal fall, data proved that this treatment gave the highest records in microscopical measurements of leaf anatomical features Table (6) and Figure 2 (A and D). Comparing to the control, leaves of the treated plants showed an increase in midvein and lamina thickness by 66.7% for both. The increase in lamina thickness was mainly due to the prominent increase of about 50.0% in palisade tissue and 80.0% in spongy tissue comparing with the control. However, the main vascular bundle of the midvein increased in length by 87.0% and in width by 80.0% over the control. Furthermore, leaves of treated plants showed an abundant increase in thickness of both xylem and phloem tissues. These increases amounted to 172.7% and 60.0% over the control for both xylem and phloem, respectively. Likewise, such treatment increased vessel diameter by 63.6% as compared with the control.

Table (6): Measurements (μ) and its relative percentages to the control of grapefruit leaf blade as treated by calcium nitrate and /or GA_3.

Treatments / Measurements (μ)	Control	$Ca(NO_3)_2$ Each 30 days	±% to control	GA_3 at 75% PF	± % to control	$Ca(NO_3)_2 + GA_3$ at 75% PF	± % to control
Midvein thickness	672.0	896.0	+ 33.3	980.0	+ 45.8	1120.0	+ 66.7
Lamina thickness	210.0	224.0	+ 6.7	294.0	+ 40.0	350.0	+ 66.7
Palisade thickness	42.0	43.4	+ 3.3	46.2	+ 10.0	63.0	+ 50.0
Spongy thickness	140.0	156.8	+ 12.0	210.0	+ 50.0	252.0	+ 80.0
Main bundle dimen.							
Length	322.0	448.0	+ 39.1	532.0	+ 65.2	602.0	+ 87.0
Width	420.0	728.0	+ 73.3	672.0	+ 60.0	756.0	+ 80.0
Xylem thickness	77.0	140.0	+ 81.8	168.0	+ 118.2	210.0	+172.7
Phloem thickness	35.0	42.0	+ 20.0	44.8	+ 28.0	56.0	+ 60.0
Vessel diameter	15.4	18.2	+ 18.2	19.6	+ 27.3	25.2	+ 63.6

The importance of leaves to the inflorescences for fruit set is well known, and has been reported for many varieties and species of citrus by a number of investigators (Erner and Bravdo,1983). The correlation found between carbohydrates and fruit set has been claimed to be the major factor in the enhancement of fruit set (Goldschmidt and Golomb ,1982). The preferential set on shoots bearing leaves and flowers has been explained mainly in terms of the supply of photosynthate to the developing fruits from the adjacent young leaves , an optimal supply being needed to enhance fruit set (Moss *et.al.*,1972). Leaves have been known to induce and control the development of vascular tissues along the plant axis by means of steady polar flow of auxins (Aloni, 1992)

It is clear from the anatomical study that the used treatments fundamentally affected the anatomical features of both the juncture of pedicel and fruitlet as well as the leaf. These changes in different characters produced by treatments occurred to different extents. As a remarkable trend of increments was observed in microscopical measurements. The lowest was caused by calcium nitrate treatment, followed by GA_3 treatment, and finally the largest changes were found as a result of the combined treatments of both calcium nitrate and GA_3 treatment. Thus, the anatomical study may give an indication for the previously obtained results concerning the effect of spraying 0.1% calcium nitrate and 25 ppm GA_3 separately or in their combination, on both quantity and quality of grapefruit yield.

The present study emphasizes the importance of the fruitlet-pedicel juncture for fruit set and provides a possible explanation for the better set (quantity and quality) on grapefruit trees treated with calcium nitrate and / or gibberellic acid comparing with the untreated control.

REFERENCES

Aloni, R. (1987). Differentiation of vascular tissues.Annual Review of Plant Physiology.38:179-204.

Aloni, R. (1992). The control of vascular differentiation.International Journal of Plant Science.153:590-92.

Anonymous (1977). Official Methods of Analysis of the Association of Official Agricultural Chemists (A.O.A.C.). Washington, D.C.USA. PP. 528-530.

