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*Full Length Research Paper*

# Impact of Pre-Harvest Chitosan Foliar Application on Washington Navel Orange Tree Growth and Chemical Composition Across Diverse Environmental Conditions

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**The present study was carried out during 2012 and 2013 seasons to study the effect of pre-harvest foliar application of chitosan (a natural beta-1-4-linked glucosamine polymer) at two concentrations 250 and 500 ppm on vegetative tree growth, fruit yield and quality as well as leaves chemical composition of Washington navel orange trees grown under two locations. As for growth parameters (shoot length, leaves number, and leaves area), the results revealed that chitosan treatments had insignificant effect. Meanwhile, it had a significant improvement on most of the studied fruit characters and leaf chemical constituents, that is, pigments, sugars, total soluble phenols, total free amino acids, endogenous plant hormones "IAA, ABA and $GA_3$" as well as leaf nutritional status "N, P, K, Zn, Ca, B and Si". Generally, pre-harvest chitosan applications mostly had pronounced positive effects on improving navel orange quality, that is, fruit weight, firmness and T.S.S.%, especially at the rate 500 ppm.**

**Key words:** Citrus, chitosan, growth characters, fruit quality, total chlorophyll, sugar, total soluble sugar (TSS).

## INTRODUCTION

Citrus is the most economically important fruit crop in the world. It is considered as one of the main sources of Vitamin C., carotenoids and an extensive array of secondary compounds with pivotal nutritional properties such as "vitamin E, pro-vitamin A, flavonoids, limonoids, polysaccharides, lignin, fibers, phenolic compounds and essential oils (Iglesias et al., 2007). Navel orange is a popular fresh fruit for (i) its seedless fruits, flavor and aroma, and (ii) yield are in important source of early season income for citrus growers at some commercial citrus areas of the world (Wardowski et al., 1985).

Trees production is erratic and usually low in some

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**Table 1.** Physical and chemical analysis of El-Qalubia and El-Sadat orchards soil.

| Physical properties | El- Qalubia | El-Sadat |
|---|---|---|
| Sand (%) | 19.39 | 85 |
| Clay (%) | 63.64 | 5 |
| Silt (%) | 16.97 | 10 |
| Texture | Clay loam | Loamy sand |
| Chemical properties | | |
| pH (Extract 1/2.5 $H_2O$) | 8.10 | 7.74 |
| EC 20°C ($dsm^{-1}$) | 0.29 | 0.305 |
| Available elements (mg/kg) | | |
| N | 538.4 | 60 |
| P | 22 | 43.68 |
| K | 278 | 1.2 |
| Ca | 5 | 32.4 |
| Mg | 3.5 | 6.8 |
| Na | 38.8 | 8.0 |
| Zn | 39 | 8.26 |
| Mn | 7 | 19.93 |
| Fe | 9.2 | 68.93 |
| Cu | 5 | < 2.50 |

regions; these may be due: (i) to lack functional pollens; (ii) rarely produce viable ovules and (iii) weakly parthenocarpic (Krezdorn, 1965). Moreover, flowers and fruits drop of navel orange occurred at three phases (Villafane et al., 1989).

Chitosan is a polysaccharide resulting from the deacetylation of chitin, the linear polymer of (1-4)-β-linked N-acetyl-D-glucosamine. It is obtained from the outer shell of crustaceans such as crabs and shrimps (Ruiz-García and Gómez-Plaza, 2013; Sandford and Hutchings, 1987; Sandford, 1989). Chitin and chitosan are polysaccharides, chemically similar to cellulose differing only by the presence or absence of nitrogen (Freepons, 1991). The positive charge of chitosan confers to this polymer numerous and unique physiological and biological properties with great potential in a wide range of industries such as cosmetology (lotions, hair additives, facial and body creams) (Lang and Clausen, 1989), food (coating, preservative, antioxidant, antimicrobial) (Sapers, 1992; Pennisi, 1992; Fang et al., 1994; Roller and Covill, 1999; Benjakul et al., 2000; Shahidi et al., 2001),
biotechnology (chelator, emulsifier and flocculent) (Hirano, 1989; Sand-ford, 1989) pharmacology and medicine (fibers, fabrics, drugs, membranes and artificial organs) (Muzarelli, 1989; Kulpinsky et al., 1997; Nishimura, 1997; Liu et al., 2001) and agriculture (soil modifier, films, fungicide, elicitor) (Hoagland and Parris, 1996; Lafontaine and Benhamou, 1996; Makino and Hirata, 1997; Ren et al., 2001).

