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RESPONSE OF VALENCIA ORANGE TREES GROWN UNDER SANDY SOIL TO MITIGATION OF HEAT STRESS BY MELATONIN, GIBBERELLIN AND SALICYLIC ACID APPLICATION

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Abstract

Rise in temperature stress can be detrimental to trees, resulting in reduced fruit yield and increased incidences of fruit disorders. Some compounds may play a definite role in solving the problem of poor yielding in citrus through enhancing growth, nutritional statues, and quality. Therefore, this study was carried out during two successive growing seasons (2017 and 2018) to reduce the impact of heat stress on the growth, productivity and quality of Valencia orange trees grown in the National Research Centre farm for research and production in Al-Nubaria region, Al-Behira Governorate, Egypt by foliar spray of hormonal substances and antioxidants i.e. (melatonin "MEL", gibberellic acid "GA_3" or salicylic acid "SA") each at concentration (25 mg/L). It can be concluded that spraying with gibberellic acid showed the best vegetative growth of trees and led to the highest crop followed in descending order by spraying with melatonin. All spray materials affected the leaf content of major and minor elements. Most characteristics of the resulting fruits did not differ significantly between all treatments, especially in the first season.

Keywords: Valencia orange, melatonin, gibberellin, salicylic acid, growth, nutrients status, yield, fruit quality.

Introduction

Temperature is the most significant factor affecting on fruits. Temperature sensitive crops include perennial crops such as almonds, grapes, berries, citrus and stone fruits (Lobell and Field, 2011). Different citrus varieties have varied responses to abiotic and biotic stresses which can show negative responses to heat/ light. Warmer spring temperatures can result in reduced fruit yields in the form of trees shut down, reduced photosynthesis, flowers drop and cause fruit sheds, smaller fruit and lower yields, poorer quality fruit (Pope 2012 and Beppu and Kataoka, 2011).

Melatonin is an indolic compound (biogenic indoleamine) structurally related with other important substances, such as tryptophan, serotonin, indole-3-acetic acid (IAA). It can act as a potential modulator of plant growth and development in a dose-dependent manner (Li *et al*., 2012). Indoleamine is currently understood an ubiquitous and conserved compound, found in evolutionarily distant organisms: bacteria, mono- and multicellular algae, fungi, higher plants, invertebrates and vertebrates (Posmyk and Janas,2009)The dual role of melatonin in plants have recognized as a protector against abiotic and biotic stresses (Wang *et al*., 2012). Melatonin was involved in many plant functions as delaying flower induction (Kolár *et al*., 2003) and protection against chlorophyll degradation in aging leaves of barley "*Hordeum vulgare* L." (Arnao and Hernández-Ruiz, 2009) and detached leaves of apple "*Malus domestica* Borkh. cv. Golden Delicious" protecting the photosystems from damage (Wang *et al*., 2012). Melatonin functions in plants can be recognized into three categories: firstly; growth promoters as auxins (Kolár and Machácková, 2005); secondly; antioxidants for free radicals and serve as a first-line defense against oxidative stress (Van Tassel *et al*., 2001); thirdly; In plants, MEL is considered to be involved in many physiological processes, *e.g.* root and shoot development (Park, 2011), flowering, flower and fruit development or delaying leaf senescence (Kolár *et al*., 2003), ion homeostasis (Sarropoulou *et al*., 2012) and other functions (signal molecules for regulation of flower development, or maintenance of developmental stages in fruit tissues) (Paredes *et al*., 2009). Arnao and Herna´ndez-Ruiz (2006) hypothesized that exogenous melatonin might cause changes in the concentration of endogenous free IAA. The chemical structure of melatonin (indoleamine) is similar to auxin-IAA hormone. This indolamine also increased photosynthetic efficiency of chlorophyll in plants (Tan *et al*., 2012).

