



## Effect of Some Natural Sources for Anti-Stresses Compounds on Washington Navel Orange Tree Productivity and Fruit Quality under Mid Egypt Conditions



**Randa E. Y. Habasy**

*Citric Research Department, Horticulture Research Institute, Agricultural Research Centre, Giza, Egypt.*

**T**HIS investigation was carried out during two successive seasons (2019 & 2020), to study effect of some natural sources for Anti-stress compounds namely: Chitosan at (100 ppm), Ascorbic acid at (100 ppm), Prolin at (30 ppm) and Vitamin E at (50 ppm) as foliar applications on Washington Navel orange trees for elucidating their effects on tree vegetative growth & physiological performance, initial fruit set & retention and yield & fruit quality which grown Ehnasia restrict / "Beni- Suief" Governorate conditions. Experimental treatments were spraying at three times at the 1<sup>st</sup> weeks of : May, July & September for two seasons. Results indicated that, all the natural sources for Anti-stress compounds positively improved Washington navel orange tree (growth & physiological performance, initial fruit set & retention and yield as :a number & weight & fruit quality. Whereas, Chitosan compound has a superior effect with significant difference on the above parameters, when compared to other treatments under this study for both seasons. Moreover, anti-stresses compounds under study could be arranged Chitosan, Ascorbic acid, Prolin and Vitamin E respect., as for their effectivness.

**Keywords:** Navel orange, Anti-stress, Chitosan, Proline, Ascorbic acid, Vitamin E.

### Introduction

Citrus fruits occupy the 1<sup>st</sup> rank among economic fruit crops in Egypt. Navel oranges is one the most popular citrus fruits due to it's delicious taste, richness in Vit. C contents, organic acid and minerals. It has a significant importance not only in the local market but also for export. Beni-Suief governorate have 2166 fed. fruiting Orange Cvs., Navel orange cultivar occupied about 654 fed. "30.19 %" of total fruiting orange Area "Statistics of the Egyptian Ministry of Agriculture - Fruit Crops 2020".

Under Beni-Suief conditions, Navel oranges trees productivity in terms of both quantity and fruit quality is rather poor thus representing serious economic limitations for producers. Flowers abscission , impaired fruit- set and fruit

drop percentages are the main contributors to the poor productivity. Many biotic and a biotic stress factors are responsible for the development of these problems. Altered environmental conditions (e.g., air temperature and relative humidity) and miss applications of fertilizers in orchards over the season leads to undesirable physiological changes that ultimately diminish the ability of tree to achieve the suitable flowering and fruit setting, fruit retention, and fruit quality. In addition to, the widespread of free radicals produced through photosynthesis, respiration and other metabolism processes accompanied by destroying plant cells which ultimately have negative effect on fruit – set percentage and fruit quality.

Anti-stresses compounds are materials that are able to reduce transpiration process, protect

the molecules from oxidation. It can oxidized in favor of saving important cellular constituents, or enhance the endogenous antioxidant system. Redox reactions in the biological systems can produce free radicals which are poorly controlled resulting in an increased oxidative stress and subsequent cell damage. Free radicals initiate chain reactions the damage important molecules and end up with cells death. Fortunately, exogenous anti-stresses materials can mitigate these effects by removal of free radicals, hence terminating the deleterious cycle of oxidative chain reactions (Kumar et al., 2013). Chitosan [a derivative of N-acetyl-glucosamine, is a natural Bio-polymer combined derived by de-acetylation of chitin, a major component of the shells of crustacean sea animals (e.g., crab, shrimp and crawfish) (Sanford, 2002), it's an important anti-stress compound for it's antioxidant role (using accompanied with blocking relative oxygen species) which protecting the trees from their damage (Park et al., 2004).

Recently, using anti-stresses materials are a common agricultural practice to protect plants against deleterious effects of both, bio-tic or abiotic stresses. Vitamins are very effective anti-stress (as antioxidants) in the fight plant cell senescence and their related disorders, (Oretili, 1987). Generally, adequate anti-stresses treatments are expected to enhance cell division, promote the biosynthesis of natural plant hormones (e.g., GA<sub>3</sub> and IAA) and pigments, nutrient bioavailability, optimize Cytokinin levels, support the photosynthetic processes, enhance water uptake, and the

biosynthesis of biopolymers such as : proteins. In addition to, biosynthesis of  $\alpha$ -keto-glutaric acid (an important precursor for amino acid and protein synthesis), (Samiullah et al., 1988), (Foyer & Lelandias, 1993) and (Singh, et al., 2001).

Proline builds up in various plant tissues under various physiological and environmental conditions (Yang, et al., 1999 and Mansour, 2000), it play a pivotal role in cellular osmotic regulation and adaptation to stress-ful environmental conditions such as salinity (Aspinall and Paleg, 1981).

The present investigation examined the impact of spraying some exogenous anti-stresses from different natural sources on the Washington Navel trees terms of growth, yield, and fruit quality performance.

