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RESEARCH ARTICLE

Effect of using bio-stimulants and foliar spraying of anti-stressors for counteract the negative effects of climate changes on growth and fruiting of Balady mandarin trees.

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Abstract

This study was conducted during seasons 2019 and 2020 at a private farm situated at Nag Hammadi district, Qena Governorate, Egypt, to study stimulate the ability of the Balady Mandarin tree to tolerate climate changes and improve the growth, yield, and characteristics of the fruits through the appropriate agricultural practices by supplying trees with bio-stimulants via soil in addition to spraying some anti-stress substances. The results showed that adding bio-stimulants to trees via soil at twice in the (end of February and mid-May) in addition to foliar spraying with some anti-stressors such as (amino acids or potassium silicate or hydroxybenzoic acid). At 0.1% four times in the first of March and middle of (May, June, and July) led to enhancing the tree's ability to withstand environmental disturbances and improving in all vegetative growth characteristics and leaf content of chlorophyll, nitrogen, and phosphorous as well as the nutritional status of trees and this was positively reflected on increasing the yield and fruit quality characteristics compared to untreated trees which recorded the lowest values for all studied traits. And there were no significant differences between the three anti-stressors. So, it can be recommended to use bio-stimulants via soil and spraying of any of the anti-stress that studied as part of an agricultural strategy to overcome the negative effects of climate change, which is highly dependent on plant nutritional status.

Key words: Abiotic stress; Anti-stressors; Bio-stimulants; Climate changes; Mandarin.

1. Introduction

In recent years, the agricultural sector has been exposed to a great challenge with climate changes such as (high and low in temperature, drought, frost and rising level of carbon dioxide) and which become represent a major threat to food security in the world, because of the various a biotic stresses it causes to plants and the occurrence of morphological, physiological and chemical changes in the plant lead to decreasing plant growth and food productivity.(IPCC, 2007; Huang, *et al*., 2013; Kai and Koh, 2014; Singh and Takhur 2018; bo batta, 2019).

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Citrus is considered the backbone of fruit crop cultivation (Salama, 2015). Mandarin (*Citrus reticulate* Blanco) fruits stand as the second variety for exportation after orange fruits in Egypt (Mohamed, *et al.*, 2019) and having significant value in the local market due to the easy peeling and eaten as compared to other types of citrus. (Obenland, *et al*., 2011). Continuous climate changes led to a change in behavior of citrus trees and negatively affected on growth, yield and fruit quality, and therefore reduced growers income. (Ahmad, *et al*., 2017; Balfagon, *et al.*, 2018; Abo batta, 2020).

In Upper Egypt, Heat stress has become an important determining factor for mandarin production. As temperature fluctuation at the beginning of the growing seasons and that rising of day/night temperature above optimum range in the period from June to August (above 40 °C) impact on growth, flowering, setting and productivity of mandarin. Mandarin performs best at temperatures between 15.6 and 30 °C (Parsons and Beck, 2004).

Can be prevent the effect of abiotic stresses by amelioration the conditions of plant growth through improve the nutritional status of plants. (Kai and Koh, 2014; Yakhin, *et al*., 2017; Sharma and Manjeet, 2020).

Plant bio-stimulants are natural or synthetic substances are efficient at small concentrations have similar actions to the plant hormones, such as auxins, gibberellins, and cytokinins which differ in containing the variety of organic and bio compounds such as hormones, humic acid, plant growth promotion bacteria and seaweed extracts. (Du-Jardin, 2015; Du-Vasconcelos and Chaves, 2019).This bio-stimulating substances effects on plant growth and cause changes in vital processes to relieve the effects of abiotic stress through improving the nutrient uptake, water use efficiency as well as enhance reactive oxygen species (ROS) responses and role it as co-factors in the enzymes antioxedative and therefore increasing the growth of plant and it productivity.(Hegab *et al.,* 2005; El- Shamma *et al.,* 2013; Barakat *et al.,* 2012; Sharma *et al.,* 2014; Battacharyya *et al.,* 2015; Brown and Saa, 2015; Georgia *et al.,* 2017; Yakhin *et al* ., 2017; Van-Oosten *et al.*, 2017).

Amino acids play important role in plant resistance to abiotic stress by different mechanisms such as regulation of pH, cellular osmotic modification to maintain cellular-water, increasing the photosynthesis, acting as compatible osmolytes, action a reserve of nitrogen and carbon, provider of mechanical strength to plant cell, organizing stomatal opening and closing. (Rai, 2002; Ashraf and Foolad, 2007; Hassan- Huda, 2014; Sadak *et al*., 2015; Souri, 2016).

Antioxidants has a very important role in enhancing water and nutrients uptake, cell division, photosynthesis, the biosynthesis of plant hormones such as IAA, GA_3 and cytokinin and biosynthesis of proteins, decreasing production of ROS which reduces oxidative stress and improve flowering and fruit setting as well as protecting plants from stress. (Samiullah *et al*., 1988; Hayat and Ahmed, 2007).