Arnon, D.I (1945). Copper enzymes in isolated chloroplasts polyphenoloxidase in *Beta vulgaris*. Plant physiology.Vol.24, No.1:1949.

Aysha Khan (1996). Role of auxin, Kinetin, gibberellic acid and L-methionine on cell division and tracheary element differentiation in *Citrus limonum* juice vesicle culture. Phytomorphology , Vol.46,No.4,pp.349-56.

Brion, P.W. and H.G. Hemming (1958). Physiology of plant growth and development .Ed .by M.B. Williams, MC-graw Hill, London, pp30-45.

Brown, I. D. and M. Lilliand (1946). Rapid determination of potassium and sodium in plant material and soil extracts by Flame Photometry. Proc. Amer. Soc. Hort. Sci., 46: 341-346.

Bustan, A.; Erner, Y. and E. Goldschmidt (1995). Interactions between developing citrus fruits and their supportive vascular system. Annals of Botany, 76:657-66.

Casagrande, J.G.; Bianchi, J. R.; Strelow, V.J. and J.C. Fachinello (1999). GA_3 influence on maturation of tangerine cv. 'Tapuari'. Revista Cientifica Rural 4(2): 34-37 (Hort.Abst.2000 Vol. 70, No.9).

Cronquist, A. (1981). An integrated system of classification of flowering plants. Columbia University press, N.Y., p. 1142-1147.

Dai, E. T. (1995): Study on the effects of mineral element nutrition on the flower bud formation of Pongan mandarin during the stage of flower bud differentiation. China Citrus, Vol.24, No.3, pp.20-21.

Davies, F. S.; Campbell, C. A.; Zalman, G. R. and M. Fidelibus (1999). Gibberellic acid application timing effect on juice yield and peel quality of 'Hamlin ' oranges .In proceeding of the 112th annual meeting of the Florida state hort. soc. Stuart. Florida, USA, 31 Oct.– 2 Nov. proceeding No.112, 22, 24.

El – Wazzan, R.A., Salem, S.E. and S.S. Moustafa (1993). Effect of GA_3 and vapor–guard in leaf hydration, yield and fruit quality of Washington navel orange trees. Research Bulletin, Fayoum J. Agri., Res. &Dev. vol. &, No .2, July.

Erner,Y. and B. Bravdo (1983). The importance of inflorescence leaves in fruit setting of ' Shamouti' orange. Acta Horticulturae, 139:107-112.

Esau, Katherine (1976): Anatomy of seed plants (2$^{\underline{nd}}$ Ed.). John Wiley& Sons, New York, 550.

Evnhuis, B. and D.e.- Waard (1980). Principles and practices plant analysis .FAO. Soil Bull. 39 (1): 152-162.

Farmahan, H. L. (1999). Effect of GA_3, on alternate bearing in Kinnow mandarin. South Indian Horticulture, 45 (1/2) 30-33 (Hort. Abst. ,5405).

Fidalski, J.; P. A. Auler and V. Tormem (2000). Relations among Valencia orange yields with soil and leaf nutrients in northwestern Parana, Brazil. Brazilian Archives of Biology and Technology Vol. 43, No. 4, PP.387-391.

Goldschmidt, E.E. (1976). Endogenous growth substances of Citrus tissues. Hort. Science , 11:95-99.

Goldschmidt, E.E. and A. Golomb (1982). The carbohydrate balance of alternate-bearing citrus trees and the significance of reserves for flowering and fruiting. Journal of the American Society for Horticultural Science , 107:206-208.

Guinn,G.(1984). Boll abscission in cotton. Crop Physiology Ed.U.S.Gupta.Pab: by Mohan for Oxford and IBH Publishing Co. Printsmon Press, Fridabed .P.:177-225.

Hollman,T.A . (1995):Control of yield characteristics of citrus and tree response to chemical treatments . Soil Crop Science Society of Florida Proceedings , 33 : 93-99 .

Huber, D. M. (1980). The role of mineral nutrition in defense. Vol V: 381 -406. Academic press, Inc.

Ibrahim, T. A.; Salem, S. E. and L. F. Guindy (1994). The influence of Gibberellic acid and promalin on the yield and fruit quality of Washington navel orange. Bull. Fac. Agric Univ. Cairo, Vol. 45 (3) July.

