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Study effect of Chitosan and Gibberellic Acid on Growth, Flowering, Fruit Set, Yield and Fruit Quality of Washington Navel Orange Trees

Shaimaa A. Mohamed and H. S. Ahmed

Citriculture Department, Horticulture Institute, Agricultural Research Centre, Cairo, Egypt

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ABSTRACT

The current study was carried out during successive seasons of 2016 and 2017 on 40- years old Washington navel orange trees, budded on Volkamer lemon rootstock grown in a private orchard at Qalub district, El Qalubia Governorate, Egypt. The study was conducted to find the effect of foliar spray application with agrochemical GA_3 at 30 ppm and a natural one such as chitosan at two doses of 2 g/L and 4 g/L. The treatments were sprayed either once during mid- January and mid- April or twice on mid- January and April. The results revealed that all the examined aspects were significantly affected by GA_3 or chitosan foliar applications and the spraying application time, beside the interaction between them as compared to control water spray. GA_3 spraying once either during mid- April or mid- January increased vegetative growth parameters and leaf C/N ratio or leaf DM%. Foliar chitosan applications with two doses of 2 g/L and 4g/L had more impacts on leafy inflorescence%, fruit set % and Canopy yield as weight (kg/m^3), as well as leaf pigments (chl.a, chl.b and its total) and carotenoid, when sprayed during both mid- January and mid- April. Moreover, chitosan at 2g/L during the same previous date participated in improving physical and chemical fruit properties. However, no significant difference were found between the two applied doses of chitosan so, it was suggested that chitosan at 2g/L could be instead of 4g/L under experiment conditions.

Keywords: Washington Navel orange, Chitosan (cht), Gibberellic acid (GA_3), Foliar sprays, vegetative growth, leafy inflorescence %, fruit set %, Yield, Fruit quality

Introduction

Egypt has auspicious conditions to occupy an advanced rank in the production of citrus which to expand to foreign export markets. This is due to enable the local environments suitable for high productivity and quality. Navel orange is considered the most important citrus variety as total fruitful area reached to about 156514 Fed. with a production of 10.844 tons/Fed. (Ministry of Agric. And Land Reclamation/Annual Report 2015). In the recent years, the period of flowering of citrus trees has been characterized by continuous waves of unorthodox temperatures, which led to large percentages of dropping flowers and fruit set, in particular parthenocarpic varieties (Iglesias *et al.*, 2007)

Gibberellic acid (GA_3), a plant hormone stimulating plant growth and development, plays an important role in stem or internodes elongation via motivates cell division and expansion. Moreover, it regulates flower initiation and development and it is essential for male and female fertility not for differentiation of floral organs (Gupta and Chakrabarty, 2013). Gibberellin is applied in citrus production for many purposes including reduction of flowers, increasing fruit setting and improving maturity and quality properties of fruits (Hifny *et al*., 2017). The benefits of GA_3 application were reported by (Agusti *et al*., 1982; Abd El-Migeed, 2002 and Sayed *et al*., 2004) on oranges, (El-Sese *et al*., 2005) on mandarin, (Mostafa *et al*., 2001) on pear and (Sharkawy and Mehiesen, 2005) on guava.

Appling of biostimulators is one of the strategies to alleviate negative effect of abiotic stress, stimulate plant growth and improve yield and quality of crops for instance chitosan (Jabeen and Ahmad, 2012). Chitosan is a natural, low toxic and economical compound, consist of an amino polysaccharide obtained by alkaline deacetylation from chitin and other decomposable substances of crabs and shrimps shells, therefore it is considered as an eco-friendly product (Mohamed et al., 2018). It act as a growth promoter through enhances nutrient absorption capacity in plant (Malekpoor *et al*., 2016 and Balal *et al*., 2017).

The goal of this search was to examine the effects of certain spraying chemicals such as GA_3 or a natural one such as chitosan at either different concentrations or application times to increase flowering, fruit set%, yield and fruits quality of Washington navel orange trees.

Corresponding Author: Shaimaa A. Mohamed, Citricultural Division, Horticultural Research Institute, ARC, Egypt. E-mail: shem1610@yahoo.com

Material and Method

Experimental site and plant materials

This study was carried out during two successive seasons, 2016 and 2017 on Washington Navel orange [*Citrus sinensis* (L.) Osbeck]. The trees were budded on Volkamer lemon rootstock grown in a private orchard at Qalub, El-Qlubia governorate, Egypt. Thirty six trees of 40 years old Navel orange trees, planted at 5 × 5 m apart in a sandy- loamy soil, under flooding irrigation system. The tree were carefully selected as being healthy and uniform as much as possible in vigor and size, receiving regularly the same horticultural practices. The purpose of this work is to study the influence of foliar spray applications with Gibberellic acid (GA_3) at 30 ppm and Chitosan (chit) at 2 and 4 g/L during three spray dates; 15th January, 15th April and on both previous two spray dates on growth, flowering, fruit set, yield and fruit quality.

Treatments and statistical design

Thirty six trees were laid out in Randomized Complete Block Design (RCBD) with two factors in a factorial arrangement with three replications. Factor A represented four foliar sprayed Agrochemicals of T1: Control (spraying with water only), T2: GA_3 at 30 ppm, T3: Chitosan (cht) at 2g / L and T4: Chitosan (cht) at 4g /L. Factor B occupied three spraying dates of D1: spraying in mid-January, D2: spraying in mid- April and D3: spraying in both mid- January and April. GA solution: To prepare the desired concentration of GA_3 solution 1 g of fine powder gibberelline (Berelex, ICI Soplant) was dissolved in 1L of distilled water and kept at low temperature (4°C) till used. The purified commercial product of chitosan (2-amino-2-deoxy-ˇ-d-glucosamine) namely Chito-Care® with a deacetylation degree 85% was used. Chitosan was dissolved in 1%acetic acid to get the desired concentrations of 2 and 4 g L^{-1}. The pH of the solution was adjusted to 6.5 with sodium hydroxide.