Benzoic acid has plays a regulatory role in resistance stress, It is effective at a low concentrations compared to salicylic acid in enhance the plant tolerance to chilling, heat and drought. Where low concentrations of it acts as a key regulator in several physiological processes and involved in the tolerance of plants to stress by improve anti-oxidative defense system as well as alters of many proteins involved in process of photosynthesis consequently, improve the nutritional status of the plant (Senaratna *et al*., 2003; Harvath *et al*., 2007; Abd El- Rahman and El- Masry, 2012; Al- Wasfy, 2012; Ahmed *et al*., 2013). Silicon performs many functions in the plant, may be physiological, protective or act as a structural component increased cell rigidity making them more tolerance to biotic and abiotic stress and enhance tolerance to (temperature, drought, wind, frost and soil salinity). (Aziz *et al.*, 2002 ; Ma and Takahashi, 2002; Liang *et al*., 2007; Guntzer *et al*., 2012). Furthermore, the beneficial effects of Si include improve the nutritional status of plant through increasing uptake nutrients and water, increased photosynthetic rates, regulation of transpiration, leaf gas exchange and which positively effect on growth and yield. (Ma, 2004; Currie and Perry, 2007; Guntzer *et al*., 2012; Ahmed *et al*., 2013 Ibrahiem and Al- Wasfy, 2014; Chanchal *et al*., 2016; Abd-Elall and Hussein, 2018).

The objective of the present investigation was to study the effect of agricultural bio-stimulants and anti-stressors substances in reducing the negative effects of climate changes and improve the growth and productivity of Balady mandarin trees as well as make agriculture more sustainable.

2. Materials and methods

The present investigation was carried out during the two successive seasons of 2019 and 2020 at a private Farm situated at Nag Hammadi, Qena Governorate, Egypt, on Balady mandarin trees (*Citrus reticulate* Blanco) budded on sour orange (*Citrus aurantium* L.) rootstock the trees were 15 years old, were planted at 5×5 meters apart in clay loam soil (Table 2) under surface irrigation system to study the effect of adding bio-stimulants with spray some anti-stressors on growth and productivity to alleviation the adverse effects of heat stress and improving the yield and fruit quality. The trees were selected as uniform as possible in growth, vigor, fruiting, and free from any disease.

The meteorological data of the studied period during studied seasons of 2019 and 2020 were presented in (Fig 1).

All the trees were fertilized with the recommended fertilizers doses (RFD) of NPK. The other cultural practices were applied on all trees. The experiment included eight treatments arranged in a split plot in a complete randomized block design, where each treatment contained three replicates with one tree each. The main plots were used for bio-stimulants and the anti-stressors were randomly arranged in the sub plots, as follow:

Main plot**:** factor (A) bio-stimulants**.**

1- Untreated.
2- Bio-stimulants: Soil adding of 10g/tree

Sub-plot**:** factor (B) Foliar spray of anti-stressors:

1- Control (spraying of water).
2- Amino acids.
3- Potassium silicate (K_2SiO_3).
4- Hydroxybenzoic acid.

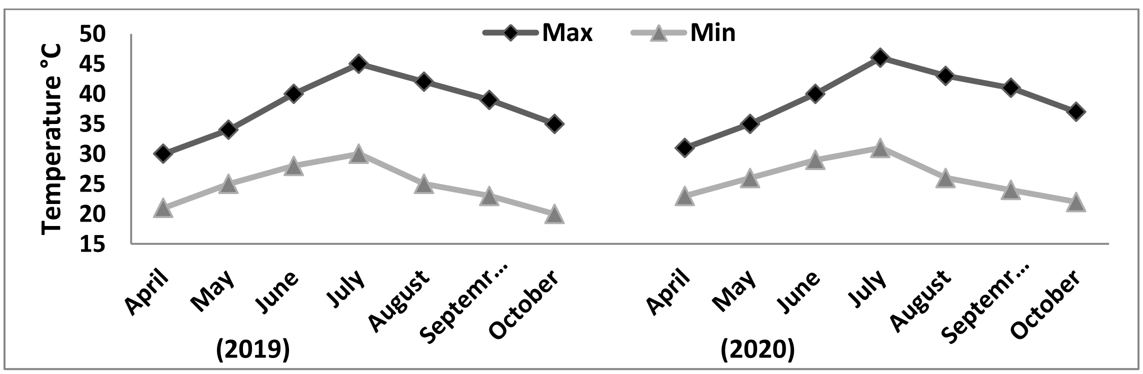

Fig 1. Mean of maximum and minimum temperature data for Nag Hammadi area during 2019 and 2020 seasons (source, meteorological station at Nag Hammadi (latitude 26°03'N, longitude 032°14'E)