Chitosan has been widely used for stimulation of plant defense (Bautista-Baños et al., 2003). Chitosan oligomers enter most regions of the cell, and subsequently induced changes in: Cell membranes, chromatin, DNA, calcium, MAP kinase, oxidative burst, reactive oxygen species (ROS), pathogenesis related (PR) genes/proteins, and phytoalexins (Hadwiger, 2013). Pre-harvest chitosan applications have been noted to be effective in controlling postharvest fungal infection in strawberries (Reddy et al., 2000). Moreover, plants treated with chitosan may be less prone to stress evoked by un-favorable conditions, such as drought, salinity and low or high temperature (Lizarraga-Pauli et al., 2011; Jabeen and Ahmad, 2013).

Therefore, this experiment was conducted to investigate the effect of pre-harvest foliar spray of chitosan (250 and 500 ppm) on tree growth and leaves composition as well as fruit-quality and production of navel orange grown in two different regions.

## MATERIALS AND METHODS

The present study was carried out during the two successive seasons (2012 and 2013) at two private citrus orchards; (I) Kalube centr El-Qalyobia Governorate, Egypt. Washington navel orange trees (*Citrus sinensis* lin, Osbek) 40 years- old budded on sour orange rootstock (*Citrus aurantium*) grown on clay loam soil at 5 × 5 m. (II) Cairo-Alex. desert road "El-Sadat City region-El-Monofia Governorate, Egypt. The trees were about 11 years - old budded on Sour orange rootstock (*citrus aurantium*) grown in reclaimed soil at 4 × 6 m. Soil samples were collected from the two orchards at depths (0-30 cm), physical and chemical properties (Table 1) were

**Table 2.** Chemical analysis of irrigation water of El-Qalubia and El-Sadat regions.

| Chemical analysis | El-Qalubia | El-Sadat |
|---|---|---|
| EC mmhos/cm at 25°C | 288 | 541.64 |
| pH | 7.10 | 7.87 |
| **Soluble ions (meq/l)** | | |
| Cations $Ca^{+2}$ | 1.10 | 1.75 |
| $Mg^{+2}$ | 0.80 | 1.10 |
| $Na^{+}$ | 2.50 | 2.27 |
| $K^{+}$ | 0.10 | 0.14 |
| Anions $Cl^{-}$ | 3.80 | 1.76 |
| $SO_4^{-2}$ | 0.30 | 0.47 |

analyzed according to Piper (1950). The 1st orchard, trees were under basin irrigation system and received about 5000 to 6000 $m^3$ of irrigated water/fed/year. While the 2nd orchard, trees were under drip-irrigation system and received about 3500 to 4000 $m^3$ of irrigated water/fed/year. In both orchards, chemical composition of used water, that is, pH, EC, $Ca^{+2}$ and $Mg^{+2}$, $Cl^{-}$ and $SO_4^{-2}$ concentrations were determined (Table 2).

Environmental factors such as air temperature (°C) (max. and min.), relative humidity (R.H. %) and evapotranspiration rate (mm.) were collected and analyzed (Table 3) for the two regions beside the El-Nubaria region "which consider the best area for citrus production in Egypt" as a control. Fertilization and pests control programs for the two regions were applied as recommended from the Ministry of Agriculture, Egypt. In the two experimental seasons, three pre-harvest foliar treatments were used as follows:

1. Control treatment sprayed with 0.5% acetic acid.
2. Chitosan foliar treatment at the rate of 250 ppm dissolved in acetic acid (0.5 %) according to (Bautista-Baños et al., 2006).
3. Chitosan foliar treatment at the rate of 500 ppm dissolved in acetic acid (0.5 %) according to (Meng et al., 2010).

Pre-harvest foliar spray were applied twice: at one month before the beginning of fruit color break (the 1st week of September) and the 2nd at one month before harvest (the 3rd week of November).

A complete randomized block design was used. Each treatment was replicated three times with one tree for each replicate.

1. Tree growth parameters: At September for new developed twigs of spring cycle; the following growth characters were tabulated:

a. Twig length (cm)
b. Number of leaves/ twig
c. Leaf area ($cm^2$) which estimated by leaf area meter (model CL-203 area meter CID, Inc., USA).
d. Flowering and fruit characters.
e. The total number of flowers.
f. Fruit set percentage (%) = (Number of fruits/ Total number of flowers) × 100
g. Number of fruits/ tree
h. Fruit drop (%) = (Total number of fruits at petal-fall stage – number of fruits in late July) / Total number of fruits ×100

**Leaves chemical constituents**

1. Leaf pigment contents: Sample of fresh leaves at the 1st of September were extracted with dimethyl formamide to determine chlorophylls a, b and carotenoids concentrations according to Moran (1982) formula.

Ethanol extract of leaves was used for the determination of total sugar (Dubois et al., 1956), total free amino acids (Moore and Stein, 1954) and total soluble phenols concentrations (Swain and Hillis, 1959).