Measurements and Analysis

Four branches of one year old were chosen from each tree. Four shoots from the current spring growth cycle were labeled to follow up the effects of the differential investigated treatments which evaluated through the response of the following measurements:

I- Vegetative growth:

1. Spring vegetative flushes:

a) Shoot length and diameter (cm), as well as leaf area (cm^2) were estimated according to the method of Radwan, (1973).

b) Leaf dry matter (%): non- fruiting shoots of the spring growth cycle on each tree were tagged in April in both seasons. Thereafter, samples of 40 leaves from these shoots were randomly collected from each tree in late September of each season. Leaves were washed with tap water, rinsed with distilled water then weighted after air drying. The leaves were oven dried at 60-70°C till a constant weight and leaf dry matter percentage was calculated.

II- Some fruiting aspects:

a) Fruit set (%): Total number of flowers and total number of fruitlets were counted then fruit set percentage was calculated according to the equation:

Fruit set (%) = (Total number of fruitlets/ Total number of flowers) ×100

b) Leafy inflorescences (%) : Total number of inflorescences and total number of leafy inflorescences were counted then leafy inflorescences percentage was calculated according to the equation:

Leafy inflorescences (%) = (Total number of leafy inflorescences/ Total number of inflorescences) ×100.

III - Physiological parameters:

a) C / N ratio was determined at mid-September where samples of mature non-fruiting spring shoots were taken, defoliated and weighed then, were oven dried at 70°C. Total carbohydrates (g/100g DW) were determined by acid hydrolyzing of the powdery samples and the resulting data of the signing sugar designated as available carbohydrates was determined by the di-nitro-salicylic acid (DNS) method of Fisher and Stein, (1961). Total nitrogen was determined in mature leaves using Evenhunis (1976). Then C/ N ratio was calculated according to the equation:

C/ N ratio = Total carbohydrates in shoots / Total nitrogen in leave

b) Leaf pigments content(chlorophyll a and b and total carotenoids): disks (2.5 cm²/ area) from the third leaf at the top of spring shoot were extracted with dimethyl formamid-e (D.M.F.) solution [$HCON(CH_3)^2$] and placed overnight at cool temperature (5°C).Chlorophyll a and b as well as carotenoids were measured by Spectrophotometer Beckman Du 7400 at wavelengths of 663,647 and 470 nm, respectively, according to the equation described by Nornai (1982) and calculated as mg/100g FW as follows :

$$\text{Chl. a} = 12.70\ A_{663} - 2.79\ A_{647}$$
$$\text{Chl. b} = 20.76\ A_{647} - 4.62\ A_{663}$$
$$\text{Total chl.} = 17.90\ A_{647} + 8.08\ A_{663}$$
$$\text{Total carotenoids} = 1000 \times A_{470} - 3.72\text{Chl.a} - 104\ \text{Chl. b} / 229$$

IV- Tree yield efficiency as kg/m^3 canopy:

Fruit yield was recorded annually. Yield in relation to tree volume was considered as a measure of tree efficiency where Tree efficiency = kg of fruits /m3 canopy of tree (Castle and Philips, 1980).

V- Fruit quality:

A sample of 10 fruits per each replicate was selected in the first week of January in both seasons to determine fruit quality as follows:

a) Fruit physical characteristics: average of fruit shape Index and peel thickness (cm) were determined.

b) Fruit chemical characteristics: Juice TSS (%), T.AC (%), TSS/T.AC ratio and V.C (mg/ 100 g. juice) were determined (A.O.A.C., 1990).

Statistical analysis

The variance of each individual factor and their interactions were compared by least significant differences (LSD) test, the means having the same letters are not significantly different according to Steel and Torrie, 1980. The statistical analysis was performed using MSTAT- C Package (1996).

Results and Discussion

Vegetative growth

Data concerning vegetative growth parameters, showed that shoot length and diameter, leaf area and leaf dry matter (DM) were increased by either foliar applications by GA_3 (30 ppm), chitosan 2 and 4 g /L or dates of spraying as a single effect, regardless of each other. GA_3 treatment was significantly prior in improving growth indices when compared with those chitosan using either dose i.e. 2 or 4 g/L or control. Significant differences between chitosan treatments and control were detected in both study seasons. Spraying on mid-April was more effective in increasing shoot length and diameter and leaf area, as well as spraying twice in mid-January and April increased leaf area. The data was cleared that dry matter % was affected positively to spraying once in mid-January. With respect to the interaction between treatments and date of spray foliar applications, it was obvious that GA_3 foliar application during mid-April only revealed statistical superiority in promoting shoot length and diameter and leaf area in both years. However, GA_3 spraying in both of mid- January and April had similar effect on shoot length and leaf area in 2016 only and both seasons, respectively. Hence, spraying with GA_3 gave the highest values of shoot length (9.90 &9.73) and shoot diameter (0.813 & 0.843 during mid- April only and leaf area (31.22 & 32.54) during mid- January and April, as well as leaf DM (43.11 & 43.78%) during mid- January only in 2016 and 2017, respectively. No significant differences were observed between chitosan spraying at 2 and 4g /L, whereas they made significant progress over control.

Table 1: Effect of GA_3 chitosan spraying on vegetative growth aspects (shoot length and diameter, leaf area and leaf dry matter %)