### Materials and Methods

This study was carried out on 26- year-old Washington Navel orange trees grafted onto sour orange rootstock and grown at the experimental orchard in Ehnasia restrict "Beni-Suief" Governorate / Egypt during (2019& 2020) successive seasons. Trees were planted at 6X6 m in well drained clay soil with surface irrigation system, Nile water was used. Fifteen uniform trees were selected for this investigation and normal horticultural practices recommended by the Ministry of Agriculture and Land Reclamation were applied. Analysis of the tested soil was conducted according to the procedures that outlined by (Wilde et al., 1985). Table (1) .

**TABLE 1. Analysis of the tested soil samples**

Constituents	Values	Constituents	Values
Sand (%)	4.4	N (%)	0.08
Silt „	23.6	P (ppm)	4.8
Clay „	72.0	K „	606.0
Texture	Clay	Mg „	5.8
PH ( 1:2.5 extract)	7.6	Fe „	4.6
Ec(1:2.5extract) (dsm)	0.77	Zn „	3.9
Total CaCO <sub>3</sub> (%)	1.78	Mn „	6.2
O.M „	1.89		

This experiment comprised five foliar treatments as follow :

The control	(spraying with water) .
Chitosan	at 100 ppm.
Ascorbic acid	at 100 „
Prolin	at 30 „
Vit. E	at 50 „

Anti-stresses under study were sprayed 3 times during / season at the 1<sup>st</sup> week of: May “Petal fall stage”, July “final fruit set” and September “mature stage”. Triton B as a wetting agent was added at 0.05%. A few drops of 0.1 N NaOH was added to Chitosan solution to facilitate its solubility. Spraying was done till run off.

*At mid of September the following parameters were determined :*

*Vegetative growth characters*

At the mid of February and on the basic directions of the tree four limbs (about ½ inch) one / direction were selected and 10 vegetative spring flushes were tagged during the 1<sup>st</sup> week of May, then, at mid of September shoot : [length & thickness] (cm) and leaf area (cm)<sup>2</sup> Were measured (according to (Ahmed and Morsy, 1999) methods .

*leaf pigments and total carbohydrates contents were extracted, determined and calculated as follows*

- Chlorophylls a&b and tot. Carotenoids as (mg / 1.0 g F.W.) : mature fresh leaves samples, representing each treatment (0.5 g) were homogenized with acetone (85% v/v) in the presence of the little amounts of NaCO<sub>3</sub> and silica quartz, then filtration “Bokhner funnel G4”. The residue was washed several times with acetone until being free from pigments. Each filtrate was made up to 250 ml and measured colorimetric at wave length 662 and 644 µm to determine both chlorophylls a and b and 440 µm for Carotenoids as B-Carotene, respectively, according to Saric et al.,(1967) and Calculated as the formula:

$$\text{Chl.a} = 12.70 A_{663} - 2.79 A_{647} = x1$$

$$\text{Chl.b} = 20.76 A_{647} - 4.62 A_{663} = x2$$

$$\text{B. Carotene} = 4.695 A_{440} - 0.268 (x1 + x2)$$

- Total Carbohydrates (g/100g DW) and tot. & reducing sugars (%) : A known weight (0.1g) of dried sample was placed in a test tube, 1N HCl acid (10 ml.) was added. The tube was sealed and placed for 6 hours in an oven at 100°C. The solution was then filtered and clarified by the leading and de-leading method using lead acetate solution (137 g/l.) and the excess of lead salt was precipitated using potassium oxalate solution. The extract was measured into a measuring flask (50 ml.). The combined filtrate

was completed to the mark with distilled water. Total Carbohydrates, tot. & reducing sugars (%) were determined according to the method of Dubois et al. (1956).

- Leaf minerals contents: at mid of September for both studied seasons mature leaves samples were taken from vegetative spring shoots for (N, P, K and Mg) % determination as follows:
- Nitrogen (N %): Leaf N content (g./100g D. wt) was determined in the digested solution by the modified micro-kjeldahl method as described by Plummer (1971).
- Phosphorus (P %): Leaf P content was measured calorimetrically, using the molybdenum blue method by using Beckman Du 7400 spectrophotometer according to Murphy and Riley (1962).
- Potassium (K %): Leaf potassium contents (g/100g D. wt) were determined against a standard using flame-photometer (JENWAY – pfp7 Flame Photometer) according to Piper, (1950).
- Magnesium: (Mg %): were determined in plant digest by titration with the verse Nate solution according to Richards (1954).
- leaf Proline content: was extracted and calculated according (Bates et al., 1973).

*Initial & fruit set (%) and tree yield:*

- Initial & fruit set (%): At the 1<sup>st</sup> week of March, 4 mature branches (about ½ Inch in thickness) were tagged at the original directions of the Earth / tree. At full-bloom stage, flowers / branch were counted and recorded. Then, after flowers petal-fall (at mid of May” the average of the number of small fruits was recorded. Initial fruit set % was determined by formula:

$$\text{Initial Fruit set \%} = \frac{\text{Total No. of Fruit lets}}{\text{Total No. Flowers}} \times 100$$

At the 4<sup>th</sup> week of June total number of fruits were recorded and fruit set % were calculated as the formula:

$$\text{Final fruit set \%} = \frac{\text{Total No. of fruits retained}}{\text{Total No. Flowers}} \times 100.$$