Gibberellic acid (GA3), Gibberellins are natural growth hormones playing a primary role in stimulating the auxin reaction, that help in growth and development of many plants as well as its direct effect on internode elongation, flowering, fruiting, quality and yield. Such, has numerous physiological effects on germination, stem elongation, leaf expansion, growth, flowering and cell expansion (Taiz and Zeiger, 2010). Exogenous application of GA3 to plants causes the increase in the activities of many key enzymes and photosynthesis (Aftab *et al*., 2010). Spraying the olive trees with GA3 at 75 ppm on first December led to an increase in vegetative growth and to improve in fruit characteristics (Abd El-Naby *et al*., 2012).

Salicylic acid is an endogenous growth regulator of phenolic nature and acts as potential non-enzymatic antioxidant that participates in the regulation of many physiological processes in plants, such as stomatal closure, photosynthesis, ion uptake, inhibition of ethylene biosynthesis, transpiration and stress tolerance (Khan *et al*., 2003 and Simaei *et al*., 2012). Salicylic acid stimulates flowering, increases flower life, retards senescence, and increases cell metabolic rate (Bhupinder and Usha, 2003). It can regulate plant growth (Amanullah *et al*., 2010). SA application influences a wide variety of plant processes, induction of antioxidant synthesis (Yordanova and Popova, 2007). SA is a key signal molecule for expression of multiple modes of plant stress resistance which has been recognized as a regulatory signal mediating plant response to abiotic stresses such as heat (Larkindale *et al.,* 2005), and osmotic

stress (Borsani *et al.,* 2001). The efficacy of SA application depending greatly on plant species, stage of growth, concentration, method and time of application and environmental conditions as reported by Senaratna *et al*. (2000).

This work aimed to investigate the growth promoting activity of some compounds (Gibberellic acid or salicylic acids or Melatonin,) on growth of Valencia orange trees and its reflection on yield and yield components as well as fruit quality.

Materials and Methods

Plant

Valencia orange (*Citrus sinensis* Osbeck) trees, grafted on volkamer lemon rootstock (*C. volkameriana,* L), were about twelve years old, and in healthy and uniform condition, planted in a system of 3.5 x 5 meters, and grown on sandy soil in the National Research Centre farm for research and production in Al-Emam Malek village, Al- Nubaria region, Al-Behira Governorate, Egypt. This study was carried out during two successive growing seasons (2017 and 2018).

Foliar spray compounds

The use of hormonal substances and antioxidants (gibberellic acid "GA_3", salicylic acid "SA" melatonin "MEL") as foliar spray was in each season in mid of April and at concentration (25mg/L)to reduce the impact of heat stress on the growth, productivity and quality.

GA_3 (product from Science Lab. com, Inc. Chemical Laboratory Equipment, 14025, Smith Road, Houston, Texas 77396, USA).

SA (product from Bio-World 4150 Tuller Rd., Ste 228Dublin, OH43017, USA). Salicylic acid was initially dissolved in a few drops of Dimethyl sulfoxide and the final volume was reached by adding distilled water, then the pH was adjusted at 6-7 with NaOH (1.0N).

MEL (product from ScienceLab.com, Inc. Chemical Laboratory Equipment, 14025, Smith Road, Houston, Texas77396, USA). Melatonin is soluble in both water and lipid so it may act as a universal hydrophilic and hydrophobic antioxidant.

This was done comparing with the trees sprayed with water (control). All spray solutions contained 0.1% triton B as a wetting agent to avoid the surface tension and sprayed till run off. Trees each treatment received 20 L of the applied solution, in the morning hours of the day in mist form. A complete randomized block design was adopted in this experiment with four treatments, where each treatment contained three replicates with one tree each.

Other horticultural practices were similar for all trees and also as recommended from national campaign for improving citrus productivity in Egypt. Drip irrigation system was used to irrigate all the trees.

Measurements

Growth and yields: in early September, leaves area were measured using the formula of 0.608 constant x (maximum leaf length x maximum leaf breadth) according to Shrestha and Balakrishnan (1985). Number of shoots /one meter branch, Number of leaves/shoot and shoot length were measured. Leaf chlorophyll content was determined as CCI (Chlorophyll Content Index) using Chlorophyll content Meter 003109(CCM-200 plus Opti -Sciences). At commercial harvest in early April; yield as weight and number of fruit per tree were recorded. Canopy volume of trees was measured in early December which tree shape was considered as a one-half of a probate sphere (volume = 4/6 x π x height x radius2 "which π = 22/7") as described by Roose *et al*. (1989). Cropping efficiency was calculated by dividing the fruit yield weight by the tree canopy according to Whitney *et al*. (1995).