Table 1. Chemical composition of the materials which used in this study

the material	composition	value	composition	value
Amino acids	Aspartic acid	2.40%	Leucine	1.05%
	Proline	3.14%	Arginine	2.20%
	Serine	3.12%	Lysine	0.62%
	Glutamic acid	3.40%	Histidine	0.32%
	Glycine	2.42%	Threonine	1.60%
	Tyrosine	0.39%	Phenylalanine	1.10%
	Alanine	1.51%	Organic nitrogen	10%
	Valine	2.02%	Potassium oxide	5%
	Isoleucine	0.87%	Total amino acids	26.2%
Anti-oxidants	Hydroxybenzoic acid	25%	Ascorbic acid	2%
	Riboflavin	1.2%		
K. silicate	K_2O 11%		SiO_3 22%	
Bio-stimulants	Humic acid	80%	Seaweed extract	5%
	Bacillus subtilis	5%		
	a little of *Trichoderma hazrianum*, *Ascophilum nodosum*, Amino acid,Vitamins, Fulvic acid, Auxin and cytokinin			

Table 2. soil analysis according to (wilde, *et al.*1985)

Soil properties	values	Soil properties	Values
sand	28.4	EC(1:2.5) dS m^{-1}	0.6
silt	29.5	Total N %	0.19
clay	42.1	Available p (ppm)	46.35
texture	Clay loam	Available k (ppm)	483
pH(1:2.5)	7.55	$CaCO_3$%	3.48
O. M %	1.35		

Bio-stimulants were applied at a rate of 10g/tree/season at twice in the (end of February and mid-may) in holes around the trunk of the tree and were directly irrigated after covering with soil.

Foliar spray substances were applied four times in each season in the first of March and middle of (May, June and July) at a concentration (0.1 %) to reduce the impact of heat stress on growth, productivity, and quality. Triton B at 0.1 % as a wetting agent was added to spraying solution. Trees have been sprayed even till runoff in the morning hours of the day in mist form in addition to control which was only sprayed with water. The following parameters were measured during the two growth seasons.

2.1. Vegetative growth traits and Leaf chlorophyll content

In the end of growth season, twelve shoots were taking from four directions (three shoots/ each) from spring growth cycle which labeled in February to determine the growth traits in both seasons .

Shoot length (cm), number of leaves/ shoot, leaf area (cm^2) according to (Shrestha and Balakrishnan, 1985) was measured and leaf chlorophyll content was estimated by using chlorophyll meter (Minolta, SPAD 502 plus) by using mature using leaves (from the fourth to fifth leaf of the labeled shoot base).

2.2. Leaf content of N, P and K, total carbohydrates and nitrogen in shoots

Forty mature leaves were randomly selected (from non-fruiting shoots of the spring cycle) in end September to determine N, P and K in leaves according to (Wilde *et al*., 1985). Total Nitrogen and carbohydrates were determined in shoots by using non fruiting shoots seven month old from the spring growth cycle according to standard methods outline by (Wilde *et al*., 1985; Smith *et al.*, 1956).

2.3. Yield and its components

At the beginning of the season, eight branches were randomly selected (two/ direction) and labeled, then number of flowers were counted / each branch and recorded it. One week before the harvest, the number of fruits for each branch labeled was counted. The fruit retention % was calculated according to equation

$$\text{fruit retention\%} = \frac{number\ of\ retained\ fruits}{total\ number\ of\ flowers} x100$$

At harvesting time, in the end of December The fruits/ tree were harvested and weighed as a whole and recorded the yield / tree (kg), the number of fruit per tree was counted.

2.4. Fruit quality properties

Twenty fruits were taken randomly from each tree (5 fruit/ each direction) to determine the fruit properties. Average fruit weight (g) and fruits chemical properties were determined according to (A.O.A.C., 2005).

2.5. Sunburn fruits percentage

At harvest time, sunburned fruits from all treatments were counted; the percentage of sunburned fruits was calculated.

$$\text{Sun burn\%} = \frac{number\ of\ sunburned\ fruits}{total\ number\ of\ fruits} x100$$

2.6. Statistical analysis

The data obtained were statistically analyzed and the differences between means were compared according to Gomez and Gomez (1984) by using the new L.S.D.

3. Results

3.1. Vegetative growth traits

Changes values in vegetative growth traits (shoot length, number of leaves and leaf area) as affected by application of bio-stimulants, anti- stressors and their interactions (comparing to untreated trees) on the mandarin trees during 2019 and 2020 seasons are presented in Table (3).

The results indicated that the highest values in all studied traits in both seasons were recorded on the trees that treated by bio-stimulants in addition to recommended dose of chemical fertilizers compare to control treatment (trees treated by recommended dose of mineral fertilizers only). Also, Data presented in Table 3 show the effect of foliar applications to Anti-stressors on vegetative growth parameters of Balady mandarin trees. It is clearly that all applications of anti-stressors foliar significantly increased of all studied growth traits (shoot length, number of leaves and leaf area).There are no significant differences between treatments of different anti-stress. However, the superiority of spraying amino acids over the rest of the anti-stressors agent, as it recorded the highest values of vegetative growth characteristics followed by spraying potassium silicate). Also, Table 3 show the affected of abovementioned studied traits by spraying of Balady mandarin trees with the different anti-stressors substances with two nutrient applications via soil (common fertilization rates only and common fertilization rates with bio-stimulants application). It is clearly noted that all anti-stressors substances treatments combined with the bio-stimulants gave statistically increase values in all vegetative growth traits compared to application of anti-stressors with the common fertilization practice treatments only (anti-stressors x RFD). Using amino acids with treated trees by bio-stimulants gave the maximum values of growth traits indicating the efficient role application of bio-stimulants to protect plants against heat stress.