Treatment	2016				2017			
	Jan.	Apr.	Jan + Apr.	Mean	Jan.	Apr.	Jan + Apr.	Mean
				Shoot length (cm)				
Control	5.53^{f}	5.54^{f}	5.55^{f}	5.54^{d}	5.80^{f}	5.79^{f}	7.79^{f}	5.79^{c}
GA_3 (30 ppm)	5.60^{ef}	9.90^{a}	8.53^{a}	7.71^{a}	5.80^{f}	9.73a	8.93^{b}	8.16^{a}
Chit (2g /L)	6.03^{def}	7.03^{bc}	6.53^{cd}	6.53^{c}	6.40^{ef}	7.53^{cd}	6.94^{de}	6.96^{b}
Chit (4g /L)	6.23^{de}	7.63^{b}	7.05^{c}	6.97^{b}	6.33^{ef}	7.70^{c}	7.30^{cd}	7.11^{b}
Mean	5.85^{c}	7.30^{a}	6.92^{b}		6.08^{c}	7.69^{a}	7.24^{b}	
				Shoot diameter (cm)				
Control	0.520^{g}	0.523^{g}	0.523^{g}	0.522^{c}	0.550^{g}	0.547^{g}	0.547^{g}	0.548^{c}
GA_3 (30 ppm)	0.560^{efg}	0.813^{a}	0.747^{b}	0.707^{a}	0.593^{efg}	0.843^{a}	0.780^{b}	0.739^{a}
Chit (2g /L)	0.550^{fg}	0.623^{cd}	0.597^{de}	0.590^{b}	0.560^{fg}	0.653^{cd}	0.627^{de}	0.613^{b}
Chit (4g /L)	0.580^{fed}	0.650^{c}	0.570^{ef}	0.600^{b}	0.597^{efg}	0.680^{c}	0.607^{fed}	0.628^{b}
Mean	0.552^{c}	0.652^{a}	0.609^{b}		0.575^{c}	0.681^{a}	0.640^{b}	
				Leaf area (cm^2)				
Control	18.60^{e}	18.61^{e}	18.62^{e}	18.61^{c}	18.72^{f}	18.70^{f}	18.71^{f}	18.71^{c}
GA_3 (30 ppm)	20.48^{de}	30.60^{a}	31.22^{a}	27.43^{a}	20.73^{e}	30.94^{a}	32.54^{a}	28.07^{a}
Chit (2g /L)	21.45^{d}	24.40^{bc}	25.09^{b}	23.65^{b}	22.47^{de}	24.51^{bc}	25.12^{b}	24.03^{b}
Chit (4g /L)	22.46^{cd}	24.85^{b}	25.42^{b}	24.24^{b}	23.17^{cd}	25.09^{b}	25.69^{b}	24.65^{b}
Mean	20.75^{b}	24.62^{a}	25.09^{a}		21.27^{b}	24.81^{a}	25.52^{a}	
				Leaf dry matter %				
Control	28.65^{h}	28.66^{h}	28.66^{h}	28.66^{c}	29.76^{g}	29.68^{g}	29.70^{g}	29.72^{c}
GA_3 (30 ppm)	43.11^{a}	30.58^{gh}	39.90^{b}	37.86^{a}	43.78^{a}	31.20^{fg}	40.31^{b}	38.43^{a}
Chit (2g /L)	36.56^{cd}	31.67^{g}	33.87^{ef}	34.03^{b}	37.18^{cd}	33.47^{ef}	34.19^{e}	34.95^{b}
Chit (4g /L)	38.08^{bc}	32.58^{fg}	34.81^{de}	35.16^{b}	38.87^{bc}	33.92^{e}	34.91^{de}	35.90^{b}
Mean	36.60^{a}	30.87^{c}	34.41^{b}		37.40^{a}	32.07^{c}	34.78^{b}	

The above results are in harmony with those of Mohamed *et al*. (2010) who found that foliar spraying of Sour orange and Volkamer lemon rootstocks with GA_3 at 200 and 400 ppm significantly increased stem length and diameter and leaf area in comparison with untreated seedlings. Also, foliar application by GA_3 at 20 and 30 ppm either alone or in combination with NAA at 20 or 25 ppm through starting from pea stage of fruit development (after fruit set) significantly improved shoot length and leaf area of Sweet orange or Washington navel orange trees relatively over control (Ghosh *et al*., 2013 & Hifny *et al*., 2017). In addition, they explained that the obtained increase in vegetative growth as a result of GA_3 application might be due to the effect of GA_3 and NAA which are critical growth regulators for the plant, since they are playing an essential role in cell division and cell wall elongation and lead to shoot elongation increase. The vegetative growth including the percentages of increment of plant height and diameter and leaf area, leaf fresh and dry weights of sour orange seedlings were affected significantly by chitosan spraying at different concentrations (50, 100, 150 ppm) as compared to untreated control water spray (Mohamed *et al*., 2018), beside they revealed that chitosan may additionally provide a few amino compounds required for plant growth, that led to increase total N content increasing in leaves or higher capacity of plant absorption of N from soil as chitosan would possibly increase key enzymes of nitrogen metabolism and promote transportation of N within the functional leaves. Also, chitosan may increase the availability, uptake and transport of essential nutrients via adjusting cell osmotic pressure and thereby progresses plant growth and development e.g. number of leaves or shoots, leaf area and total leaf area per plant that reversing in increasing its fresh and dry weight. Also, Uthairatanakij *et al*. (2007) suggested that chitosan may induced a signal to synthesize plant hormones such as gibberellins, moreover it possibly enhance growth and development via signaling pathway related to auxin biosynthesis by a tryptophan-independent pathway.

Leafy inflorescence and fruit set percentages

The results in Table (2) showed that the percentages of leafy inflorescence and fruit set significantly responded to the used foliar applications and in all dates of application as a singl factor or their interactions. Chitosan treatment with 4g/L increased significantly leafy inflorescence % and fruit set % compared to chitosan at 2g/L, GA_3 and control and the variations among the last three treatments were significant, except no substantial difference were found between GA_3 and control in promoting leafy inflorescence % in both seasons. Moreover, leafy inflorescence % and fruit set % were significantly affected by date of spraying twice on mid- January and April, in addition mid-January only improved appreciably leafy inflorescence % in both study years. These criterions followed a regular pattern in both study seasons; chitosan spraying at 4g /L during mid- January and mid- April attained the highest values of leafy inflorescence and fruit set.