Leaf mineral composition: leaf samples were collected in early September and were mature fully expand from non-fruiting non flushing spring cycle growth (5 old month) according to Jones and Embleton (1960), then washed, dried at 70°C until a constant weight, ground and digested using an acid mixture consisting of nitric, perchloric and sulfuric acids in the ratio of 8:1:1(v/v), respectively according to Chapman and Pratt (1978). Nitrogen was measured by semi-micro Kjeldahl method of Plummer (1978). Phosphorus was determined using a spectrophotometer at 882-OVV by the method outlined by Jackson (1973). Potassium, calcium, sodium were determined by a flame photometer "Jenway PFP7". Magnesium, iron, manganese, zinc, copper were determined using atomic absorption Spectrophotometer "Perkin Elmer 1100" (Cottanie *et al*., 1982). These measurements were performed in Agricultural Services Unit and Laboratory Analysis of Research Project (Micronutrients and Other Plant Nutrition Problems in Egypt) in NRC.

Fruit quality: ten fruits were randomly sampled per each tree for determination of weight, diameter, peel thickness, then from the juice, total soluble solids percentage (TSS Brix %) determined by Carl Zeiss hand refractometer; total acidity as anhydrous citric acid % and vitamin C. was expressed as mg ascorbic acid per 100 ml juice according to A.O.A.C.(1995).

Statistical analysis: The data obtained in each season were analyzed by ANOVA according to Snedecor and Cochran (1982). Means were separated by Duncan (1955) and multiple range test using a significance level of $P<0.05$.

Results

Table (1) shows that the values of the estimated characteristics (number of branches produced, number of leaves formed and leaf area) under all sprayed materials on orange trees have improved in the second season comparing with the first season.

It also explained that spraying orange trees with GA_3 gave the best growth represented by the largest number (of shoots/one meter branch and leaves /shoot) and the highest chlorophyll content in the leaf and also the leaf area during the two seasons of the study.

Whereas spraying MEL was at the same level significantly in affecting the number of shoots, the length of the shoot and the leaf area, especially in the second season, and the chlorophyll content of the leaf, especially in the first season.

Whereas the effect of spraying with SA was in the third place compared to the trees that were sprayed with water.

Table 1: Mitigation of heat stress effects by using gibberellic acid, salicylic acid and melatonin on vegetative growth characteristics and leaf chlorophyll content of Valencia orange trees during 2017 and 2018 season.

characteristics / Seasons / Treatments	No. of shoots / one meter branch		Shoot length (cm)		No. of leaves/ Shoot		Leaf area (cm^2)		chlorophyll content index (CCI)	
	2017	2018	2017	2018	2017	2018	2017	2018	2017	2018
Gibberellic Acid	18.670 A	22.667 A	48.330 A	71.667 A	28.090 A	47.667 A	19.807 B	26.893 A	111.58 A	95.700 A
Salicylic Acid	14.670 B	18.667 A	45.000 B	76.667 A	22.850 B	28.667 B	19.170 B	23.293 B	89.58 A	78.933 B
Melatonin	14.000 B	22.333 A	49.830 A	78.333 A	22.610 B	35.000 AB	20.003 B	27.670 A	104.28 A	84.933 B
Control (water spray)	12.170 C	13.333 B	37.993 C	41.667 B	21.150 C	23.333 B	27.623 A	24.703 B	78.40 A	81.867 B

Values followed by the same letter/s over each column didn't significantly differ at 5% level

In Table (2), it was found that the materials used in spraying orange trees maintained the appropriate level for the values of the major elements in the leaf, spraying GA_3 showed significantly higher values of (N) in the first season and (K-Ca-Mg) in the two seasons. Spray SA showed the highest values of (Ca) in the first season and (P-Mg) in the two seasons. While spraying MEL showed the highest values of (Ca) in the first season and (K) in the second season.