For hormones analysis, leaves of navel orange were extracted twice, each 3 h, with 80% methanol and again twice with 40% methanol, each 2 h (Sadeghian, 1971). The aqueous fraction was adjusted to pH 2.6 by the addition of 1 N HCl and was partitioned three times with ethyl acetate. Gibberellic acid ($GA_3$), indole-3-acetic acid (IAA) and abscisic acid (ABA) were measured using HPLC according to the method described by Müller and Hilgenberg (1986).

2. Leaf mineral contents: Digestion of plant materials were carried out using sulphuric and per-chloric acids as described by Piper (1950).
3. Nitrogen (%) was determined by the micro-kjeldahl as described by Schouwenburg and Walinga (1978).
4. Phosphorus (P %) was determined colorimeterically as described by King (1951).
5. Potassium (K %) was determined by using flame photometer (Corning 410).
6. Calcium (%), zinc (ppm) and boron (ppm) were determined by using atomic absorption spectrophotometer (Thermo-Jarrellash, AASCANI).
7. Silicon (Si %) was determined according to Schuffelen et al. (1961).
8. Yield, fruit physical and chemical characters: At the end of November, yield of each tree as Kg and number of fruits / tree were estimated as well as the following fruit physical characters were taken as follows:

a. Fruit weight (g)
b. Fruit size ($cm^3$): it was measured by water displacement in graduate jar.
c. Fruit shape index: Fruit length and diameter (cm) were measured by a Vernier caliper and fruit shape index (length/ diameter ratio) was calculated.
d. Fruit firmness: Fruit firmness of the skin was recorded by LFRA texture analyzer instrument model TS-091000 stainless stell needle, using penetrating cylinder of 1 mm of diameter to a constant distance 5 cm inside the skin to the flesh by a constant speed 2 mm/s. The results were expressed as the resistance force to the penetrating tester in fruits of pressure g/$cm^2$ (Harold, 1985).

5. Fruit juice %.
6. Peel thickness (mm): it was were measured by a Vernier caliper.
7. Fruit chemical properties: that is, T.S.S. %, titratable acidity (mg of citric acid/100 ml juice). Vitamin C (mg/100 ml juice).

**Statistical analysis**

A complete randomized block design was used. The obtained data were subjected to the analysis of variance according to Snedecor and Cochran (1972). Differences between treatments means were compared using the L.S.D. at 0.05 level.

## RESULTS AND DISCUSSION

### Vegetative growth

Data in (Table 4) indicated that spraying of both chitosan

**Table 3.** Air temperature (°C), relative humidity (%) and evapotranspiration (mm) in El-Qalubia, El- Sadat and El-Nubaria regions during season 2013.

| Location | Month | Air temperature (°C) | | | Relative humidity (%) | Evapo-transpiration (mm) |
|---|---|---|---|---|---|---|
| | | Max. | Min. | Average | Average | Average |
| El-Qalubia | January | 15.7 | 4.7 | 10.2 | 56 | 1.1 |
| | February | 18.9 | 9.9 | 14.4 | 55 | 1.5 |
| | March | 20.7 | 15.2 | 17.95 | 57 | 2.2 |
| | April | 25 | 16.8 | 20.9 | 49 | 3.0 |
| | May | 32.1 | 19.5 | 25.8 | 49 | 3.6 |
| | June | 35.9 | 22.7 | 29.3 | 52 | 3.8 |
| | July | 39.8 | 25.8 | 32.8 | 55 | 4.2 |
| | August | 40.9 | 26.9 | 33.9 | 55 | 3.4 |
| | September | 33.3 | 23.2 | 28.25 | 53 | 2.5 |
| | October | 25.9 | 15.9 | 20.9 | 60 | 1.8 |
| | November | 20.2 | 12.8 | 16.5 | 72 | 1.3 |
| | December | 17.5 | 7.2 | 12.35 | 74 | 1.0 |
| El-Sadat | January | 11.5 | 1.9 | 6.7 | 57 | 0.1 |
| | February | 13.8 | 3.8 | 8.8 | 59 | 1.3 |
| | March | 18.9 | 9.5 | 14.2 | 58 | 3.7 |
| | April | 38.5 | 11.8 | 25.2 | 46 | 6.9 |
| | May | 37.6 | 25.2 | 31.4 | 47 | 8.0 |
| | June | 40.4 | 26.6 | 33.5 | 48 | 8.4 |
| | July | 45.3 | 28.9 | 37.1 | 54 | 8.1 |
| | August | 47.7 | 28.7 | 38.2 | 50 | 8.4 |
| | September | 38.9 | 22.8 | 30.9 | 54 | 6.2 |
| | October | 35.7 | 10.1 | 22.9 | 65 | 4.0 |
| | November | 18.6 | 8.7 | 13.7 | 77 | 2.2 |
| | December | 12.6 | 3.1 | 7.9 | 79 | 1.5 |
| EL-Nubaria (Behera) | January | 17.5 | 9.6 | 11.8 | 81 | 1.5 |
| | February | 19.9 | 9.2 | 13.5 | 75 | 2.3 |
| | March | 23.4 | 12.0 | 16.6 | 69 | 3.6 |
| | April | 24.1 | 13.0 | 18.3 | 69 | 4.4 |
| | May | 28.4 | 17.8 | 23.4 | 69 | 5.0 |
| | June | 29.8 | 20.5 | 24.9 | 70 | 5.4 |
| | July | 29.5 | 22.3 | 27.7 | 75 | 5.8 |
| | August | 30.8 | 23.1 | 30.2 | 80 | 6.1 |
| | September | 29.3 | 21.0 | 22.5 | 75 | 5.6 |
| | October | 26.0 | 17.4 | 15.0 | 72 | 2.5 |
| | November | 24.4 | 15.5 | 14.5 | 69 | 2.2 |
| | December | 19.0 | 10.4 | 12.8 | 70 | 2.0 |