Table 2: Effect of GA_3 and chitosan spraying on Leafy inflorescence and fruit set percentage

Treatments	2016				2017			
	Jan.	Apr.	Jan + Apr.	Mean	Jan.	Apr.	Jan + Apr.	Mean
				Leafy inflorescence %				
Control	56.52^{c}	56.53^{c}	56.53^{c}	56.53^{c}	56.64^{d}	56.64^{d}	56.64^{d}	56.64^{c}
GA_3 (30 ppm)	57.37^{c}	55.75^{c}	55.83^{c}	56.32^{c}	59.00^{d}	56.64^{d}	58.28^{d}	57.98^{c}
Chit (2g /L)	68.09^{b}	59.00^{c}	71.29^{b}	66.13^{b}	69.40^{b}	62.56^{c}	71.23^{b}	67.73^{b}
Chit (4g /L)	75.48^{a}	59.13^{c}	77.66^{a}	70.76^{a}	77.99^{a}	60.65^{cd}	78.40^{a}	72.35^{a}
Mean	64.36^{c}	57.60^{b}	65.33^{a}		65.76^{a}	59.12^{b}	66.14^{a}	
				Fruit set %				
Control	1.61^{h}	1.62^{h}	1.62^{h}	1.62^{d}	1.55^{h}	1.56^{h}	1.55^{h}	1.55^{d}
GA_3 (30 ppm)	1.66^{h}	2.20^{g}	2.30^{f}	2.03^{c}	1.75^{g}	3.97^{c}	3.75^{d}	3.16^{c}
Chit (2g /L)	3.20^{e}	3.19^{e}	3.95^{b}	3.45^{b}	4.00^{c}	3.09^{f}	4.14^{b}	3.75^{b}
Chit (4g /L)	3.41^{c}	3.33^{d}	4.26^{a}	3.67^{a}	3.55^{d}	3.44^{e}	5.26^{a}	4.08^{a}
Mean	2.47^{c}	2.58^{b}	3.03^{a}		2.71^{c}	3.02^{b}	3.68^{a}	

These results are in line with Ahmed *et al*., (2016) who studied pre-harvest foliar application of Washington navel orange tree by chitosan at two concentrations of 250 and 500 ppm. They declared that there was a significant increase in total number of flowers /tree and fruit set % over controls especially at high concentration. The positive effect of chitosan in stimulating flowering and increasing its number was reported by Wanichpongpan *et al*. (2001) on gerbera and Ramos-Garcia *et al*. (2009) on gladioli, beside Salachna and Zawadzińska (2014) who reported that "Gompey" freesia corms treated with 0.5% chitosan solution had more leaves, flowered earlier and formed more flowers. According to what was previously mentioned that chitosan may provide a few amino compounds that led to increasing total N content in leaves or higher capacity of plant absorption of nitrogen from soil and also the suggestion of Iqbal *et al.* (2004) who stated that the rate of leafy infolerscence formation and its ovaries growth was determined by various nitrogenous compounds since these show a higher polyamine content, as well as Abdel-Aziz and El-Azazy (2016), since NH_3ammonia and NH_4^+ammonium ion accumulated during stress-as winter low temperature (that induced flowering in citrus) resulted in stimulation of new arginine biosynthesis and the accumulation of putrescine at an early stage of floral organogenesis, followed by rapid metabolism of these compounds during flower development. Moreover, many investigators observed reported that exogenous application of gibberellins had a promotive effect on increasing fruit set of citrus trees; (Soost and Burnett, 1961) on Clementine mandarin, (Agusti *et al*., 1982) on sweet orange, (El-Sese, 2005) on Balady mandarin, (Abd El-moneim *et al*., 2007 & Abd El-Rahman *et al.*, 2012) on washington navel orange and (Baghdady *et al*., 2014) on Valencia orange. Plant growth regulators (PGR) particularly gibberellic acid and naphthline acetic acid had an important role on flowering and fruit set of different crops, since it promote fruit set and reduce fruit drop in many citrus species and varieties (Hikal, 2013), therefore, it could be attributed to that the application GA_3 increased availability of nutrients from leaves (Somwanshi *et al*., 2017 &Ullah *et al*., 2014). On contrary, GA_3 foliar applications had insignificant effect on Navel orange inflorescence types when sprayed at tree blooming stages (Hassanain *et al*., 2018).

Leaf chlorophyll contents and Leaf total carotenoids

Regarding leaf chlorophyll contents (a, b and its total) and total carotenoids shown in Table (3) were significantly influenced by the tested treatments and dates of foliar applications in both seasons.

The highest values of both chlorophyll (a, b and its total) and total carotenoids were displayed from spraying chitosan at 2 and 4g /L without significant differences between them followed by GA_3 (30ppm) comparing with the lowest values obtained by control. Likewise, spray foliar application during both mid- January and April recorded the highest values of these variables and the lowest was obtained from spraying on mid- January only, whereas spraying once on mid- April scored intermediate values in between them with pronounced differences in both years. It has been emerged clearly, interactions effect on these traits did not take a different direction of the pervious singly effect of treatments and time of applications, therefore the significant highest values of chlorophyll (a, b and its total) and carotenoids achieved in responding to chitosan treatment which applied twice at 2 and 4g/L during mid- January and April compared to the other interactions value. In plants, photosynthesis happens basically in leaf cells in organelles called chloroplast containing chlorophyll a, b and carotenoids.

Table 3: Effect of GA_3 and chitosan spraying on Physiological parameters; leaf chl. a &b, total chl. and carotenoids