Table 2: Effect of using gibberellic acid, salicylic acid and melatonin on some minerals content of Valencia orange leaf during 2017 and 2018 seasons.

Characteristics	N(g/100g)		P(g/100g)		K(g/100g)		Ca(g/100g)		Mg(g/100g)	
Seasons / Treatments	2017	2018	2017	2018	2017	2018	2017	2018	2017	2018
Gibberellic Acid	2.38 B	2.540 A	0.360 BC	0.320 B	1.71 A	1.84 A	5.90 A	6.40 A	0.470 A	0.450 A
Salicylic Acid	2.48 A	2.360 B	0.500 A	0.480 A	1.24 C	1.08 B	6.00 A	5.90 AB	0.380 B	0.460 A
Melatonin	2.38 B	2.400 B	0.400 B	0.380 AB	1.48 B	1.96 A	5.78 A	6.15 AB	0.390 AB	0.370 B
Control (water spray)	2.08 C	2.047 C	0.300 C	0.320 B	1.76 A	1.95 A	6.00 A	5.15 B	0.450 AB	0.480 A

Values followed by the same letter/s over each column didn't significantly differ at 5% level

Adequate ranges for citrus leaf were :2.4-3.5 (N), 0.15-0.3 (P), 1.2-2.0 (K), 3-7(Ca),0.25-0.7(Mg). (Werner, 1992).

Table (3) showed that all spray treatments of orange trees maintained the appropriate level for the values of the micronutrients in the leaf. Where spraying MEL showed higher values of significance in the leaf content of (Fe- Mn) as it was significantly on the bar with spraying SA in the values of (Mn - Na) during the two seasons of study. While spraying GA_3 gave the highest values of Zinc. Thus, the values varied between the sprayed and the non-sprayed trees.

Table 3: Effect of using gibberellic acid, salicylic acid and melatonin on some minerals content of Valencia orange leaf during 2017 and 2018 seasons.

characteristics	Fe(ppm)		Mn(ppm)		Zn(ppm)		Cu(ppm)		Na(g/100g)	
Seasons / Treatments	2017	2018	2017	2018	2017	2018	2017	2018	2017	2018
Gibberellic Acid	93.00 B	80.00 C	29.00 A	27.70 B	14.00 AB	14.00 A	6.00 B	4.40 C	0.090 B	0.390 A
Salicylic Acid	119.0 A	82.00 C	34.40 A	33.10 A	15.00 A	13.00 AB	6.00 B	7.00 B	0.320 A	0.390 A
Melatonin	122.0 A	172.0 A	32.00 A	30.70 A	14.00 AB	11.00 B	8.00 A	8.00 B	0.070 B	0.190 B
Control (water spray)	120.0 A	114.0 B	27.80 A	26.50 B	11.00 B	12.00 AB	8.00 A	11.00 A	0.380 A	0.210 B

Values followed by the same letter/s over each column didn't significantly differ at 5% level

Adequate ranges for citrus leaf were: 35-135 (Fe), 19-50 (Zn), 19-100 (Mn), 5-15 (Cu) (Wutscher and Smith 1994).

Table (4) showed that the orange trees which were sprayed with GA_3 showed an increase in the number of fruits and their weight and consequently the yield compared to those sprayed with water during the two seasons of the study. Also, the trees which were sprayed with MEL came in the second place. Likewise, the size of the growing tree under these treatments increased that those sprayed with water. Thus, the crop efficiency was higher when spraying with GA_3 was used during the two seasons.