Generally, it could be concluded that the interactions between application of bio-stimulants and anti-stressor gave the highest values of vegetative growth and decreased the negative effect of climate changes on Balady mandarin trees compared to spraying of anti-stressors alone and untreated trees.

3.2. Leaf content of nutrients and chlorophyll

data presented in Table (4) clear that the changes in the leaf content values of macronutrients (N, P, K) and Chlorophyll% as a result of the application bio-stimulants and foliar spraying of anti-stressors and their interactions (comparing to untreated trees) on the mandarin trees grown under Upper Egypt condition during 2019 and 2020 seasons.

The results indicated that highest values of leaves content of Leaves from nutrients and chlorophyll were recorded in the trees that treated with bio-stimulants in both seasons comparing with the control treatment (untreated ones). Overall, it can be concluded that an appropriate supply the trees by bio-stimulants is crucial to achieve the best growth and increase the nutrients in leaves.

Also, Data presented in Table (4) show the effect of foliar applications of anti-stressors on leaves content of (N, P, K) and chlorophyll of trees under study. It is clearly that all foliar applications of anti-stressors significantly increased the leaves content of (N, P, K macronutrients) and Chlorophyll. Sprayed trees with amino acids gave the highest values in the studied traits i.e., N, P, Chlorophyll in both seasons compared to untreated trees (control) followed by potassium silicate in both seasons while, potassium silicate it outperformed on all anti-stress treatments in the leaves potassium content in the two seasons.

Generally, the three studied anti-stressors led to improving the leaves content from N, P, K and chlorophyll at both seasons. It is known that heat stress affects directly on growth of mandarin trees .Therefore, the control trees showed the lowest values for all the studied traits, this is to far extent proved that these trees were greatly affected by environmental disturbances.

Also, data in table (4) show the effect of the interactions between application of bio-stimulants and foliar spray of anti-stressors on the leaves content from (N, P, K and chlorophyll) of Balady mandarin trees. The obtained data clear that the trees which sprayed with any of anti-stress substances with application bio-stimulants gave the highest values in content of the leaves of Balady mandarin trees from N, P, K and Chlorophyll compared to the application of anti-stressors on the trees untreated with bio-stimulants or control (untreated tress by any bio-stimulants and anti-stressors). The highest values in all traits studied were recorded upon treatment of trees by bio-stimulants and foliar spray by amino acids in both seasons, except leaf content from k in which potassium silicate spray with bio-stimulants application gave the highest values in both season.

Table 3. Effect of bio-stimulants and anti-stressors on shoot length, number of leaves and leaf area of Balady mandarin trees during 2019&2020 seasons.

treatments	parameters	length(cm) Shoot		Number of leaves		Leaf area (cm^2)	
		2019	2020	2019	2020	2019	2020
Effect of application bio-stimulants							
Without Bio-stimulants		43.40	42.13	35.48	34.33	8.01	7.93
Bio-stimulants		53.30	51.46	38.53	36.95	8.48	8.44
L.S.D		2.07	2.14	2.18	2.19	0.17	0.19
Effect of spraying anti-stressors							
Water spray		44.85	43.15	35.36	33.35	7.95	7.83
Amino acid		50.90	49.56	37.96	36.95	8.43	8.45
Potassium silicate		49.26	47.66	37.60	36.50	8.35	8.32
Hydroxybenzoic acid		48.40	46.83	37.10	35.75	8.25	8.15
L.S.D		2.31	2.40	2.23	2.28	0.19	0.22
Effect of interactions between bio-stimulants and anti-stressors							
Without Bio-stimulants	Water spray	40.30	39.20	34.30	32.19	7.65	7.50
	Amino acid	45.20	43.91	36.32	35.41	8.21	8.15
	K- silicate	44.21	43.16	35.90	35.40	8.12	8.10
	H.B.A	43.90	42.26	35.40	34.30	8.05	7.96
Bio-stimulants	Water spray	49.39	47.10	36.41	34.50	8.25	8.15
	Amino acid	56.60	55.20	39.60	38.49	8.65	8.70
	k- silicate	54.30	52.15	39.30	37.60	8.58	8.55
	H.B.A	52.90	51.40	38.80	37.20	8.45	8.35
L.S.D		2.51	2.49	2.59	2.64	0.32	0.37

3.3. Nutrients status (shoot total carbohydrates, nitrogen percentage and C/N ratio

Data presented in table (5) showed that the effect of application of bio-stimulants, foliar spraying with anti-stressor and their interaction on the nutritional status of Balady mandarin trees during the two seasons of study 2019&2020. The results revealed that the total nitrogen, total carbohydrates and C/N ratio in shoots of balady mandarin trees significantly improved by supplying trees of bio-stimulants compared to un-treatment trees (control). These results could be attributed to the important role of bio-stimulants in increasing the nutrient uptake from the soil.