	2016				2017			
Treatments	**Jan.**	**Apr.**	**Jan + Apr.**	**Mean**	**Jan.**	**Apr.**	**Jan + Apr.**	**Mean**
				Leaf chl. a (mg/100g Fw)				
Control	0.860^{e}	0.870^{e}	0.867^{e}	0.866^{c}	0.920^{de}	0.913^{de}	0.883^{e}	0.906^{c}
GA_3 (30 ppm)	0.923^{de}	1.02^{bc}	1.04^{b}	0.996^{b}	0.977^{cd}	1.04^{bc}	1.09^{b}	1.04^{b}
Chit (2g /L)	0.950^{cd}	1.05^{b}	1.29^{a}	1.10^{a}	0.973^{cd}	1.11^{b}	1.31^{a}	1.13^{a}
Chit (4g /L)	0.927^{de}	1.02b^{bc}	1.28^{a}	1.08^{a}	0.980^{cd}	1.04b^{bc}	1.29^{a}	1.10^{a}
Mean	0.915^{c}	0.989^{b}	1.12^{a}		0.962^{c}	1.03^{b}	1.14^{a}	
				Leaf chl. b((mg/100g Fw)				
Control	0.350^{e}	0.350^{e}	0.363^{de}	0.354^{c}	0.357^{de}	0.323^{e}	0.327^{e}	0.336^{c}
GA_3 (30 ppm)	0.380^{de}	0.420^{bc}	0.427^{b}	0.409^{b}	0.380^{cd}	0.410^{bc}	0.430^{b}	0.407^{b}
Chit (2g /L)	0.390^{cd}	0.430^{b}	0.527^{a}	0.449^{a}	0.380^{cd}	0.430^{b}	0.510^{a}	0.440^{a}
Chit (4g /L)	0.380^{cd}	0.417^{bc}	0.523^{a}	0.440^{a}	0.387^{cd}	0.410^{bc}	0.500^{a}	0.432^{a}
Mean	0.375^{c}	0.404^{b}	0.460^{a}		0.376^{b}	0.393^{b}	0.442^{a}	
				Leaf total chlorophyll				
Control	1.89^{d}	1.90^{d}	1.90^{d}	1.90^{c}	1.99^{e}	1.93^{e}	1.95^{e}	1.96^{c}
GA_3 (30 ppm)	2.03^{c}	2.24^{b}	2.29^{b}	2.19^{b}	2.12^{d}	2.26^{c}	2.37^{b}	2.25^{b}
Chit (2g /L)	2.09^{c}	2.30^{b}	2.83^{a}	2.41^{a}	2.11^{d}	2.40^{b}	2.83^{a}	2.45^{a}
Chit (4g /L)	2.03^{c}	2.24^{b}	2.82^{a}	2.37^{a}	2.13^{d}	2.25^{c}	2.80^{a}	2.40^{a}
Mean	2.01^{c}	2.17^{b}	2.46^{a}		2.09^{c}	2.21^{b}	2.49^{a}	
				Leaf carotenoids (mg/100g Fw)				
Control	0.677^{e}	0.687^{de}	0.687^{de}	0.683^{c}	0.720^{efg}	0.693^{g}	0.717^{fg}	0.710^{c}
GA_3 (30 ppm)	0.727^{de}	0.803^{bc}	0.823^{b}	0.784^{b}	0.770^{def}	0.820^{bcd}	0.860^{bc}	0.817^{b}
Chit (2g /L)	0.747^{cd}	0.827^{b}	1.01^{a}	0.862^{a}	0.767^{def}	0.873^{b}	1.03^{a}	0.890^{a}
Chit (4g /L)	0.727^{de}	0.803^{bc}	1.01^{a}	0.847^{a}	0.773^{de}	0.817^{cd}	1.02^{a}	0.869^{a}
Mean	0.719^{c}	0.780^{b}	0.883^{a}		0.758^{c}	0.801^{b}	0.906^{a}	

Chlorophylls and carotenoids have essential roles in photosynthesis, for example, the generation of sugars and organic molecules by fixation of CO_2 and water according to Dzung *et al*. (2011), additionally they confirmed our results where they stated that the application of chitosan and chitosan oligomer on coffee plant leaves enhanced the content of chlorophylls and carotenoids. Moreover, they proved that chitosan treatments resulted in a significant increasing of magnesium and total nitrogen in the leaves which are very important elements in composition of chlorophylls. These results supported by Mohamed *et al*. (2018) who found that foliar chitosan application significantly improved leaf chlorophyll content in sour orange seedling at different concentrations, since chitosan may be play an important role in increasing number of chloroplasts per cell and cell size and number per unit area, as well as stimulate synthesis of chlorophyll. Regarding results of GA_3, the data are in harmony with the findings of Hassanain *et al.* (2018) on Washington navel orange and Mazher *et al*. (2014) on *Schefflera arboricola* plants. They reported that leaf chlorophyll a, b and carotenoids content increased as a result of GA_3 applications at three levels (100, 200 and 300ppm) or during three intervals of application, whereas our results disagreed with those of Mohamed *et al*. (2010) who demonstrated leaf chlorophyll (a & b) content of sour orange and Volkamer lemon significantly decreased by increasing GA_3 concentrations as compared to control (untreated seeds and seedlings).

Leaf Carbohydrate-nitrogen ratio (C:N)

Both C and N supplements are essential for different cellular functions, hence sufficient supply of these two elements are important for plant growth, development and production , as well as reaction to a various stresses. It is clear from the obtained data in Table (4) that foliar spray with GA_3 improved the ratio of carbon to nitrogen and appeared a significant superiority when compared with other treatments. Spraying chitosan at 4 g/L followed chitosan at 2g/L with significant differences between the last two treatments as compared to control which recorded the lowest ratio. As for spraying time, regardless the tested treatments, the results revealed that the highest ratio of C:N occurred when spraying was applied either during mid - April only in both years or mid- January and April in 2016 only. However, the lowest ratio was observed from treatment applied on mid - January and the variations were significant among three dates especially in 2017. Meanwhile, GA3 foliar application once on mid- April in 2016, or twice on mid- January and April in both seasons resulted in the highest value compared with other interactions.

Table 4: Effect of GA_3 and chitosan spraying on C: N ratio, Yield and fruit physical properties (Fruit shape & Fruit peel thickness)