- Tree Yield: expressed as number and weight (kg) / tree as follow : At harvest stage of Navel orange under Beni- Suef Governorate conditions (mid of December for both seasons, the number of fruits / replicate were counted and weight ( kg)

#### *Fruit Physical & chemical characteristics:*

- Fruit Physical parameters: at harvest stage 10 fruits / replicate were randomly picked, washed and air dried for about one hour. Then , weighed (g.) , fruit height and diameter were measured and shape index as fruit height / diameter Ratio was calculated , by using digital Pacolez peel thickness (cm) was measured , peel weight % (w/w) according to (A.O.A.C.1995)
- Fruit Chemical characteristics as: Juice TSS % , total and reducing sugars %, total acidity % ( as g . citric acid/ 100 ml juice) and Vit. C (as mg/ 100 ml juice) were determined and recorded according to (Lane &Eynon, 1965) and (A.O.A.C., 2000).
- Experimental design: Randomized complete block design (RCBD) was used, Each treatment was replicated three times(one tree per replicate) .

#### *Statistical analysis*

The obtained data were subjected to analysis of variance (ANOVA) according to (Snedecor and Cochran, 1990). M. Static program and Duncan test were used to compare between means of

treatments according to (Waller and Duncan, 1969) at probability of 5%.

### **Results and Discussions**

#### *Vegetative growth characteristics*

Data in Table (2) demonstrated that foliar application of some anti-stresses compound improved navel orange trees vegetative growth but induced different results. Chitosan was the superior anti-stress effect which significantly gave the best results the highest shoots (6.60 & 6.85) cm., thickest shoots (0.23 &0.24) cm. and largest leaf area (26.11 &26.19) cm<sup>2</sup>. Values were for both seasons respectively whereas, the control showed the lowest results (4.91 &5.16) cm., (0.14 &0.18) cm. and (24.29 &24.30)cm<sup>2</sup>. for the considered parameters and both seasons respect.

Navel orange trees grown under Beni Suef climates suffer from a biotic stresses as (high temperature with low Relative Humidity) which negatively reduced the tree growth in terms of shoot length & thickness and leaf area. Spraying anti-stresses in general and Chitosanin specific alleviated abiotic stresses and there by enhanced trees growth.

These findings are in agreement with those obtained by: (Limpanuvech et al., 2008 and Pongprayoon et al., 2013) whom mentioned that the effect of Chitosan can be explained by its ability to enhance the tolerance of plants to a abiotic stresses through improved cell membrane and reducing leaf transpiration rate.

**TABLE 2. Effect of different anti-stresses natural sources on Navel orange tree vegetative growth characteristics during (2019 & 2020) seasons.**

Treatment	Aspects	Shoot length (cm.)		Shoot thickness (cm.)		Leaf area (cm <sup>2</sup> )	
		S1	S11	S1	S11	S1	S11
The control		4.91e	5.16e	0.14c	0.18b	24.29d	24.30d
Chitosan (100 ppm)		6.60a	6.85a	0.23a	0.24a	26.11a	26.19a
Ascorbic acid (100 ppm)		6.30b	6.43b	0.19b	0.21ab	26.00a	26.04a
Proline (30 ppm)		5.85c	5.92c	0.17bc	0.19b	25.72b	25.80b
Vit.E (50 ppm)		5.30d	5.48d	0.15c	0.19b	25.05c	25.11c
New LSD 0.05		0.29	0.31	0.03	0.03	0.19	0.21

*Leaf pigments & total carbohydrates contents*

Concerning leaf Chl. a & b , Carotenoids and total Carbohydrates, presented data in Table (3) illustrate that, in spite of, both Chitosan or Ascorbic acid significantly improved navel orange leaf chl.a & b and carotenoids contents in compared to other treatments. Yet, Chitosan had a more superior effect attained the highest chl. a (0.91&0.97) , chl. b (0.41 &0.43), carotenoids (0.38 &0.39) (mg/100 g f.w) respect., and total carbohydrates (16.90 & 17.10) percentage for both seasons respectively. Whereas, the control attained the lowest during the two seasons of this investigation.

It's well known that anti-stresses compounds play an important role in improving the metabolic processes within the plant, which increases its' tolerance to a biotic stress. This well reflects on the leaves' content of substances that increase the efficiency of photosynthesis (plant pigments), which leads to an increase in the food stock in the plant tissues. These results attained are in harmony with those obtained by : Oretili, (1987), Foyer & Lelandias , (1993) and Singh et al., (2000), whom noticed that an important positive functions of vitamins on cellular metabolism, photosynthesis and translocation of sugars building of plant pigments and proteins which are reflected on enhancing growth and tree nutritional status, yield and fruit quality.

*Leaf total & reducing sugars (%) and Prolin (mg/ 1.0 g f.w) content*

As for the navel orange trees responsibility to anti-stresses compounds applications for total and reducing sugars and Proline content. Data tabulated in Table (4) illustrated that both Chitosan or Ascorbic acid resulted in the highest with in significant difference of leaf total and reducing sugars percentages. Moreover, Proline treatment significantly gave the highest leaf proline percentage in compared to other treatments under study. Whereas, the control treatment was the lowest for both seasons.