with regard to the application of foliar spraying of anti-stressor substances, the data illustrated in the same table show that all treatments of foliar spraying with anti-stresses led to significant increased in total nitrogen, total carbohydrates and C/N ratio in shoots of Balady mandarin trees in the two seasons of the study 2019 and 2020 comparing to spraying trees water only (check treatment) and there were no significant differences among of the three anti-stress. The highest values of total carbohydrates and C/N ratio in shoots were recorded on trees that sprayed with potassium silicate (11.77 and 10.12) and (9.14 and 8.56), respectively followed by amino acids which recorded (11.71 and 10.0) and (9.02 and 8.35), respectively during the two seasons of study. While the maximum values of total nitrogen in shoots were recorded on the trees sprayed with amino acids. (1.30 and 1.20) in both seasons, respectively. So, it can be said that foliar spraying of anti-stress led to enhance the nutritional status of Balady mandarin trees which reflected on the vigor and health of trees, and thus their increased resistance of abiotic stress. All interactions between the factors of studied (bio-stimulants and anti-stress) showed a positive effect on the total nitrogen, total carbohydrates and C/N ratio in the shoots of Balady mandarin trees compared to un-treated trees. The maximum values of total carbohydrates and C/N ratio in the shoots were recorded in the trees that have been supplied with bio stimulants plus foliar spray by potassium silicate. While the minimum values in this traits were recorded on the untreated trees

Table 4. Effect of bio-stimulants and anti-stressors on contents of N, P, K and Chlorophyll% in the leaves of Balady mandarin trees during 2019&2020 seasons.

treatments	parameters	N %		P %		K %		Chlorophyll%	
		2019	2020	2019	2020	2019	2020	2019	2020
Effect of application bio-stimulants									
Without Bio-stimulants		2.42	2.56	0.26	0.27	1.40	1.44	69.93	70.35
Bio-stimulants		2.67	2.77	0.31	0.34	1.56	1.63	81.73	83.13
L.S.D		0.08	0.09	0.01	0.01	0.05	0.06	1.68	1.74
Effect of spraying anti-stressors									
Water spray		2.23	2.37	0.23	0.25	1.37	1.45	70.90	73.00
Amino acid		2.71	2.81	0.33	0.34	1.52	1.55	78.60	79.80
Potassium silicate		2.65	2.76	0.30	0.33	1.55	1.63	77.55	77.70
hydroxybenzoic acid		2.61	2.72	0.28	0.30	1.48	1.52	76.25	76.45
L.S.D		0.11	0.10	0.02	0.02	0.07	0.08	1.26	1.29
Effect of interactions between bio-stimulants and anti-stressors									
Without Bio-stimulants	W. spray	2.11	2.25	0.19	0.21	1.24	1.31	65.9	67.7
	Amino acid	2.59	2.71	0.30	0.31	1.45	1.47	72.1	72.4
	K- silicate	2.51	2.66	0.28	0.29	1.48	1.54	71.3	71.1
	H.B.A	2.48	2.61	0.27	0.26	1.41	1.42	70.4	70.2
Bio-stimulants	W. spray	2.35	2.48	0.26	0.29	1.49	1.58	75.9	78.3
	Amino acid	2.82	2.91	0.36	0.37	1.58	1.63	85.1	87.2
	k- silicate	2.79	2.86	0.32	0.36	1.62	1.71	83.8	84.3
	H.B.A	2.73	2.81	0.29	0.34	1.55	1.61	82.1	82.7
L.S.D		0.13	0.14	0.03	0.04	0.11	0.13	1.98	2.04

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Table 5. Effect of bio-stimulants and anti-stressors on total carbohydrates%, total nitrogen % and C/N ratio in shoots of Balady mandarin trees during 2019 & 2020 seasons.

treatments	parameters	Shoot total carbohydrates %		Shoot total nitrogen %		C/N ratio	
		2019	2020	2019	2020	2019	2020
Effect of application bio-stimulants							
Without Bio-stimulants		10.01	8.66	1.20	1.13	8.35	7.64
Bio-stimulants		12.22	10.56	1.32	1.18	9.25	8.97
L.S.D		0.69	0.51	0.06	0.07	0.19	0.17
Effect of spraying anti-stressors							
Water spray		9.47	8.57	1.18	1.08	7.99	7.94
Amino acid		11.71	10.00	1.30	1.20	9.02	8.35
Potassium silicate		11.77	10.12	1.29	1.18	9.14	8.56
hydroxybenzoic acid		11.50	9.75	1.28	1.17	9.00	8.35
L.S.D		0.57	0.61	0.05	0.07	0.35	0.35
Effect of interactions between bio-stimulants and anti-stressors							
Without Bio-stimulants	Water spray	8.28	7.71	1.13	1.09	7.33	7.07
	Amino acid	10.62	8.98	1.23	1.16	8.64	7.74
	K- silicate	10.67	9.15	1.22	1.15	8.75	7.96
	H.B.A	10.45	8.78	1.21	1.13	8.63	7.77
Bio-stimulants	Water spray	10.65	9.43	1.23	1.07	8.66	8.81
	Amino acid	12.79	11.01	1.36	1.23	9.40	8.95
	k- silicate	12.87	11.08	1.35	1.21	9.53	9.16
	H.B.A	12.55	10.71	1.34	1.19	9.37	8.93
L.S.D		1.38	1.28	0.08	0.09	0.71	0.58