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concentrations had non-significant effect on most of the studied growth characters in the two successive seasons as well as in the two different orchards. This result was obtained also by El Hadrami et al. (2010), who found that foliar application of chitosan did not affect maize or soybean height, leaf area and total dry mass.

On the other hand, a contradict results were obtained by Mahdavi (2013) who mentioned that length and weight of roots and shoots were increased in Isabgol (*Plantago ovata* Forsk) plants pretreated with chitosan under salt stress. Also, El-Miniawy et al. (2013) working on strawberry plants (*Fragaria x ananassa* Duch.) revealed that all tested foliar applications of chitosan increased all vegetative growth characteristics.

In this respect, Bittelli et al. (2001) suggested that chitosan might be an effective as anti-transpiring to

**Table 4.** Effect of pre-harvest chitosan spray on growth characters of Navel orange tree grown in El-Qalubia and El-Sadat orchards in 2012 and 2013 seasons.

| Location | Chitosan | El-Qalubia | | El-Sadat | |
|---|---|---|---|---|---|
| **Growth character** | **Conc. (ppm** | **2012** | **2013** | **2012** | **2013** |
| Twig length (cm | Control (0.0) | 5.08 | 8.60 | 9.10 | 7.63 |
| | 250 | 5.27 | 9.25 | 9.17 | 7.90 |
| | 500 | 5.50 | 9.67 | 9.07 | 8.00 |
| | L.S.D. 0.05 | n.s. | 0.03 | n.s | n.s. |
| Number of leaves / twig | Control (0.0) | 5.12 | 6.90 | 6.13 | 5.37 |
| | 250 | 5.48 | 7.02 | 6.23 | 6.02 |
| | 500 | 5.42 | 7.17 | 6.51 | 6.05 |
| | L.S.D. 0.05 | n.s. | n.s. | n.s. | 0.92 |
| Average leaf area ($cm^2$) | Control (0.0) | 6.62 | 11.06 | 5.91 | 10.18 |
| | 250 | 6.95 | 11.67 | 6.20 | 10.52 |
| | 500 | 7.72 | 12.18 | 6.40 | 10.87 |
| | L.S.D. 0.05 | n.s. | 0.08 | 1.74 | 0.36 |

preserve water resources used in agriculture.

## Flowering and fruit set

From the obtained results in Figure 1, it could be noticed that increase of total number of flowers /tree over control was non-significant in the first season but was significant in the second one as sprayed with both concentrations of chitosan in El-Qalubia region. In El-Sadat orchard, there was a significant effect at the first season but was not in the second one.

In this concern, Ohta et al. (1999) found that flower number of *Eustoma grandiflorum* was greatest in plants grown in chitosan treated. A stimulating effect of chitosan on the number of flowers was observed in plants such as gerbera (Wanichpongpan et al., 2001) and gladioli (Ramos-Garcia et al., 2009). Salachna and Zawadzińska (2014) working on 'Gompey' freesia, reported that the chitosan-treated plants (0.5%) had more leaves and flowered earlier as well as had higher relative chlorophyll content.

Concerning fruit set%, it was found non-significant effect of chitosan in the first season and significant one in El-Qalubia orchard in the second season. Meanwhile, a significant increase in fruit set % was found with the increase concentration of chitosan as compared with control for the two successive seasons in El-Sadat orchard (Figure 1).

In this concern, Ghoname et al. (2010) observed that foliar application of chitosan on sweet pepper significantly increased the number of fruits per plant and the mean weight of fruit, as well as fruit quality characteristics.

Regarding the effect of chitosan on the drop of navel orange fruits %, it was found significant decrease in the second one with either chitosan concentration in both orchards (Figure 1).

In this respect, it could suggest that chitosan might alter the hormonal balance in ways that are in harmony with observed decreases in fruit abscission. However, the data of fruit yield showed a non-significant effect under foliar application of both chitosan treatments as compared with non-sprayed control trees in both regions (Figure 1).