Treatments	2016 Jan.	Apr.	Jan + Apr.	Mean	2017 Jan.	Apr.	Jan + Apr.	Mean
				C/N R				
Control	10.94^{e}	10.95^{e}	10.96^{e}	10.95^{d}	11.86^{h}	11.87^{h}	11.89^{h}	11.87^{d}
GA_3 (30 ppm)	11.42^{de}	24.89^{a}	24.39^{a}	20.23^{a}	11.70^{h}	27.37^{a}	25.18^{b}	21.42^{a}
Chit (2g /L)	12.17^{de}	17.52^{c}	16.14^{c}	15.28^{c}	12.62^{h}	18.13^{e}	16.71^{f}	15.82^{c}
Chit (4g /L)	12.62^{d}	20.76^{b}	21.17^{b}	18.18^{b}	13.62^{g}	20.87^{d}	22.50^{c}	19.00^{b}
Mean	11.79^{b}	18.53^{a}	18.17^{a}		12.45^{c}	19.56^{a}	19.07^{b}	
				Yield efficiency(kg/m3)				
Control	0.967^{i}	0.970^{i}	0.973^{i}	0.970^{d}	1.02^{e}	1.00^{e}	1.00^{e}	1.01^{c}
GA_3 (30 ppm)	1.01^{h}	2.25^{e}	1.98^{g}	$1. 75^{c}$	1.01^{e}	2.28^{c}	2.02^{d}	1.77^{b}
Chit (2g /L)	2.70^{c}	2.17^{f}	3.39^{a}	2.76^{b}	2.81^{b}	2.20^{cd}	3.47^{a}	2.82^{a}
Chit (4g /L)	2.61^{d}	2.31^{e}	2.84^{b}	2.59^{a}	2.74^{b}	2.39^{c}	3.32^{a}	2.82^{a}
Mean	1.82^{c}	1.93^{b}	2.30^{a}		1.90^{b}	1.97^{b}	2.45^{a}	
				Fruit shape				
Control	1.09^{c}	1.09^{c}	1.09^{c}	1.09^{b}	1.08^{bcd}	1.09^{bc}	1.09^{bc}	1.09^{b}
GA_3 (30 ppm)	1.16^{ab}	1.17^{a}	1.10^{c}	1.14^{a}	1.15^{a}	1.17^{a}	1.11^{a}	1.14^{a}
Chit (2g /L)	1.10^{c}	1.11^{bc}	1.13^{abc}	1.12^{a}	1.07^{cd}	1.05^{de}	1.09^{bc}	1.07^{b}
Chit (4g /L)	1.09^{c}	1.10^{c}	1.10^{c}	1.10^{b}	1.06^{de}	1.03^{e}	1.05^{de}	1.04^{c}
Mean	1.11^{a}	1.12^{a}	1.11^{a}		1.09^{a}	1.09^{a}	1.08^{a}	
				Fruit peel thick.(cm)				
Control	1.10^{bc}	1.08^{bc}	1.11^{bc}	1.10^{ab}	1.17^{bc}	1.17^{bc}	1.17^{bc}	1.17^{a}
GA_3 (30 ppm)	1.23^{ab}	1.47^{a}	1.00^{bc}	1.23^{a}	1.21^{b}	1.54^{a}	1.03^{bcd}	1.26^{a}
Chit (2g /L)	0.900^{c}	0.917^{c}	1.00^{bc}	0.939^{c}	0.787^{de}	0.760^{e}	0.917^{cde}	0.821^{b}
Chit (4g /L)	0.900^{c}	0.900^{c}	1.08^{bc}	0.961^{bc}	0.777^{de}	0.680^{e}	0.813^{de}	0.757^{b}
Mean	1.03^{a}	1.09^{a}	1.05^{a}		0.986^{a}	1.04^{a}	0.981^{a}	

Our results are in line with Mohamed *et al*., (2010) who reported that GA_3 application increased leaf total carbohydrates, also there is a positive correlation between vegetative growth and carbohydrate accumulation in leaves, this correlation was clear in the current study on shoot length and diameter and leaf area as shown it Table(1). Leaf total carbohydrates were increased significantly in response to foliar chitosan application at 250 ppm on cowpea plants or 100 and 150 ppm on sour orange seedlings according to Farouk and Ramadan (2012) and Mohamed *et al.* (2018). Therefore it could be expected that the improvement of leaf total carbohydrate was reflected on raising the ratio of C: N. These results are in line with Scholefield *et al*. (1985) who mentioned that maximum levels of starch occurred in early spring and decreased sharply from then until autumn. During this period of rapid decline of carbohydrate reserves, flowering and early fruit growth were taking place as well as two vegetative growth flushes. Vegetative growth ceased and fruit development was almost completed by late autumn and the reserves of starch began to accumulate rapidly through autumn and winter to reach maximum levels again in spring.

Yield efficiency (kg/m^3)

Canopy yield as weight (kg / m3) is shown in Table (4), revealed that this variable increased gradually in an ascending order; where spraying by water as control,GA_3 at 30 ppm, chitosan at 4g / L and chitosan at 2g / L. However, no significant difference was detectable between the last two foliar applications in 2017. On contrary, the values obtained concerning time of application were in a descending order; spraying on mid- January and April, on mid- April only and on mid- January only. Hence, the sprayed tree with chitosan 2g /L during mid- January and April scored the highest canopy yield (kg/ m3) in both years, as well as chitosan at 4g/L on mid- January and April in 2017 only. However, the lowest one was achieved by control spray during the three dates of spraying in both seasons. Hence, it is observed that the yield efficiency followed to great extent the same trend pertaining leafy inflorescence% and fruit set%. These results are supported by Zagzog *et al.* (2017) where they study the effect of nano- chitosan at 2.5 and 5 ml L^{-1} on fruit set and yield of two cultivars of mango and found that chitosan treatment significantly increased the yield as number of fruits or weight per tree as compared to control of mineral oil at 1.5%. Moreover, application of Chitosan at 500 ppm recorded higher significant values of yield per vine in comparison with tap water control in accordance with those obtained by El-Kenawy *et al.* (2017). On the other hand, our results disagreed with Ahmed *et al.* (2016) who mentioned that fruit yield as kg / tree of Washington navel orange showed a non-significant effect under foliar application of chitosan treatments at different concentrations as compared with non-sprayed control trees. Also, the present findings are in conformity by other reports published earlier by Abd El-moneim *et al.*(2007), Abd El-Rahman *et al.*(2012), Hikal (2013), Hifny *et al.*(2017) and Hassanain *et al.*(2018) on Washington navel orange, beside Baghdady *et al.* (2014) on Valencia orange and Ullah *et al.*(2014) on Sweet orange. They all decided that GA_3 foliar application spray at different concentrations ranged between 10 and 100 ppm significantly increased the yield expressed as a number of fruits or weight per tree , as well as tree yield efficiency as number of fruit per m3 of canopy. It could be concluded that the previously mentioned enhancement in yield via spraying with chitosan and GA_3 may be attributed to the improvement in leafy inflorescence % and fruit set % and ultimately resulted to promote yield in comparing to water spray control.