Table 4: Mitigation of heat stress effects by using gibberellic acid, salicylic acid and melatonin on yield and its components of Valencia orange trees during 2017 and 2018 season.

characteristics Seasons / Treatments	Number of fruits(No.)		Fruit weight (g.)		Yield (Kg/tree)		Tree canopy (m^3)		Crop efficiency (Kg/m^3)	
	2017	2018	2017	2018	2017	2018	2017	2018	2017	2018
Gibberellic Acid	196.67 A	220.00 A	171.39 A	299.90 A	33.466 A	65.862 A	60.093 AB	81.913 A	0.5707 A	0.8233 A
Salicylic Acid	145.00 AB	177.00 B	193.89 A	315.83 A	27.955 AB	55.756 B	65.273 AB	93.091 A	0.4407 AB	0.6000 B
Melatonin	163.33 AB	207.00 AB	191.11 A	309.16 A	31.066 A	63.917 A	83.947 A	88.382 A	0.3693 B	0.7230 AB
Control (water spray)	130.00 B	135.00 C	166.94 A	242.77 B	21.265 B	32.735 C	45.183 B	46.141 B	0.4697 AB	0.7127 AB

Values followed by the same letter/s over each column didn't significantly differ at 5% level

It was noted in Table (5) that in the first season, all the compounds used did not cause a change in the characteristics of the fruits produced except that spraying GA_3 slightly improved the total soluble solids, but with spraying all the materials in the second season, increased the fruits diameter and decreased the total soluble solids, acidity and vitamin C..

Table 5: Mitigation of heat stress effects by using gibberellic acid, salicylic acid and melatonin on fruit characteristics of Valencia orange trees during 2017 and 2018 season.

Characteristics Seasons / Treatments	Fruit diameter (cm.)		Peel thickness (cm.)		Total soluble solids (%)		Titratable acidity (%)		Ascorbic acid (mg/100 ml juice)	
	2017	2018	2017	2018	2017	2018	2017	2018	2017	2018
Gibberellic Acid	6.6633 A	8.2660 A	0.4667 A	0.5880 A	11.930 A	11.333 B	1.8100 A	2.3333 B	36.667 A	34.600 C
Salicylic Acid	7.0267 A	8.2330 A	0.4887 A	0.5520 B	11.443 AB	11.100 B	1.7733 A	2.1333 B	36.240 A	38.400 BC
Melatonin	7.0867 A	8.3997 A	0.5443 A	0.5330 B	10.953 B	10.167 C	1.9067 A	2.0667 B	35.360 A	39.000 B
Control (water spray)	6.6600 A	7.5663 B	0.5333 A	0.5990 A	11.873 A	13.300 A	2.0767 A	2.9000 A	48.400 A	43.800 A

Values followed by the same letter/s over each column didn't significantly differ at 5% level

Discussion

There, problems associated with rise in temperature stress can be detrimental to trees, resulting in reduced fruit yield and increased incidences of fruit disorders. Which, induction of reactions, and hence results in decline of existing and augmented antioxidant activities (R´eblov´a, 2012). Also, during plant development, high temperatures can affect photosynthesis, respiration, water relations, and membrane stability as well as plant hormone levels, primary and secondary metabolites (Magan *et al*., 2011). SO, using of some compounds may play a definite role in solving the problem of poor yielding in citrus through enhancing growth, nutritional statues and yield. As well as, biostimulators are different kinds of non-toxic substances of natural origin that at low concentrations improve and stimulate plant life processes otherwise than fertilizers or phytohormones, which they can stimulate the synthesis of phytohormones, facilitate the uptake of nutrients from the substrate and contribute to a higher yield and improve its quality. As well as they are agents which increase resistance of plants to unfavorable conditions as extreme temperature, drought, heavy metals etc. (Basak 2008). Gibberellic acid is used widely in various horticultural crops for improving growth and productivity. Many investigators studied the role of GA_3 on some plants and coming to the opinion that, GA_3 caused an enhancement in plant growth and productivity (Pavlista *et al*., 2012; Rathod *et al*., 2015). Rajput and Singh (1982) reported that shoot length, leaf area, fruit weight and yield were increased when 16-year-old trees of the ber cv. Banarasi Karaka were treated with GA_3 at 20 ppm . Çolak (2018) investigated the effects of hormones application at different doses on fruit quality and yield ,which treated the leaves of jumbo blackberry with melatonin and gibberellic acid hormones, found that number and weight of fruit were most increased with the treatment of MEL + GA_3 10 ppm and MEL 10 ppm and in terms of the fruit size(length and width), the maximum efficiency was achieved with GA_3 5 ppm and MEL 10 ppm. It could conclude that, GA_3 spraying promotes vegetative growth through its effect of inhibiting flowering and increases the hormones responsible for vegetative growth suggests that the balance between endogenous inhibitors and gibberellins may be one of the factors associated with flower induction and differentiation in the olive. Therefore, the possibility of transition from floral to vegetative buds or retard the floral transition by exogenous GA_3 in "on" year may be useful to overcoming on alternate bearing phenomenon. (Abd El-Naby *et al*., 2012). Due to the fact that, melatonin possesses both lipophilic and hydrophilic properties, it may be easy for the molecule to cross morpho- and physiological barriers with minimal difficulty, resulting in the rapid transport of the molecule into plant cells, which One melatonin molecule may scavenge up to 10 free radicals (Tan *et al*., 2007). Melatonin antioxidant activity may manifest itself in several ways: (i) direct free radical scavenging, (ii)elevating the antioxidant enzyme activity, (iii) protecting antioxidant enzymes from oxidative damage, (iv) increasing the efficiency of mitochondrial transport chain and (v) reducing the generation of free radicals (Tan *et al*., 2010). Abd El-Naby *et al*. (2019) revealed that melatonin treatment was the most pronounced treatment on vegetative growth of apricot. Moreover, by boosting photosynthetic rate in response to enhanced antioxidant enzyme activities, it therefore appears that SA can generally be used as a growth regulator to enhance plant growth, nutritional status and yield (Ghasemzadeh and Jaafar, 2013). In addition, (Vazirimehr and Rigi, 2014) explaining that the promotive effect of salicylic acid could be attributed to its bio regulator effects on physiological and biochemical processes in plants such as