## Leaf chemical constituents

### *Leaf pigments*

Data concerning chlorophyll a, b and total chlorophyll as well as total carotenoids of navel orange leaves in both orchards indicated that total chlorophyll, especially chl. a showed a significant increase by chitosan application as compared to the control in both gardens, especially the higher chitosan concentration. On the contrary, the total carotenoids concentrations were decreased in leaves of both orchards (Table 5).

These results are consistent with El-Tantawy (2009) reported that application of chitosan on tomato plant increased photosynthetic pigments thereby the net photosynthesis increased. Again, Mondal et al. (2012) reported that chlorophyll content was increased in leaves of chitosan applied okra plants (100 ppm) than control.

On the other hand, a reverse trend was detected by El-Miniawy et al. (2013) who reported that there was no significant effect for the chitosan treatments on leaf of strawberry chlorophyll content. Therefore, it could suggest that exogenous chitosan might alleviate abiotic stresses between both regions by increment chlorophyll

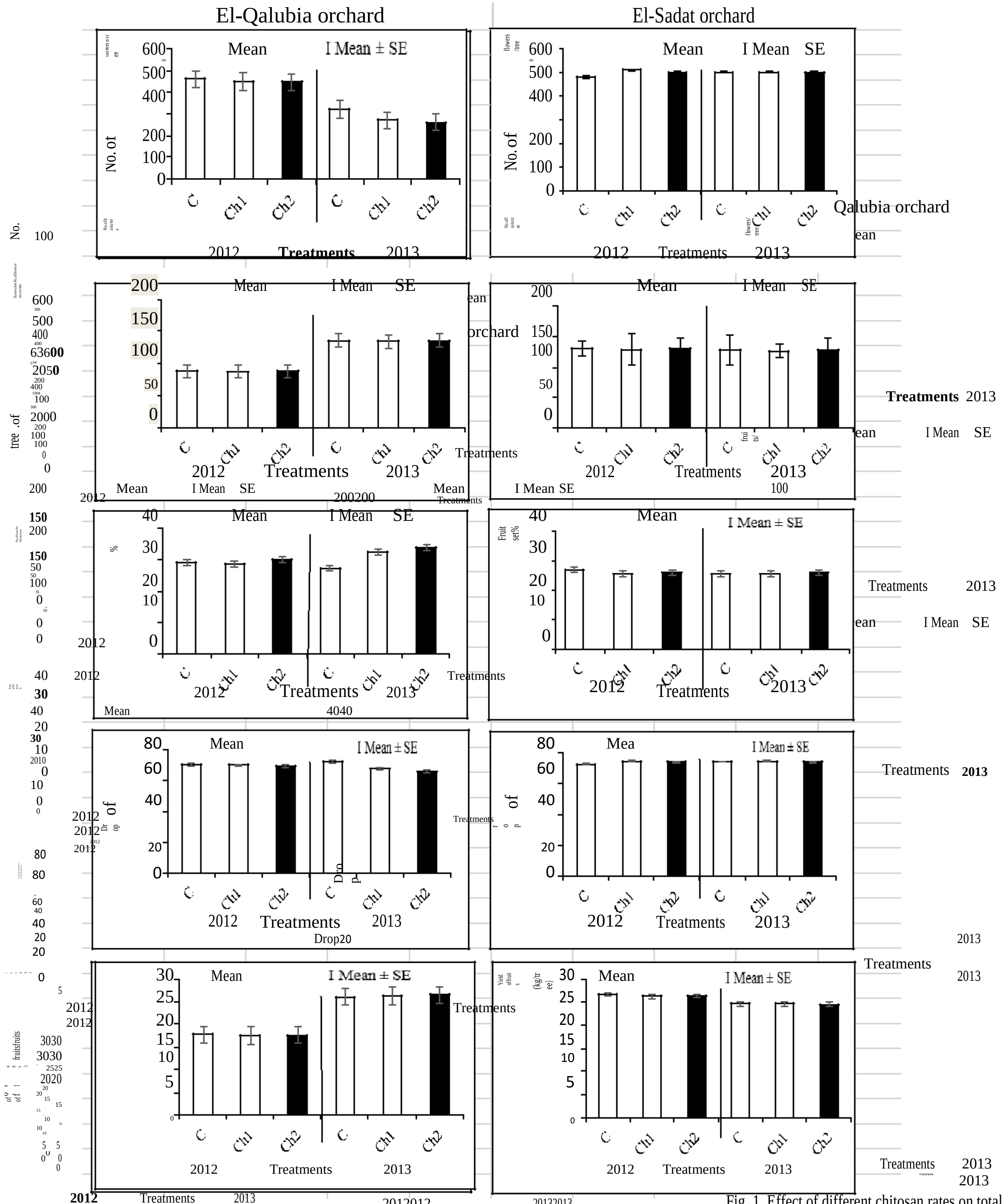


Fig. 1. Effect of different chitosan rates on total no. of flow

**Figure 1.** Effect of different chitosan rate on total no. of flower and no. of fruit per tree, fruit andset%,yield(kg/tree)atfruit El-Qalubia and El-Sadat orchard