John, P. and A. Bower (2000). Water stress and its alleviation. Hort. Sci. School of Agric. Sci. and Agribusiness, Univ. of Natal. ISC Congress 2000 Orlando Florida programs and abstracts. Dec 3-7 P.77.

Jones, A. M. (1990): Do we have the auxin receptor yet? Physiol. Plant .80: 154 -158.

Kim, Y. ; Young, M. D. and K. hanyong (2000). Effect of foliar application of ethychlozate mixed with Calcium formulae on a fruit quality of Satsuma mandarin. Korean Journal of Hort. Sci. & Technology (2000) 18 (5) 605 -611 (Hort. Abst Vol. 71 No .6, 2001).

Koller, O. C.; Schafer, G.; Sartori, I. A.; Sohwarz, S. F. and J .G. Lima (2000). Production of navel orange cv.monte parnaso with branch girdling and application of plant growth regulators. Revista Brasileira de Fruticultura 22 (special Issue) 68 -72.

Lavon, R.; Goldschmidt, E. E.; Salomon, R. and A. Frank (1995). Effect of potassium, magnesium, and calcium deficiencies on carbohydrate pools and metabolism in citrus leaves. J of, Amer. Soc. for Hort. sci. Vol. 120, No. 1, PP.54 -58.

Li, J. ; Li, X. ; Peng, J. and Z. Guoqiang (1999). Effect of five mineral elements on nutritional state of Navel orange tree. J. Of Hunan Agric Univ. 25 (1) 36-39, China.

Matthew, W. and S. D. Frederick (2000). Gibberellic acid application timing effects on 0brix and peel color of processing oranges. Dep. of Hort. Sci. Univ. of Florida. ISC Congress 2000 Orlando Florida program and abstracts. Dec 3-7- 2000 p.154.

Mazumdar, D.C . and D.N. Bhatt (1977). Effect of pre –harvest application of GA_3 and ethrel on sweet orange fruits. Progressive Hort., 8(2): 89-91(Hort.Abst.47:9877).

Moss , G. ; Steer, B. and P. Kriedemaun (1972). The regulatory role of inflorescence leaves in fruit-setting by sweet orange (*Citrus sinensis)* . Physiologia Plantarum , 27:432-38.

Murphy, J. and J. P. Riley (1962). A modified single solution method for the determination of Phosphate in natural water. Anal. Chim. Acta, 27:31-36.

Ouyang Guochun. (1998). Experiment of fertilizing for *Satsuma mandarin.* South China fruit, Vol .27, No.2 P.21.

Patil, B. S., Yoo, K. S. and L.M. Pike (2000). Post harvest storage effect on grapefruit functional compounds. Texas A&m University Kingsville citrus Center , ISC Congress 2000. Orlando Florida program and abstracts. Dec. 3-7- 2000 p.164.

Pregl, F. (1945). Quantitative organic analysis .4th ed., J. A. Churchill, Ltd., London.

Sachs,T. (1991). The canalization of vascular differentiation. In:Patteren formation in plant tissues. Cambridge University Press,70-88.

Sass,J.E.(1967). Botanical Microtechnique. Oxford and IBH Publishing Co. Calcutta, 3 rd ed.

Shawki, A., El –Tomi, A. and A. Nasr (1978). Effect of urea, gibberellic acid and 2,4-d spray on navel orange. Egypt, J. Hort. Vol. 5, No.2 115-121.

Shishova, M. and S. Lindberg (1999). Auxin cytosol acidification in Wheat leaf protoplasts depends on external concentration of Ca^{++} .J of Plant Physiol. 1552, 189-196 (C.F. Crop Physiol Abst.25 .703).

Smith, P. F. (1964). Effect of K level and substrat. Lime on growth, fruit quality, and nutritional status of valencia orange trees. Plant analysis and fertilizer problems 4:pub .by Amer. soc . Hort. Sci., E. Lansing , Mich ., 332 -343.

Snedecor, G. W. and W. G. Cochran (1972). Statistical methods, 6th ed. the Iowa st. Unvi.press. Ames, U.S.A pp. 593.