Undoubtedly, spraying of chitosan has a positive effect on plant pigments contents in leaves, which will be reflected on the photosynthesis efficiency which positively reflected on leaf carbohydrates and total sugars contents. As for ascorbic acid, it has an effective role in reducing sugars, which its' increase in the cells. On the other hand, spraying Proline leads to an increase in its accumulation in the plant leaves. These results are compatible with those obtained by: Ezz (1999), (Mansour, 2000) and (Takeuchi et al., 2008) whom demonstrated that free amino acids as proline improve cellular growth, total store of carbon, nitrogen, and energy. In addition, this amino acid improves cellular growth, total store of carbon, increased the percentage of fruits juice and its ascorbic acid content nitrogen, and energy.

**TABLE 3. Effect of different anti-stresses natural sources on Navel orange tree leaf pigments contents (mg/100 g f.w)& Carbohydrates (%) during (2019 & 2020) seasons.**

Treatment \ Aspects	Chl. a (mg/100 g f.w)		Chl. b (mg/100 g f.w)		Carotenoids (mg/100 g f.w)		Carbohydrates %	
	S1	S11	S1	S11	S1	S11	S1	S11
The control	0.75c	0.76b	0.26c	0.27c	0.23c	0.25c	14.5e	14.5e
Chitosan (100 ppm)	0.91a	0.97a	0.41a	0.43a	0.38a	0.39a	16.9a	17.1a
Ascorbic acid (100 ppm)	0.88ab	0.89ab	0.36ab	0.37ab	0.33ab	0.34ab	16.3b	16.4b
Proline (30 ppm)	0.81abc	0.83b	0.32bc	0.34bc	0.29bc	0.31abc	15.8c	15.9c
Vit. E (50 ppm)	0.79bc	0.81b	0.29bc	0.30bc	0.27bc	0.29bc	14.9d	15.0d
New LSD 0.05	0.11	0.13	0.07	0.08	0.06	0.07	0.3	0.4



**TABLE 4. Effect of different anti-stresses natural sources on Navel orange tree leaf tot.& reducing sugars (%) and Prolin (mg/ 1.0 g f.w) during the two seasons.**

Aspects Treatment	Total sugars %		Reducing sugars %		Prolin (mg/ 1.0 g f.w)	
	S1	S11	S1	S11	S1	S11
The control	8.8c	8.9c	4.7c	4.8b	42.8d	43.2c
Chitosan (100 ppm)	10.8a	10.9a	5.6a	4.7b	51.9b	52.2b
Ascorbic acid (100 ppm)	10.5a	10.6a	5.3ab	5.4a	48.7c	49.0b
Proline (30 ppm)	9.9b	10.1b	5.1b	5.2a	53.5a	53.8a
Vit. E (50 ppm)	8.6c	8.8c	4.6c	4.7b	46.2d	46.9d
New LSD 0.05	0.5	0.6	0.3	0.4	0.9	1.0

*Leaf minerals( N,P,K & Mg) % contents*

Regarding the effect of different anti-stresses natural sources spraying on Navel orange tree leaf N, P, K & Mg percentage contents . Data presented in Table (5) reveal that Chitosan treatment significantly induced the highest leaf N (1.89 & 1.89) , P (0.27 & 0.27) , K (1.70 & 1.71) and Mg ( 1.11 & 1.00 ) percentages respect., when compared to other treatments under study. Also, the control treatment was the lowest for both seasons.

Naturally, Chitosan as an anti-stress compound which is a derivative of N-acetylglucosamine, is a natural biopolymer combined derived by de-acetylation of chitin, a major component of the shells of crustacean sea animals (e.g., crab, shrimp, and crawfish) “shrimp scales source”, when applied as a foliar applications on trees will improve leaf minerals contents . On the other hand , other anti-stresses compounds (Ascorbic acid , Proline and Vit.E) under study consider an “organic sources”, thus it's may be have indirect and lower effect for these elements. These findings are confirmed with those obtained by :Robinson, 1973, Foyer and Lelandias(1993) and Singh et al. (2000) whom demonstrated the promoting effects of vitamins and amino acids on the uptake of water and nutrients, especially zinc and boron, securing a balanced nutritional supply to the trees and hence reflected on enhancing tree growth and improved the nutritional status. Shehata et al. (2012) found that foliar spray

of chitosan significantly increased N and P concentrations in cucumber leaves. 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. On the other hand they indicated that all tested chitosan sprayings not only increased phosphorus content but also potassium content of leaf tissues . Nguyen Van et al. (2013) demonstrated that spraying with chitosan (600 kDa) on coffee seedlings increased the content of nitrate and phosphorus in leaves.

*Fruit set and tree yield:*

Data in Table (6) showed that Chitosan applications has a superior effect on navel orange trees Initial (2.98 & 3.05) & final fruit set( 1.36 & 1.39) (%) respect., and the final tree production [number of fruits ( 332 & 335) & weight ( 79.7 & 81.0) kg/tree) when compared to other treatments. Ascorbic acid treatment came 2<sup>nd</sup> rank in it's effect with various degrees of significance when compared with Chitosan. The control treatment significantly was the lowest for both seasons .