. Effect of differenterent chitosanrates on total no. of flowers and no. of fruits per tree, fruit se %, fruit drop%

[ control , chitosan (250 ppm) and

drop and yield (kg/tree)

(data are the mean ± standard error of nine replicates

[ control , ,chitosan(250 ppm) andchitosan(500(500ppm)ppm) ] ]

standard error of nine

(data are thereplicates)meann±±standard errorofofnininereplicates)

**Table 5.** Effect of both pre-harvest chitosan rates on plant pigments (chl. a, chl. b, total chls. and total carotenoids) concentrations (mg/g f.w.) in navel orange leaves of El-Qalubia and El-Sadat orchards during 2013 season.

| Location | Chitosan Conc. (ppm) | Plant pigment (mg/gf.w.) | | | |
|---|---|---|---|---|---|
| | | Chlorophyll a | Chlorophyll b | Total chlorophylls | Total carotenoids |
| El-Qalubia | Control (0.0) | 1.25 | 0.48 | 1.73 | 0.35 |
| | 250 | 1.45 | 0.50 | 1.95 | 0.22 |
| | 500 | 1.60 | 0.58 | 2.18 | 0.30 |
| | L.S.D. 0.05 | 0.12 | 0.07 | 0.20 | 0.02 |
| El-Sadat | Control (0.0) | 1.11 | 0.41 | 1.52 | 0.51 |
| | 250 | 1.68 | 0.58 | 2.26 | 0.20 |
| | 500 | 1.62 | 0.75 | 2.37 | 0.18 |
| | L.S.D. 0.05 | 0.13 | 0.07 | 0.19 | 0.08 |

**Table 6.** Effect of both pre-harvest chitosan rates on total sugar, total free amino acids, total soluble phenols (mg/g f. wt.) and plant hormones concentrations ($GA_3$, ABA and IAA) as µg/100 g f. wt. in navel orange leaves of El-Sadat and El-Qalubia orchards during 2013.

| Location | Chitosan conc. (ppm) | Total sugar (mg/g f. wt.) | Total free amino acids (mg/g f. wt.) | Total soluble phenols (mg/g f. wt.) | Plant hormone ( µg/100 g f. wt.) | | |
|---|---|---|---|---|---|---|---|
| | | | | | $GA_3$ | ABA | IAA |
| El-Qalubia | Control (0.0) | 3.68 | 1.88 | 1.95 | 4.06 | 2.68 | 1.01 |
| | 250 | 3.78 | 2.20 | 1.88 | 6.15 | 3.17 | 1.20 |
| | 500 | 4.06 | 2.74 | 2.05 | 7.08 | 4.01 | 1.40 |
| | L.S.D. 0.05 | 0.06 | 0.11 | 0.06 | - | - | - |
| El-Sadat | Control (0.0) | 4.53 | 2.29 | 2.02 | 4.57 | 3.24 | 1.22 |
| | 250 | 4.25 | 2.87 | 1.90 | 5.05 | 3.65 | 1.56 |
| | 500 | 5.30 | 4.18 | 2.17 | 7.85 | 4.60 | 1.82 |
| | L.S.D. 0.05 | 0.22 | 0.20 | 0.06 | - | - | - |

concentration, decreasing the stomatal and non-stomatal transpiration as well as improve water use efficiency.

### *Organic components*

The data in Table 6 revealed that there were significant increases in total sugar, total free amino acids and total soluble phenols concentrations in leaves of navel orange trees sprayed by chitosan, especially at higher concentration in both orchards as compared with control trees.

These results are in harmony with No et al. (2003) who reported that application of chitosan increased carbohydrates in soybean leaves. Cai et al. (2014) reported that chitosan enhanced the production of phenolic acids by 1.5 to 2.0-folds after 3 days of cell suspension cultures of *Malus* × *domestica* Borkh. El-Miniawy et al. (2013) reported that total carbohydrates of strawberry were increased as a result of chitosan spraying. Also, Mathew and Sankar (2014) mentioned that chitosan foliar application increased phenolic compounds as well as antioxidant activity in plants.

It appears that chitosan increased the concentration of simple organic molecules such as, sugar, free amino acids and total soluble phenols, playing a role in regulation of plant osmosis and consequently better plant growth and yield under un-favorable environmental conditions recorded in both orchards locations (Table 3). Furthermore, chitosan might play an important role in scavenging the free radicals thus lead to mitigate the adverse impact of stress and improve growth, productivity and quality of plants.

Earlier reports showed that chitosan triggering highest total phenolic content in cell cultures (Chakraborty et al., 2009); low concentration of chitosan (50 mg/l) was found to trigger the highest secondary metabolite content in O. *gratissimum* (Mathew and Sankar, 2014).