3.4. Yield and its components

Yield and its components per tree of Balady mandarin trees during 2019 and 2020 seasons in response to application of bio-stimulants and foliar spraying of anti-stressors (amino acids, potassium silicate and hydroxybenzoic acid) and interaction of them are given in Table (6). From the obtained data it could be concluded that supplying trees with bio-stimulants had a significant effect on fruit retention, number of fruits and yield/ tree. Where trees treated with bio-stimulants were recorded the highest values of fruit retention%, number of fruit and total yield per tree. While the lowest values of these traits were recorded on the untreated trees. The results took a similar trend in the two seasons of study. The positive effect of the bio-stimulants on growth and nutritional status of trees surely reflected on enhancing the fruit retention%, number of fruits and yield per tree.

With regard the effect of anti- stressors namely, (amino acids, potassium silicate and hydroxybenzoic acid) on the yield and its components per tree. Data in the same table show that fruit retention%, number of fruit and yield per tree significantly affected by foliar spraying of these materials compared to non-application (control). Foliar spraying of anti-stressors increased the remaining of fruit %, fruits number and yield/ tree.

The superiority of foliar spraying of amino acids over the other spraying treatments in increased the yield and its components per tree. The highest values of fruit retention%, number of fruits and yield per tree were recorded on the trees sprayed four times with amino acids at 0.1% (1.69 and 1.84), (350.75 and 389.80) and (54.81 and 61.85) in both seasons, respectively followed by the trees sprayed four times with potassium silicate at 0.1% which recorded (1.64 and 1.80), (344.60 and 383.59) and (52.20 and 59.93) in the two seasons, respectively. While the lowest values of these traits were recorded on water sprayed trees (1.42 and 1.57), (310.65 and 354.40) and (41.37 and 49.94), respectively, these results were obtained in the two seasons of study 2019&2020.

All the possible combinations between adding the bio-stimulants and spray of anti-stressors were significantly affected the yield and its components of Balady mandarin trees. The maximum values with regard to fruit retention%, fruits number and yield per tree were recorded on the trees that have been supplied with bio-stimulants through soil and foliar sprayed with amino acids (1.87 and 2.01), (370. 2 and 401.5) and (63.84 and 68.80) compared to the trees untreated with bio-stimulants and any of anti-stressors (1.31 and 1.45), (292.8 and 338.2) and (34.51 and 42.13) in the 2019&2020 seasons.

Table 6. Effect of bio-stimulants and anti-stressors on the yield and its components of Balady mandarin trees during 2019 & 2020 seasons.

treatments	parameters	Fruit retention %		Fruits number / tree		Yield / tree (kg)	
		2019	2020	2019	2020	2019	2020
Effect of application bio-stimulants							
Without Bio-stimulants		1.44	1.61	316.53	363.60	41.40	50.24
Bio-stimulants		1.73	1.88	356.65	389.03	58.32	64.78
L.S.D		0.09	0.11	4.60	3.81	1.25	1.31
Effect of spraying anti-stressors							
Water spray		1.42	1.57	310.65	354.40	41.37	49.94
Amino acid		1.69	1.84	350.75	389.80	54.81	61.85
Potassium silicate		1.64	1.80	344.60	383.59	52.20	59.93
hydroxybenzoic acid		1.61	1.77	340.35	377.45	51.07	58.33
L.S.D		0.16	0.18	8.58	9.32	3.04	3.28
Effect of interactions between bio-stimulants and anti-stressors							
Without Bio-stimulants	Water spray	1.31	1.45	292.8	338.2	34.51	42.13
	Amino acid	1.51	1.67	331.3	378.1	45.77	54.89
	K- silicate	1.48	1.66	323.1	372.4	43.06	52.77
	H.B.A	1.46	1.64	318.9	365.7	42.26	51.16
Bio-stimulants	Water spray	1.52	1.68	328.5	370.6	48.23	57.74
	Amino acid	1.87	2.01	370.2	401.5	63.84	68.80
	k- silicate	1.77	1.94	366.1	394.8	61.34	67.08
	H.B.A	1.75	1.89	361.8	389.2	59.87	65.49
L.S.D		0.23	0.25	11.93	13.15	4.65	5.23

3.5. Fruit quality properties

Data in table (7&8) revealed that fruit quality properties especially sunburned fruits (%) were significantly affected by application bio-stimulants and foliar spraying of the anti-stressors and its interaction during in both seasons of study 2019&2020.