Fruit quality

a-Physical properties

Fruit shape index

It could be observed from Table (4) that values of fruit shape in all the examined treatments including control increased over 1, so fruit shape tended to be ellipsoid. The highest value attained from GA_3 foliar application in both seasons and chitosan (2g/L) in 2016 only. No significant variation was found between control and chitosan (4g/L) in the first season, whereas the last treatment recorded the lowest value. Dates of spraying did not affect substantially fruit shape in both seasons. Tree sprayed with GA_3 during mid- April or mid- January produced fruits showing a significant highest value of fruit shape index in both years, as well as using chitosan at 2g/L and GA_3 on mid- January and April in 2016 and 2017, respectively. The above results are in partial agreement with those reported by Ahmed *et al*. (2016). They revealed that chitosan foliar spray had insignificant effect on fruit shape index or a significant decrease in fruit shape index with 250 ppm chitosan and control at different tested locations. On the opposite side, our results disagree with Hikal (2013) and Hassanain *et al*. (2018) who demonstrated that GA_3 foliar spray produced fruit had shape index values that did not differ than water spray control.

Peel thickness (cm)

Table 4 showed significant differences among various treatments as regard peel thickness. Trees treated with GA_3 (30ppm) had maximum peel thickness was statistically similar to tree of control spray, whereas applying chitosan either at 2 or 4g/L gave thinner peel in both study years. No appreciable effect of foliar application sprays time on this criterion in both seasons. The highest thickness of fruits was decided as a response of interaction between spraying with GA_3 (30ppm) and time of application specifically on mid- April in 2016 and 2017. Our findings are in line with those of Abd El-moneim *et al*. (2007) and Saleem *et al.* (2007). They revealed that GA3 foliar application at various concentrations did not significantly affect peel thickness in comparison with control. Similar observation regarding peel weight was reported by Hifny*et al*. (2017). On the other hand, the present findings disagreed with Abd El-Rahman *et al*. (2012) who reported that spraying GA_3 reduced peel thickness of oranges of Washington navel. Hassanain *et al*. (2018) also demonstrated that spray time of GA_3 (25 ppm) during different stages of full bloom, while the highest values were obtained from GA_3 spraying at 25% full bloom as compared with 75% or 25 &75% of full bloom.

b- Chemical properties

TSS%

It was clear from the data in Table (5) that TSS% significantly differed by the various applied spray treatments. Chitosan treatments at 2or 4g/L were statistically superior compared to GA_3 and control treatments, as well as there was a significant difference between the last two treatments in the first season and the lowest values were obtained from control in 2016. Regarding of the treatments used, this aspect did not respond to dates of spraying in both seasons. In general, TSS% was enhanced significantly via chitosan treatments applied either with dose of 2 or 4g/L, especially on mid- April only in both study years. This result was consistent with Ahmed *et al*. (2016) who stated that TSS % of navel orange fruits were affected significantly by pre-harvest chitosan spray at 250 and 500 ppm at two different locations under study in comparison with control. The same observation was reported by Abdel-Mawgoud *et al.* (2010) on strawberry plants and SaifEldeen *et al.* (2014) on artichoke. However, a reverse result was indicated by Zagzog *et al.* (2017) on mango and El-Kenawy (2017) on grape. Regarding GA_3 treatment in the present study, found to be partially agreed with Hikal (2013), Baghdady*et al*. (2014) and Hifny*et al.* (2017) reported that the GA_3 spray treatment significantly improved TSS% in comparison to the water spray control. In addition some earlier researches proved that the treatment of GA_3 did not have a clear effect than the control (Abd El-moneim *et al*., 2007; Saleem *et al*., 2007 and Abd El-Rahman *et al*., 2012). However, our results are in harmony with Hassanain *et al.* (2018) they reported that TSS% was not affected by the time of GA_3 spraying at the same concentration (25 ppm) during different stages of full bloom.

Acidity%

Concerning the data of acidity % in Table (5) it was found to follow a reverse pattern of TSS% where the control and GA_3 treatments recorded the highest acidity values with insignificant differences between them, where as chitosan at 4 g /L, while the lowest significant values were recorded from chitosan at 2 g/L in both years. As for spraying date, regardless the studied applications, it is observed that in general there is no significant effect on acidity% except between spraying once during January and twice on January and April in 2016 season. However, the highest acidity% values of the interaction between both spraying application and its date resulted from spraying GA_3 once on mid-April; in contrary spraying of chitosan (2g/L) at the same previous time, significantly recorded the lowest acidity% values in 2016 and 2017. Our results agreed with Ibrahim *et al*. (1994), Abd El-moneim *et al.* (2007) and Saleem *et al.* (2007) who indicated that GA_3 spray foliar application at concentrations ranged from 10 - 25 ppm did not significantly affect the acidity when compared with water spray as control. Meanwhile, they were partially in harmony with Hikal (2013) and Hifny *et al*. (2017) who obtained the same previous result only in one season, while the time of spraying during various full bloom stages caused slightly effect on acidity Hassanain *et al.* (2018). Otherwise our findings completely disagreed with Abd El-Rahman *et al*. (2012) and Baghdady *et al*. (2014) who revealed that no defined trend of acidity in response to GA_3 spraying treatment, moreover the representing results of chitosan were in line with Zagzog *et al*. (2017), who showed that acidity % statistically reduced by chitosan foliar application in comparison with control, whereas Ahmed *et al*.(2016) and El-Kenawy (2017) exhibited that acidity % significantly was not affect by chitosan treatment compared to control.

Table 5: Effect of GA$_3$ and chitosan spraying on chemical fruit properties (TSS %, acidity, TSS/acid ratio and V.C content).