Application of chitosan to soybean leaf tissues have been reported to cause an increase activity of pheny-lalanine ammonia lyase (PAL) and tyrosine ammonia lyase (TAL); the key enzymes of phenylpropanoid

**Table 7.** Effect of different pre-harvest chitosan rates on nutrients concentrations in navel orange leaves in El-Qalubia and El-Sadat orchards during season 2013.

| Location | Chitosan conc. (ppm) | N% | P% | K% | Ca% | Zn (ppm) | B (ppm) | Si (mg/gd.wt.) |
|---|---|---|---|---|---|---|---|---|
| El-Qalubia | Control (0.0) | 2.03 | 0.95 | 1.20 | 0.80 | 25.21 | 17.45 | 16.62 |
| | 250 | 2.13 | 1.04 | 1.96 | 0.97 | 37.15 | 10.72 | 18.05 |
| | 500 | 2.30 | 1.14 | 2.0 | 1.08 | 40.00 | 12.06 | 19.77 |
| | $LSD_{0.05}$ | 0.23 | 0.02 | 0.19 | 0.16 | 1.69 | 0.81 | 0.97 |
| El-Sadat | Control (0.0) | 2.27 | 1.07 | 1.67 | 0.70 | 12.30 | 10.46 | 13.00 |
| | 250 | 2.75 | 1.39 | 2.13 | 0.97 | 11.80 | 9.62 | 16.45 |
| | 500 | 2.89 | 1.46 | 2.97 | 1.10 | 12.25 | 10.05 | 17.06 |
| | $LSD_{0.05}$ | 0.15 | 0.10 | 0.23 | 0.10 | 0.24 | 0.32 | 0.63 |

pathway (Khan et al., 2003). The products of PAL and TAL are modified through phenylpropanoid metabolism to precursors of secondary metabolites including lignin, flavonoid pigments, and phytoalexins, all of which play key roles in a range of plant-pathogen interactions (Morrison and Buxton, 1993).

The results of plant hormones (Table 6) showed an increase in $GA_3$, ABA and IAA concentrations with the foliar application of both concentrations of chitosan comparing with the control plants. The highest concentrations of $GA_3$ and ABA might refer to the effect of chitosan on induction of terpenoids formation; $GA_3$ and ABA are among compounds belong to terpenoids formed in plants.

In this connection, Uthairatanakij et al. (2007) mentioned that chitosan might induce a signal to synthesize plant hormones such as gibberellins as well as signaling pathways related to auxin biosynthesis.

Also, those might refer to stomatal closure which reduces transpiration and transport of solutes to the aerial-parts of the plant. Iriti et al. (2009) reported that chitosan was able to reduce transpiration in bean plants and this might refer to an increase in ABA content in the treated leaves. Increasing endogenous plant hormones (ABA, $GA_3$ and IAA) as well as osmoprotectants compounds such as sugar, free amino acids and soluble phenols might improve plant tolerance to unfavorable environmental conditions prevailing in both different regions.

**Mineral elements**

The data in Table 7 revealed significant increases in N, P, K, Ca and Si concentrations of Washington navel orange leaves with chitosan foliar application as compared to control treatment in both orchards. In El-Qalubia orchard, Zn concentration was significantly increased by both chitosan treatments, whereas it was significantly decreased in leaves grown in El-Sadat orchard. Meanwhile, a significant decrease in B concentration of navel orange leaves of both regions as compared to control tree. In this respect, Shehata et al. (2012) found that foliar spray of chitosan significantly increased N and P concentrations as well as some micro-nutrients (Fe, Zn, Cu and Mn) contents in cucumber leaves. El-Miniawy et al. (2013) mentioned that nitrogen content of strawberry leaves recorded a significant increase for the tested treatments of chitosan as compared with the control plants.

Saif Eldeen et al. (2014) illustrated that receptacle contents of N, P, total sugars % and protein % of globe artichoke were greatly affected by chitosan treatments as compared to the control. Farouk and Abd El Mohsen (2011) showed that pronounce and highly significant increase in nitrogen, phosphorous and potassium percentages in the shoot due to exogenous application of chitosan (250 mg/l).

Concerning the low B concentration detected in leaves of navel orange trees sprayed with chitosan might explain the increase in total soluble phenols, total free amino acids and auxins concentrations in leaves.

In this respect, Mengel and Kirkby (1979) pointed out that when B is present the activity of the pentose phosphate pathway is decreased in favor of glycolysis.

On the other hand, when boron is deficient the pentose phosphate pathway is favored and consequently induces the accumulation of shichemic acid metabolits; among which phenolic compounds and amino acid tryptophan which act as a precursor for auxin synthesis. Similar discussion was reported by Hanafy Ahmed et al. (2008) on wheat plants.