Data discerned that a significant increase in all fruit properties that has been studied such as (fruit weight, T.S.S (%), total and reducing sugars, and V.C content) as well as significant decrease in sunburned fruits (%) and total acidity of juice due to supply the trees by bio-stimulants via soil compared to the untreated trees.

Also, from the data received in the same table it could notice that a significant improve in the fruit quality properties on trees that sprayed with any anti-stress (amino acids, potassium silicate and hydroxybenzoic acid) compared to untreated trees. Spraying the trees with amino acids gave the best results in fruit weight. While spraying the trees with potassium silicate gave the maximum values in T.S.S, sugars content and V.C as well as the lowest values of sunburned fruits (%) and juice total acidity in the two seasons of study. While the minimum values in fruit quality were recorded on the trees sprayed with water only (control). Moreover, all possible interactions between the bio-stimulants application along with spraying of anti-stress were very effective in improving fruit quality. The trees treated with bio-stimulants and sprayed with potassium silicate gave the best fruit quality and the lowest sunburned fruits (%) followed by trees treated by bio-stimulants along with sprayed amino acids. The improvement in the fruit quality and reduced the sunburned fruits (%) due to supplied the trees by bio-stimulants alongside sprayed with anti-stress can be attributed to their important role in increasing the trees resistance to abiotic stress.

Table 7. Effect of bio-stimulants and anti-stressors on fruit weight, sunburned fruits % and TSS % of Balady mandarin fruits during 2019 & 2020 seasons.

treatments	parameters	Fruit weight (gm)		Sunburned fruits (%)		T.S.S (%)	
		2019	2020	2019	2020	2019	2020
Effect of application biostimulants							
Without Bio-stimulants		131.27	138.56	9.92	8.68	10.83	11.06
Bio-stimulants		163.92	167.44	6.11	5.74	11.71	11.73
L.S.D		5.98	6.15	1.61	1.87	0.27	0.30
Effect of spraying anti-stressors							
Water spray		134.32	141.17	14.27	12.29	10.81	11.16
Amino acid		155.80	159.72	6.74	6.10	11.39	11.49
Potassium silicate		151.92	156.97	4.85	4.31	11.59	11.79
hydroxybenzoic acid		148.33	151.14	7.21	6.13	11.30	11.15
L.S.D		6.43	7.79	0.99	1.10	0.05	0.06
Effect of interactions between biostimulants and anti-stressors							
Without Bio-stimulants	Water spray	119.27	125.47	17.12	15.24	10.15	10.74
	Amino acid	138.35	146.67	8.14	7.27	11.06	11.15
	K- silicate	135.67	142.84	6.09	5.11	11.14	11.39
	H.B.A	131.79	139.27	8.33	7.08	10.96	10.96
Bio-stimulants	Water spray	149.37	156.87	11.41	9.33	11.46	11.58
	Amino acid	173.25	172.76	5.34	4.92	11.72	11.82
	k- silicate	168.17	171.10	3.60	3.51	12.03	12.18
	H.B.A	164.87	169.01	6.10	5.18	11.64	11.35
L.S.D		5.80	6.63	1.62	1.70	0.27	0.30

Table 8. Effect of biostimulants and anti-stressors on reducing, total sugars, total acidity and V.C of Balady mandarin fruits during 2019 & 2020 seasons.

treatments	parameters	Total sugars %		Reducing sugars %		Acidity %		V.C (Mg/100gm)	
		2019	2020	2019	2020	2019	2020	2019	2020
Effect of application biostimulants									
Without Biostimulants		8.17	8.07	3.15	3.08	1.20	1.24	41.78	40.41
Biostimulants		9.18	8.95	3.57	3.48	1.11	1.16	46.14	45.11
L.S.D		0.15	0.12	0.11	0.14	0.06	0.07	1.82	2.04
Effect of spraying anti-stressors									
Water spray		8.10	7.95	3.03	2.95	1.37	1.43	39.79	37.39
Amino acid		8.70	8.53	3.45	3.37	1.12	1.15	44.93	44.21
Potassium silicate		9.26	9.07	3.57	3.50	1.05	1.08	46.50	45.48
hydroxybenzoic acid		8.66	8.49	3.40	3.30	1.08	1.13	44.63	43.95
L.S.D		0.29	0.26	0.13	0.10	0.11	0.12	0.40	0.50
Effect of interactions between biostimulants and anti-stressors									
Without Biostimulants	W. spray	7.85	7.61	2.85	2.72	1.45	1.48	37.92	35.65
	Amino acid	8.25	8.15	3.25	3.15	1.15	1.20	42.85	41.66
	K- silicate	8.41	8.38	3.32	3.31	1.08	1.11	43.95	42.95
	H.B.A	8.19	8.12	3.19	3.12	1.10	1.17	42.41	41.35
Biostimulants	W. spray	8.35	8.29	3.21	3.17	1.29	1.38	41.65	39.13
	Amino acid	9.15	8.91	3.65	3.59	1.09	1.10	47.01	46.75
	k- silicate	10.11	9.75	3.81	3.69	1.01	1.05	49.04	48.01
	H.B.A	9.12	8.85	3.61	3.48	1.05	1.09	46.85	46.55
L.S.D		0.40	0.36	0.10	0.12	0.16	0.17	1.89	2.10

Therefore, it can be said that supplying trees of Balady mandarin with bio-stimulants through soil and spraying with anti-stress is very important to obtain the best yield and quality of fruits under the unfavorable environmental conditions.