Treatments	2016 Jan.	2016 Apr.	2016 Jan + Apr.	2016 Mean	2017 Jan.	2017 Apr.	2017 Jan + Apr.	2017 Mean
				TSS%				
Control	6.75^{c}	6.75^{c}	6.77^{c}	6.76^{d}	7.25^{cd}	7.24^{cd}	7.23^{cd}	7.24^{b}
GA$_3$ (30 ppm)	7.67^{b}	6.58^{c}	7.84^{b}	7.36^{c}	7.58^{cd}	7.08^{d}	7.92^{c}	7.53^{b}
Chit (2g /L)	8.83^{b}	9.37^{a}	8.83^{a}	9.01^{a}	9.00^{ab}	9.69^{a}	9.50^{ab}	9.40^{a}
Chit (4g /L)	7.58^{b}	8.83^{a}	7.83^{b}	8.08^{b}	8.75^{b}	9.75^{a}	9.50^{ab}	9.33^{a}
Mean	7.71^{a}	7.88^{a}	7.81^{a}		8.15^{a}	8.44^{a}	8.54^{a}	
				Acidity%				
Control	0.858^{b}	0.857^{b}	0.857^{b}	0.857^{a}	0.878^{b}	0.877^{b}	0.876^{b}	0.877^{a}
GA3 (30 ppm)	0.817^{b}	0.938^{a}	0.809^{b}	0.855^{a}	0.807^{c}	0.975^{a}	0.856^{bc}	0.879^{a}
Chit (2g /L)	0.709^{cd}	0.631^{e}	0.667^{de}	0.669^{c}	0.734^{de}	0.609^{g}	0.659^{fg}	0.667^{c}
Chit (4g /L)	0.810^{b}	0.687^{cde}	0.744^{c}	0.747^{b}	0.778^{d}	0.639^{g}	0.702^{ef}	0.706^{b}
Mean	0.799^{a}	0.778^{ab}	0.769^{b}		0.799^{a}	0.775^{a}	0.776^{a}	
				TSS/Acid				
Control	7.87^{e}	7.88^{e}	7.88^{e}	7.88^{d}	8.26^{de}	8.26^{de}	8.25^{de}	8.26^{b}
GA$_3$ (30 ppm)	9.39^{d}	7.02^{e}	9.69^{cd}	8.70^{c}	9.39^{d}	7.27^{e}	9.24^{d}	8.63^{b}
Chit (2g /L)	12.45^{b}	14.84^{a}	13.24^{b}	13.51^{a}	12.27^{bc}	15.90^{a}	14.41^{ab}	14.19^{a}
Chit (4g /L)	9.36^{d}	12.85^{b}	10.52^{c}	10.91^{b}	11.25^{c}	15.26^{a}	13.54^{b}	13.35^{a}
Mean	9.77^{b}	10.65^{a}	10.33^{a}		10.29^{b}	11.67^{a}	11.36^{a}	
				V.c content (mg/100ml fruit juice)				
Control	41.85^{e}	41.86^{e}	41.87^{e}	41.86^{c}	42.77^{c}	42.78^{c}	42.79^{c}	42.78^{b}
GA$_3$ (30 ppm)	38.14^{g}	44.02^{d}	39.67^{fg}	40.61^{d}	38.90^{d}	44.48^{c}	39.94^{d}	41.11^{b}
Chit (2g /L)	50.67^{b}	48.62^{c}	52.95^{a}	50.75^{a}	51.45^{ab}	49.59^{b}	53.78^{a}	51.61^{a}
Chit (4g /L)	48.44^{f}	44.16^{d}	41.48^{ef}	44.69^{b}	48.83^{b}	45.10^{c}	43.85^{c}	45.93^{b}
Mean	44.78^{a}	44.67^{a}	43.99^{a}		45.49^{a}	45.49^{a}	45.09^{a}	

TSS: acidity ratio

As for results of TSS: acid ratio displayed in Table (5), it could be inferred that there was a similar trend of TSS%; chitosan treatments significantly maximized the values of TSS: acid with pronounced difference between 2 and 4g/ L in 2016 only, followed by GA$_3$ and control, while the lowest values were obtained from control in the first year only. No statistical differences were observed among the three dates of spraying except that of treatments applied on mid- - January which recorded the lowest TSS: acid ratios as compared with the other two dates in both seasons. Spraying chitosan at 2g/L and 4g/L during mid- April reflected the highest significant values in both the study years and 2017 only, respectively, beside the lowest ratio attained from control in both season. These results were partially agreed with Hikal (2013), Hifny *et al*. (2017) and Hassanain *et al*. (2018) they showed that TSS: acid did not respond to GA$_3$ foliar application as compared to control. In addition Abd El-Rahman *et al*. (2012) and Baghdady *et al*. (2014) shared relatively the present findings, where they showed that spraying with GA$_3$ significantly reduced the ratio of TSS: acid comparing with control.

Vitamin C content

Data in Table (5) showed that the maximum values of ascorbic acid (V.C mg/100g of fruit juice) were recorded when the trees were sprayed with chitosan at 2g/L in comparison to other treatments including control in both seasons, while chitosan at 4g/L follows, then water spraycontrol and GA$_3$ with significant differences among them in 2016 only. No appreciable effect of spraying date was found on this attribute in both study years. As for the interaction between spraying treatment and its date, V.C content appeared a significant response when spraying chitosan 2g / L twice during mid- January and April in both seasons, as well as the same treatment on mid- January only in 2017 season in comparing to the rest of treatments including control. The present results completely correspond to those published by Zagzog *et al*. (2017) who stated that fruits content of V.C was statistically superior as a result of foliar chitosan application at lower concentration (25 ml/L) compared to chitosan at higher one (5ml/L) and water spray control. Likewise, the results are partially in line with Abd El-moneim *et al*. (2007), Hifny *et al*. (2017) and Hassanain *et al*. (2018), they all showed that V.C did not appear appreciable improvement by GA_3 spraying, whereas in some cases GA_3 caused a significant decrease in vitamin c as compared to water spray as control according to Saleem *et al.* (2007). The findings of Hikal (2013) and Baghdady *et al*. (2014) disagreed with the current results, where they mentioned that GA_3 spraying positively affected V.C in comparison with control.

Conclusion

From the obtained results, it could be deduced that all spraying treatments improved all the tested attributes in comparison with water spray control; GA_3 spraying once either during mid- April or mid- January only increased vegetative growth parameters and leaf C/N ratio or leaf DM%, respectively. Foliar chitosan applications with two doses of 2 and 4g/L had more impacts on leafy inflorescence, fruit set %, yield (kg/m3), as well as leaf pigments (chl.a, chl.b and its total) and carotenoids, when spraying during both mid- January and mid- April. Moreover, chitosan at 2g/L during the same previous time participated in improving physical and chemical fruit properties.

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