**Fruit physical and chemical qualities**

The data in Table 8 revealed that both chitosan treatments had a significant increase on fruit weight of navel orange grown in both regions as compared with control.

**Table 8.** Effect of both pre-harvest chitosan rates on fruit quality in El-Qalubia and El-Sadat orchard of 2013 season.

| Location | Chitosan conc. (ppm) | Fruit quality | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Fruit weight (g) | Fruit shape index | Fruit size ($cm^3$) | Fruit firmness ($g/cm^2$) | Peel thickness (mm) | Juice % | T.S. S. % | Vit.C. (mg/100 ml juice) | Acidity (mg/100 ml juice) |
| El-Qalubia | Control (0.0) | 206.2 | 1.02 | 360.0 | 179.1 | 0.35 | 60.37 | 7.89 | 53.99 | 1.08 |
| | 250 | 221.2 | 0.97 | 382.5 | 187.5 | 0.34 | 55.71 | 8.04 | 52.32 | 1.10 |
| | 500 | 218.3 | 1.00 | 396.9 | 190.7 | 0.37 | 57.40 | 8.47 | 50.28 | 0.99 |
| | L.S.D. $_{0.05}$ | 0.73 | 0.04 | 2.32 | 2.14 | 0.01 | 0.77 | 0.11 | 0.63 | n.s. |
| El-Sadat | Control (0.0) | 214.5 | 1.02 | 388.3 | 186.5 | 0.35 | 65.61 | 8.90 | 49.80 | 1.24 |
| | 250 | 226.0 | 1.00 | 390.1 | 188.7 | 0.35 | 59.41 | 9.58 | 48.10 | 1.27 |
| | 500 | 224.8 | 0.99 | 396.5 | 191.5 | 0.40 | 62.71 | 10.15 | 47.19 | 1.27 |
| | L.S.D. $_{0.05}$ | 0.63 | n.s. | 2.18 | 1.86 | 0.01 | 0.74 | 0.10 | 0.73 | n.s. |

In this concern, Reddy et al. (2000) reported that chitosan spray significantly maintained the keeping quality of strawberry fruits as compared with control.

Data concerning the fruit shape index presented in Table 8 revealed that foliar spray by chitosan showed a non-significant effect on fruit shape index in El-Sadat garden. Meanwhile, there was a significant decrease in fruit shape index with 250 ppm chitosan and control in El-Qalubia garden. Saif Eldeen et al. (2014) showed that foliar spraying with chitosan was responsible for significant improvement on head quality of Globe artichoke.

The results in Table 8 indicated that foliar application of chitosan at 500 ppm produced the highest significant increase in fruit size as compared to the other treatments in the both locations.

This result was in agreement with those reported by Mondal et al. (2012) who revealed that okra fruit size was increased with increasing chitosan concentration until 25 ppm.

Pre-harvest spray of chitosan showed a significant increase in fruit firmness with increasing the concentration of chitosan (Table 8).

The beneficial effect of the elevated chitosan concentration on firmness has been reported for peach, Japanese pear, Kiwifruit (Du et al., 1997). Reddy et al. (2000) indicated that fruits from chitosan sprayed strawberry fruits were firmer and ripened at a slower rate as indicated by anthocyanin content and titratable acidity.

On the other hand, El-Miniawy et al. (2013) revealed that chitosan spraying did not affect strawberry fruit firmness.

The results in Table 8 indicated that the pre-harvest spray of chitosan showed a significant increase in fruit peel thickness, and this increase was enhanced with increasing the concentration of chitosan. This was accompanied by a significant decrease in fruit juice %.

Concerning, the effects of pre-harvest chitosan spray on T.S.S. % of navel orange fruits; it was found that the highest recorded values were obtained by chitosan at concentration 500 ppm in both regions.

This result was consistent with Saif Eldeen et al. (2014) who showed that foliar spraying with chitosan was responsible for significant improvements on total soluble solids.

Abdel-Mawgoud et al. (2010) found that T.S.S. of strawberry fruits showed a tendency to increase in response to chitosan application. However, a revere result was obtained by El-Miniawy et al. (2013) who found that there was no significant difference in fruit soluble solids content between chitosan spray and control.

As for the effect of the foliar application of chitosan on the total acidity, there was no significant effect among treatments on orange fruits in both orchards as shown in Table 8.

## Conclusions

Generally, it could suggest that the significant increase in fruit quality obtained by chitosan foliar applications might be attributed to its roles on improving water retention, nutrients uptake and increasing osmoprotectants; sugars, total free amino acids, total soluble phenols as well as enhancing plant hormones biosynthesis of citrus trees grown under unfavorable environmental conditions recorded in both regions. Finally, further studies are needed to evaluate the effect of pre-harvest chitosan application on navel orange fruits quality after harvesting under different storage temperatures.

## Conflicts of interests

The authors have not declared any conflict of interests.

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