Undoubtedly, foliar applications of anti-stresses compounds on navel orange trees at blooming or fruit setting stages will alleviate a biotic stress ( heat or drought) effect on tree yield thus leading to a better performance under the prevailing conditions. Moreover both Chitosan or Ascorbic acid treatments play an important role in regulating leaves stomata mechanism which reducing water lost. These results are in line with

**TABLE 5. Effect of different anti-stresses natural sources on Navel orange tree leaf N, P, K & Mg(%) during the two seasons.**

Treatment	Aspects	N %		P %		K %		Mg %	
		S1	S11	S1	S11	S1	S11	S1	S11
The control		1.61e	1.63 e	0.22 d	0.22 c	1.42e	1.42e	0.82d	0.86 c
Chitosan (100 ppm)		1.89a	1.89 a	0.27 a	0.27 a	1.70a	1.71a	1.11a	1.00a
Ascorbic acid (100ppm)		1.81b	1.82 b	0.25 b	0.25 b	1.63b	1.64b	1.00b	0.98a
Proline (30 ppm)		1.77c	1.78 c	0.24 c	0.24 c	1.53c	1.56c	0.98b	0.98a
Vit.E (50 ppm)		1.68d	1.70 d	0.24 c	0.24 c	1.49d	1.51d	0.91c	0.92b
New LSD 0.05		0.03	0.04	0.01	0.02	0.03	0.04	0.04	0.04

**TABLE 6. Effect of different anti-stresses natural sources on Navel orange tree leaf initial & final fruit set (%) and tree yield (number & weight) during the two seasons.**

Treatment	Aspects	Initial fruit set %		Fruit retention %		Tree yield as a number		Tree yield (kg)	
		S1	S11	S1	S11	S1	S11	S1	S11
The control		2.72d	2.75d	1.04e	1.01 e	298d	300c	62.6d	64.5e
Chitosan (100 ppm)		2.98a	3.05a	1.36a	1.39 a	332a	335a	79.7 a	81.0 a
Ascorbic acid (100 ppm)		2.91ab	2.99ab	1.29b	1.30 b	321b	324a	75.8b	77.4b
Proline (30 ppm)		2.85bc	2.89bc	1.19c	1.21c	316b	319a	70.2c	71.8c
Vit.E (50 ppm)		2.79cd	2.81cd	1.08d	1.10 d	309c	311b	67.4c	68.4d
New LSD 0.05		0.09	0.11	0.03	0.03	7	7	2.9	3.1

those obtained by: (Zandalinas et al. 2016b). whom found that anti-stresses represents a key phytohormone which is repressed by heat stress probably avoiding stomatal closure, and keeping high transpiration rates to cool leaf surface.

#### *Fruit physical and chemical characters*

##### *Fruit physical characters*

As for the effect of some anti-stress compounds on navel orange fruit physical characters, data presented in Table (7a) resulted that Chitosan treatment significantly increased fruit weight (240.00 & 242.00) g with insignificant difference with Ascorbic acid treatment (236.00 & 239.00)

g respect., in compared to other treatment under study, whereas, the control was the lowest. Additionally, Chitosan application statistically reduced fruit peel ( 18.80 & 18.700) % (w/w) and fruit peel thickness (0.21 & 0.20) with insignificant variance with Ascorbic acid treatment (0.23 & 0.22) cm. respect., . In contrast, it increased fruit pulp (81.20 & 81.30) % (w/w) in compared to the other treatments for both seasons. On the other hand navel orange fruit shape index was > 1 and all treatments have insignificant differences in fruit shape index which seemed to be Oval shape during the two seasons.

*Fruit chemical characters*

Data in Table (7b) reveal that Chitosan application significantly increased fruit juice :TSS (12.9 & 13.0) %, TSS/ Acid Ratio (10.9 & 11.2) , Vit. C content ( 51.8 & 52.2) (mg/ 100 ml juice) and reduced acidity (1.18 & 1.17)% with insignificant difference with Ascorbic acid for TSS % ( 12.60 & 12.70) % when compared to other treatments. The control was the lowest except the acidity was the highest content for the two seasons.

It's well known that Chitosan hydrolysates and menadione sodium bisulphite (MSB) [poly-(1→4)-β-D-glucoseamine] are a partially deactivated form of chitin, a natural biopolymer from the exoskeleton of crustaceans and fungal cell walls, which is biocompatible, biodegradable

and a sustainably renewable cheap resource that has many applications, including agricultural sector. Thus, Chitosan has been widely used in agricultural applications mainly for stimulation of plant defense) Bautista-Baños *et al.*, 2003). Moreover, Chitosan oligomers enter most regions of the cell. Subsequent changes occur in: cell membranes, chromatin, DNA, calcium, MAP kinase, oxidative burst, reactive oxygen species (ROS), callose formation, pathogenesis related (PR) genes/proteins, and phytoalexins, primarily in plant defense, additionally in yield increase and induction of cell death and stomatal closing (Hadwiger, 2013). Total soluble solids (T.S.S.) of strawberry fruit showed tendency to increase in response to chitosan application. Abdel-Mawgoud *et al.* (2010). Finally, chitosan sprayed plants were