4. Discussion

The nutritional status of trees greatly affects their ability to acclimate to environmental stress conditions, where the elements macro and micro-nutrients plays a big major role in the employ of absorbed light energy and plays many roles in physiological processes within the tree, in addition to the large cycle in increasing tree resistance to environmental stresses (Marschner, 1995; Waraich *et al.*, 2011).

So, the use of plant bio-stimulants become very important and a basic practice in agriculture where the bio-stimulants activate the tree to absorb the macro and micro-elements, leading to an increase in the metabolism and an increase in the elements content in the trees and led to the stimulation of growth and cell division and development, regulation of carbohydrate metabolism, in addition to enhance the plants resilience to climate perturbations and improvement of the characteristics of vegetative growth, yield and fruits quality (Sharawy, 2005; Du-Jardin, 2015; Brown and Saa, 2015; Vandenkoornhuyse *et al*., 2015; Van Oosten *et al*., 2017; De Vasconcelos and Chaves, 2019). These results are agreement with those obtained by (Ebrahiem *et al*., 2000; El-Salhy *et al*., 2002; Abdel Hamied-Sheren, 2014; Abo batta and El-Azazy, 2020). The improvements in plant growth could be attributed to large increases in plant nutrition after bio-stimulants application. Overall, it can be concluded that an appropriate nutrient supply is crucial to attain the best growth in fruit trees. These results are agreement with (Ebrahiem *et al*., 2000; El-Salhy *et al*., 2002) on Balady mandarin trees, and Abdel Hamied-Sheren, (2014) on Valencia orange. (Waraich *et al*., 2011; El Kheshin, 2016) stated that the nutritional status of plants greatly affects their ability to acclimate to environmental stress conditions, and the elements can many roles in physiological processes within the tree, in addition to the large role in an increasing tree resistance to environmental stresses.

The anti-stressors working to stop the internal physiological disturbances which the accompanying of high temperature and therefore succeed in alleviating the deleterious effects of heat stress on the trees, due to their roles antioxidants in quench formed reactive oxygen radicals or activating enzymes related with scavenging and removing the toxic and degradable ROS away from the center of the active metabolic machinery of plant tissues i.e. peroxidase, superoxide dismutase and catalase (Abd El- Rahman and El- Masry, 2012; Al-Wasfy, 2012; Abd El-Naby *et al*., 2005; El-Tanany *et al*., 2019; Mohamed *et al.*, 2019; Abd El-Naby *et al*., 2020 a & b).

Furthermore, anti-stressors application increase water potential through decreasing water loss by transpiration and protection of plants from climatic conditions inappropriate which helps in keeping healthy plant. (Abd El-Kader *et al*., 2006; Masoud, and El- Sehrawy, 2012).

The significant increments were obtained in the growth and yield characters over the control may be due to the protective and recovered specific transporter enzymes and/or the whole machinery under stress conditions (Palta, 1990) where, foliar application of anti-stressors on the trees have the beneficial effects on the metabolic potential for synthesis of amino acids, proteins, sugars and carbohydrates, as well as, their antioxidant defensive function, corresponding with normal growth and high yielding capacity (Al- Wasfy, 2012). Nevertheless, the beneficial effects of the applied treatments (bio-stimulants application and anti-stressors foliar spray) may be explained due to the nutritional status of trees greatly affects their ability to adapt to adverse environmental conditions, and the increase in trees growth by relatively high levels may be attributed to the valuable effects of nutrients on stimulating the meristematic activity, for producing more tissues and organs, and cell enlargement, synthesis of structural proteins and other several macro-molecules, in addition to its vital contribution in several biochemical processes in the plant related to growth .

Generally, the plant nutrients have different functions in plant metabolism, accordingly, their influences on specific yield parameters is a complex, especially under conditions of abiotic and biotic stress (Al-wasfy, 2012). Specific and non-specific nutrient interactions in the soil and the plants must also be taken into account.

5. Conclusion

In light of the results obtained from this study, it can be concluded that the improvements in plant growth and increment of fruit yield and quality could be attributed to large increases in plant nutrition after bio-stimulants applications and foliar spray with anti-stressors substances which led to increase the resistance trees to abiotic stress when climate condition are not suitable. Therefore, it can be said that supplying trees of Balady mandarin with bio-stimulants through soil and spraying with anti-stress is very important to obtain the best yield and quality of fruits under the unfavorable environmental conditions.

So, it can be recommended using bio-stimulants via soil and spraying the anti-stressors as part of an agricultural strategy to overcome the negative effects of climate changes is highly dependent on plant nutritional status.

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Data presented in this study are available on fair request from the respective author.

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This work carried out at Horticulture department.

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Conflicts of Interest

declare no conflict of interest.

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