ion uptake. On the other hands, it is known that the range between beneficial and toxic effects of auxin can be quite narrow. The application of these hormones at low concentration regulates growth, and development, either by promotion or inhibition and allows physiological processes to occur at a normal rate (Naeem *et al*., 2004). It is worth to mention that all the major elements in the leaf of all the trees under the experiment were within the appropriate limits for the formation of healthy developing citrus leaf according to Werner (1992) and as that all the micro elements in the leaf of all the trees under the experiment were within the standards appropriate limits of the developing healthy citrus leaf according to Wutscher and Smith (1994). Moreover, there is reduction of some nutrients in response to some spraying treatments may be due to the increase in growth which depletes more amounts of those nutrients, besides, there are an increase in some other elements due to the availability of elements which slow release matches uptake by plant roots and prevents it from leaching. Also, this difference in the uptake of nutrients may be attributed to variation in the breakdown of some elements with time which may alter metal availability for crops (Alloway and Jackson1991). Under climate change is increased risk of nutrient leaching and future decrease in soil organic content (Olesen and Bindi 2002). Such, increase in tree canopy is important because the largest trees usually used the most water and results in highest fruit yield (Syvertsen and Smith, 1996). These results are in agreement with those reported by (Abd El-Naby and El-Sonbaty, 2016). On the other hands, Foliar application of salicylic acid has been shown to increase biomass and yield in a variety of plant species (Larqué-Saavedra and Martín-Mex, 2007; Javaheri *et al*., 2012). The effect of GA_3 on increasing T.S.S attributed to enhanced level of leaf chlorophyll in the treated grapevines ultimately resulted in increased rate of photosynthesis and accumulation of carbohydrate reserves in the vines (Khan *et al*., 2012). These auxins are known by their ability to increase the cell size (Westwood 1993 and Davis2004) and enhance fruit growth of Clementine (Agusti *et al*., 1995), date palm (Shabana *et al*., 1998 and Al-Juburi *et al*., 2001).

Conclusion

Generally, it could be summarized that spraying with GA_3 showed the best vegetative growth of plants and led to the highest crop followed by spraying with MEL. All spray materials affected the leaf content of major and minor elements. Most characteristics of the resulting fruits did not differ significantly between all treatments, especially in the first season. Therefore, we recommended It can be used these materials specially GA_3 and in descending order MEL on orange trees to obtain good growth and higher yield.

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