**TABLE 7a. Effect of different anti-stresses natural sources on navel orange fruit physical characters during the two seasons.**

Treatment \ Aspects	Fruit weight (g.)		Fruit peel % (w/w)		Fruit Pulp % (w/w)		Fruit peel thickness (cm)		Fruit Shape Index	
	S1	S11	S1	S11	S1	S11	S1	S11	S1	S11
The control	210.0b	215.0b	27.5a	27.4a	72.5e	72.6e	0.30a	0.29a	1.03	1.02
Chitosan (100 ppm)	240.0a	242.0a	18.8e	18.7e	81.2a	81.3a	0.21c	0.20c	1.02	1.03
Ascorbic acid (100 ppm)	236.0a	239.0a	21.0d	20.4d	79.0b	79.6b	0.23c	0.22c	1.02	1.03
Prolin (30 ppm)	222.0b	225.0b	23.2c	22.9c	76.8c	77.1c	0.26b	0.25b	1.02	1.01
Vit.E (50 ppm)	218.0b	220.0b	24.9b	24.4b	75.1d	75.6d	0.28ab	0.27ab	1.02	1.02
New LSD 0.05	7.9	8.1	0.8	0.9	1.1	1.1	0.02	0.02	NS	NS

**TABLE 7b. Effect of different anti-stresses natural sources on navel orange fruit chemical characters during the two seasons.**

Treatment \ Aspects	TSS %		Total acidity %		TSS/ acid R		Vit. C (mg/ 100 ml juice)	
	S1	S11	S1	S11	S1	S11	S1	S11
The control	10.8c	10.09d	1.35 a	1.32a	8.0e	8.3e	44.6d	45.8d
Chitosan (100 ppm)	12.9a	13.0a	1.18 e	1.17 e	10.9 a	11.2a	51.8a	52.2a
Ascorbic acid (100 ppm)	12.6a	12.7ab	1.23d	1.19d	10.3b	10.7b	49.1b	49.7b
Prolin (30 ppm)	12.0b	12.2bc	1.27c	1.25c	9.4c	9.8c	46.8c	47.0c
Vit.E (50 ppm)	11.6b	11.7c	1.30b	1.29b	9.0d	9.1d	45.0d	46.7cd
New LSD 0.05	0.4	0.5	0.02	0.02	0.2	0.3	1.1	1.2



firmer and ripened at a slower rate as indicated by anthocyanin content and titratable acidity than from non-treated plant, Reddy et al. (2000). And foliar spray by chitosan showed a non-significant effect on fruit shape index as compared to the control (Xia, 2003).

### Conclusion

It can be concluded that, navel orange orchards under Beni Swief region suffer from a biotic stress as: heat, drought, lower humidity, etc., this leads to good vegetative growth plus poor in tree yield production and fruit quality. Spraying some natural anti-stress compounds significantly increased tree fruit return and improved fruit physical & chemical characters.

Generally, spraying Chitosan at 100 ppm three times during fruit-let setting and growth gave the superior results when compared with other treatments under this experiment during the two studied seasons.

*Funding statements:* None

*Conflicts of interest:* None

### References

- Abdel-Mawgoud, AMR, Tantawy, A.S., El-Nemr, M.A., Sassine, Y.N. (2010) Growth and yield responses of strawberry plants to chitosan application. *Eur. J. Scientific Res.*, **39**(1), 161- 168.
- Abo- El- Komsa, E.A., Hegab, M.Y. and Amera A. Fouad (2003) Response of Balady orange trees to application of some nutrients and citric acid. *Egypt. J. Apph. Sci.*, **18** (3), 228-246.
- Ahmed, F.F. and Morsy, M.H. (1999) A new method for measuring, leaf area in different fruit crops, *Minia of measuring Agric. Res. Develop.*, **19**, 97-105.
- Ali- Ragaa, S.A. (2008) Effect of Ascorbic acid and citric acid on fruiting of Balady mandarin trees M. Sc. Thesis Fac. of Agric. Minia Univ.
- Aspinall, D. and Paleg, I. (1981) Proline accumulation physiological aspects. In Paleg, L.G. Aspinall, D. eds. *The physiology and biochemistry of Drought Resistance in plants*. Academic press Sydney, pp. 205-241.
- Association of Official Agricultural Chemists (2000) *Official Methods of Analysis* 14<sup>th</sup> ed. (A.O.A.C.) Benjamin Franklin Station, Washington D.C. U.S.A. pp. 490-510.
- Bautista-Baños, S., Hernández-López, M., Bosquez-Molina, E., Wilson, C.L. (2003) Effect of chitosan and plant extracts on growth of *Collettrichum gloeosporioides*, anthracnose levels and quality of papaya fruit. *Crop Prot.*, **22**, 1087–1092.
- Bates, L.S., Waldren, R.P. and Teare, I.D. (1973) Rapid determination of free proline for water stress studies. *Plant and Soil*, **39**, 205-207.
- Caronia, A., Gugliuzza, G. and Inglese, P. (2010) influence of L. proline on citrus sinensis L. 1<sup>st</sup> New Hall and Tarocco seire, J. fruit quality *Acta Hort.*, **884**, 423-426.
- Dubois, M., F.Smith, K.A. Gilles, J.K. Hammlton and P.A. Robers (1956) Colorimetric method to determination of sugar and related substances: *Anal. Chem.*, **28** (3), 350-356.
- El-Sayed- Esraa, M. (2017) Behaviour of prime seedless grapevines grown under sandy soil conditions top foliar application of chitosan J. product Dev., **22**, 682-696.
- El-Miniawy SM, Ragab ME, Youssef SM, Metwally AA (2013): Response of strawberry plants to foliar spraying of chitosan. *Res. J. Agric. & Biol. Sci.*, **9**(6), 366-372.
- Ezz. T.M. (1999) Eliminating chilling injury of citrus fruits by preharvest proline foliar spray. *Alex. J. Agric. Res.*, **4** (1), 213-225.
- Foyer, C.H. and Lelandias, S. (1993) The role of ascorbate in regulation of photosynthesis. In Yamamoto, Y., Smith, C.H. (Ed.), *photosynthesis responses to the environment*.
- Gamal, A.F.O. (2013) Fruiting of Washington Navel orange trees in relation to application of seaweed extract, boron and citric acid *Ph.D. Thesis*, Fac., of Agric. Minia Univ. Egypt.
- Hadwiger L.A (2013) Plant science review: Multiple effects of chitosan on plant systems: Solid science or hype. *Plant Sci.*, **208**, 42-49.