Tafazoli, E. ; Saffarian, A. and H. Rastegar (1989). Yield and quality of Valencia orange as affected by Gibberellic acid .Indian J of Hort .46:3, 319-321.

Thukral, B. R. ; Ranvir S. ; Misra, K. K. and H. R. Jaiswal (1994). Effect of plant growth regulators on growth and mineral composition of leaves of lemon (*Citrus limonum* Burm.). Annals of Agricultural Research, Vol.15, No.3, pp.306-309, India.

Treeby, M. T. ; Bower, J .P. and C. J. Lovatt (2000). Albedo breakdown (creasing) in Australia, South Africa, and California, USA: physiological Bnsis. Prediction and control. ISC Congress International Society of Citriculture, Orlando Florida, Program and Abstracts in Dec. 3-7.

Wang, H. and R. Yu (2000). Effect of spraying GA_3 on the fruit set of fingered citron. South China fruits 29 (1) 16-17. Agric.Res. Inst.,Jinhua Municipality, Zhejiang,China.

Xie, Y. H. ; Ou, Y.; Cao, Z.C. and Q. Fan (1992): Effect of pre-harvest application of calcium and IAA on the fruit quality of Jinchen (*Citrus sinensis*). J. of Southwest Agric. Unvi. China .14: 6, 543 -545.

Zhou, J. ; Liao, Y. and X. Qin (1998). Effect of B and Ca on photosynthetic physiology and root vigour in lemon (*Citrus Limonum*) seedlings. J .of Southwast Agric.Univ., 20 (4) 315-320, China (Hort. Abst. Vol. 69 No.8).

تأثير الرش بالكالسيوم وحمض الجبراليك على المحصول وصفـــات الجــودة والتســاقط لثمارالجريب فروت

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أجريت هذه الدراسة لموسمين متتاليين(١٩٩٩/٢٠٠٠-٢٠٠٠/٢٠٠١) على أشجار الجريب فروت صنـــف روبي رد لمعرفة تأثير الرش بنترات الكالسيوم بتركيز ١و٠ % وأيضا حمض الجبر اليك بتركيز٢٥ جزء في المليـون (وكان الرش إما منفردا أو خلطا بينهم) على المحصول وصفات الجودة والتساقط للثمار. اتضح من هذه الدراســة أن الرش عمل على زيادة وزن المحصول (كجم/شجرة) مقارنة بالكنترول في كلا الموسمين، وأيضا أدى الـــرش ألــي تحسين الصفات الطبيعية والكيماوية للثمار (وزن الثمار (جم) ، حجم الثمار (سم٣) ، سمك القشرة (سم) ، نسبة العصير ، نسبة المواد الصلبة الذائبة الكلية ، نسبة الحموضة ، نسبة المواد الصلبة الذائبة الكلية/الحموضة ، الســكريات الكليــة (مجم) ، حمض الاسكوربيك (مجم) ، كما زاد أيضا محتوى الأوراق من الكلوروفيل (أ،ب). وقد أشار تحليل الأوراق آن محتواها من الفوسفور والبوتاسيوم والكالسيوم والماغنسيوم والحديد والزنك والمنجنيز قد زاد معنويا مقارنة بـــالكنترول في كلا الموسمين وكانت الزيادة غير معنوية بالنسبة للنتروجين في الموسم الأول بينما كانت معنوية في الموسم الثـــاني فقط .وقد أشارت نتائج الدراسة التشريحية أن المعاملات أثرت تأثيرا ملحوظا على المعالم التشريحية لمنطقة الاتصـــال بين الثميرة والعنق وكذلك الورقة. وقد أدى الرش بالمخلوط الى الحصول على أفضل النتائج التى تؤدى الى خفض نسبة التساقط للثمار والتى تتمثل فى زيادة سمك منطقة الاتصال بين الثميرة والعنق وكذلك سمك العنق . كما أدت أيضا الـــى الحصول على أفضل اتصال وعائى وترابط للخلايا فى منطقة الاتصال ، بالاضافة الى افضل تركيب تشريحى للورقة.