- Hiscox, A. and Isrtalstam, B. (1979) Method for the extraction of chlorophyll from leaf tissue without maceration. *Can J. Bot.*, **57**, 1332-1334.
- Jabeen, N. and Ahmed, R. (2013) The activity of antioxidant enzymes in response to salt stress in safflower (*Carthamus tinctorius* L.) and Sunflower (*Helianthus annuus* L.) seedlings raised from seed treated with chitosan. *J. Sci. Food Agric.*, **93** (7): 1699-1705.
- Khafagy, O.M.M. (2019) The beneficial effects of using chitosan and glutathione on the fruiting of Red Roomy grapevines *M.Sc. Thesis*, Fac. of Agric. Minia Univ, Egypt.
- Khalil, M.A. (2021) Studies on the effect of chitosan on productivity of Flame seedless grapevines *Ph.D. Thesis*, Fac. of Agric. Al Azhar Univ. (Assiut Branch), Egypt.
- Kumar, V., Lemos, M., Sharma, M. and Shriram, V. (2013) Antioxidant and DNA damage protecting activities of *Eulophia nuda* Lindl. *Free Radicals and Antioxidants*, **3**(2), 55- 60.
- Lane, J.H. and Eynon, L. (1965) Determination of reducing sugars of means of Fehling's solutions with methylene blue as indicator. *A.O.A.C. Washington D.C., U.S.A.*
- Limponavech, P., Panavech, P., Chaiyasuta, S., Vangprommek, R., Pichyangkura, R., Khunwasim, C., Chadchawan, S., Lotrakul, P., Bunjongrate, R., Chaide, A. and Bangyeekhun, T. (2008) Chitosan effects of floral production on gene expression and anatomical changes in the *Dendrobium* orchid. *Ci. Hort.*, **116**(1), 65-72.
- Mansour, M.M.F. (2000) Nitrogen containing compound and adaptation of plants to salinity stress. *Biol. Plant* **43**, 491-500.
- Mead, R., Curnow, R.N. and Harted, A.M. (1993) *Statistical Methods in Agricultural and Experimental Biology*, 2<sup>nd</sup> ed., Chapman & Hall. London, pp. 10-44.
- Nguyen, Van S., Minh, H.D., Anh, D.N. (2013) Study on chitosan nanoparticles on biophysical characteristics and growth of Robusta coffee in green house. *Biocatalysis Agri. Biotechnol.*, **2**(4), 294-289.
- Oretili, J.J. (1987) Exogenous application of vitamins as regulators for growth and development of plants. *Pflanzenzucht*, **150**, 375-391.
- Park, P.J., Je, J.Y. and Kim, S.K. (2004) Free radical scavenging activities of differently deacetylated chitosan using an ESR Spectrometer carbohydrate polymers, **55** (1), 17-22.
- Pongrayoon, W., Rortrakul, S., Pichayankura, R. and Chadchawan, S. (2013): The role of hydrogen peroxide in chitosan induced resistance to osmotic stress in rice (*Oryza-Sativa* L.) *Plant Growth Regul.*, **70**(2), 159-173.
- Saied, H.H.M. (2005) Studies on tolerance of some mango cultivars to salinity and lime. *Ph.D. Thesis*, Fac. of Agric. Minia Univ. Egypt.
- Saied, H.H.M. and Radwan, E.M.A. (2017) Insight into the effect of chitosan on growth and fruiting of Suckery mango trees. *J. Product Dev.*, **22**(3), 781-793.
- Samiulלה, S.A., Ansori, M.M. and Afridi, R.K. (1988) B- vitamins in relation to crop productivity-Indian, *Rev. Life Sci.*, **8**, 51-74.
- Sanford, P.A. (2002) Commercial sources of chitin and chitosan and their utilization, *Advances in Chitin Science*, **6**, 35-42.
- Shehata, S.A., Fawzy, Z.F., El-Ramady, H.R. (2012) Response of cucumber plants to foliar application of chitosan and yeast under greenhouse conditions. *Aust. J. Basic & Appl. Sci.*, **6**(4), 63-71.
- Singh, D.V., Srivastava, G.G. and Abidin, M.S. (2001) Amelioration of negative effect of water stress in *Cassia angustifolia* by benzyladenine and/ or ascorbic acid. *Bidoyia plantarum*, **4**(1), 141-143.
- Snedecor, G.W. and W.G. Cochran (1990) *Statistical Method*, 6<sup>th</sup> ed. The Iowa State Univ. Press, Iowa, U.S.A. p.593.
- Summer, M.E. (1985) Diagnosis and Recommendation integrated System (DRIS) as a Guide of orchard Fertilization. *Hort. Abst.* **55**(8): 7502.
- Takeuchi, M., Arakawa, C., Kuwahara, Y. and Gemma, H. (2008) Effect of proline foliar application on the quality of Kosui Japanese pear. *Acta Hort.* **2** (800), 549-554.

- Von-Wettstein, D.V. (1957) Chlorophyll-Ithal under submikrosphische formiuechrel der plastiden celi, *Drp. Res. Amer. Soc. Hort. Sci.*, **20**, 427-433.
- Waller, A. and Duncan, D.B. (1969) Multiple range and Multiple test. *Biometrics*, **11**, 1 - 24.
- Wilde, S.A., Corey, R.B., Lyer, I.G. and Voigt, G.K. (1985) *Soil and Plant Analysis for Tree Culture*, 3<sup>rd</sup> ed., Oxford & IBH publishing Col., New Delhi, pp. 1-218.
- Xia, W.S. (2003) Physiological activities of chitosan and its application in functional foods. *J. Chin. Inst. Food Sci. Technol.*, **3**(1), 77–81.
- Yang, C.W., Line, C.C. and Kas, C.H. (1999) Endogenous ornithine and arginine content and dark induced proline accumulation in detached rice- leans. *J. Plant. Physiol.*, **155**, 665-668.
- Zandalinas, S.I., Rivero, R.M., Martínez, V., Gómez-Cadenas, A., Arbona, V. (2016b) Tolerance of citrus plants to the combination of high temperatures and drought is associated to the increase in transpiration modulated by a reduction in abscisic acid levels. *BMC Plant Biol.*, **16**, 105–121.

## تأثير بعض المصادر الطبيعية لمضادات الاجهاد على انتاجية اشجار وصفات جودة ثمار البرتقال بسرة تحت ظروف مصر الوسطى

راندا السيد يونس حباسي

باحث قسم بحوث الموالح - معهد بحوث البساتين - مركز البحوث الزراعية - الجيزة - مصر.

أجريت هذه الدراسة خلال موسمين متتاليين (٢٠١٩ و ٢٠٢٠) بهدف دراسة تأثير الرش ببعض مضادات الاجهاد (من مصادر طبيعية مختلفة) وهي : الشيتوزان بتركيز ١٠٠ جزء/المليون ، حامض الاسكوربيك بتركيز ١٠٠ جزء في المليون ، البرولين بتركيز ٣٠ جزء في المليون وفيتامين هـ بتركيز ٥٠ جزء في المليون. لقد تم رش اشجار كل معاملة ثلاثة مرات خلال موسم النمو في الاسبوع الاول من : مايو ، يوليو ثم سبتمبر) على سلوك النمو الخضري والفسولوجي والنسبة المئوية للعقد المبدئي والنهائي وكمية المحصول وجودة ثمار اشجار برتقال بسرة نامية تحت ظروف مركز اهناسيا / محافظة بني سويف.

أدى رش الاشجار بالمعاملات السابقة الى حدوث تحسن واضح في جميع الصفات (الخضرية ، الفسولوجية ، العقد الاولى او النهائي ، محتوى الاوراق من الازوت والفسفور والبوتاسيوم والمغنيسيوم والمحصول وبعض صفات الجودة الطبيعية او الكيماوية للثمار) تحت الدراسة وذلك بالمقارنة بمعاملة الكونترول وبمقارنة نتائج المعاملات ببعضها او بمعاملة المقارنة أمكن ترتيب هذه المواد حسب تأثيرها الايجابي على تلك الصفات على النحو التالي: الشيتوزان في المرتبة الاولى تلاه حمض الاسكوربيك ثم البرولين واخيرا فيتامين هـ.

**وخلصت النتائج** الي انه كي نرفع من قدرة اشجار البرتقال بسرة علي تحمل الاجهاد اللاحيوي تحت ظروف محطة بحوث البساتين بسدس ( محافظة بني سويف ) وتحسين نموها وتحقيق محصول اقتصادي تتمتع ثماره بجودة عالية فمن الضروري رش الاشجار ثلاثة مرات خلال الموسم (مايو ، يوليو وسبتمبر) بمركب الشيتوزان بتركيز ١٠٠ جزء في المليون.