



MITIGATION OF HEAT STRESS EFFECTS ON WASHINGTON NAVEL ORANGE BY USING MELATONIN, GIBBERELLIN AND SALICYLIC TREATMENTS

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Abstract

One Farm strategy for adaptation to high temperatures and increasing the production of 'Washington navel' orange on sandy soil was achieved by spray some compounds to avoid the stress. Using hormonal substances and antioxidants (melatonin "Mel", gibberellic acid "GA₃", salicylic acid "SA") as a foliar spray was carried out in mid-April at two concentrations (25 and 50 mg/L) to reduce the impact of heat stress on the growth, productivity and quality of 'Washington navel' orange trees grown in the National Research Centre farm for research and production in Al-Nubaria region, Al-Behira Governorate, Egypt. This study was carried out during two successive growing seasons (2017 and 2018). Such, the low concentration of each of GA₃ and Mel show a positive effect on most of vegetative growth characteristics followed by SA at the high concentration. Also, spraying with Mel or GA₃ at low concentration had high values of many elements such as N, P, K and Mg followed by SA at high concentration which had high values of many elements such as Fe, Mn and Cu. Fruit set percentage of Washington navel orange was improved using all spraying materials especially GA₃ and Mel, and this resulted in improving number of fruits, fruit weight and tree yield in the two experimental seasons. So, it can be observed that the high yield which resulted from GA₃ spray at low concentration was due to the high fruits number while those resulted from Mel spray at low concentration was due to the high fruit weight, this was true in the first season whatever, in the second season, high yield resulted from GA₃ spray at low concentration or Mel spray at two concentrations was due to the high fruits number and weight, as high yield resulted from SA spray at low concentration was in second order comparing with unsprayed trees. Also, all spraying materials maintained good fruit quality.

Key words: navel orange, melatonin, gibberellin, salicylic, growth, nutrients status, yield, fruit quality, crop efficiency.

Introduction

Citrus is economically an important crop in Egypt. However, citrus is exposed to a number of abiotic (high temperature, drought and salinity) or biotic (insect, pest and disease) stresses that limit its yield potential. Citrus can be grown in a variety of arid and humid climates, and can withstand temperatures ranging from -2.2 to 40.6°C, but it performs best at temperatures between 15.6 and 30°C (Parsons and Beck, 2004). Temperature sensitive crops include perennial crops such as almonds, grapes, berries, citrus and stone fruits (Lobell and Field 2011). High percent fruits of varieties such as navel orange can be lost if heat occurs early in development.

Melatonin (N-acetyl-5-methoxytryptamine) was discovered in plants during 1995. It is widely present in many higher plants, dicotyledons and monocotyledons.

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Melatonin has been detected and quantified in roots, shoots, leaves, fruits and seeds of a considerable variety of plant species (Hattori *et al.*, 1995). Many microorganisms including bacteria and fungi produce melatonin (Hardeland and Poeggeler, 2003). 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). The dual role of melatonin in plants has been 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 Hernandez-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 Machackova, 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 (Kolar *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 Hernaández-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 increases photosynthetic efficiency of chlorophyll in plants (Tan *et al.*, 2012). Exogenously, applied MEL affects developmental processes during both vegetative and reproductive growth, so it seems that it may play a similar role in plants as this hormone.

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 their direct effect on internode elongation, flowering, fruiting, quality and yield. Such, they have 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 increase in the activities of many key enzymes and photosynthesis (Aftab *et al.*, 2010). GA₃ applied at fruit set is used extensively to increase the berry size of *Vitis vinifera* seedless table grapes, and primarily affect the growth by controlling the cell elongation and division, which reflected on yield and its components and fruit quality of various grape cultivars (Pires *et al.*, 2000).

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 and induces antioxidant synthesis (Yordanova

and Popova, 2007). Recently, SA has received a particular attention because it 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 salinity and drought (Chini *et al.*, 2004), chilling (Kang and Saltveit, 2002), heavy metal tolerance (Freeman *et al.*, 2005), heat (Larkindale *et al.*, 2005), and osmotic stress (Borsani *et al.*, 2001). The efficacy of SA application depends 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 (Melatonin, Gibberellic acid or salicylic acids) at two concentrations on growth of Washington navel orange trees and its reflection on yield and yield components as well as fruit quality.

Materials and methods

‘Washington navel’ orange (*Citrus sinensis* L. 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 × 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).

The use of hormonal substances and antioxidants (melatonin “MEL”, gibberellic acid “GA₃”, salicylic acid “SA”) as foliar spray was in each season in mid-April and either at two concentrations (25, 50 mg/L) to reduce the impact of heat stress on the growth, productivity and quality.

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 (Janas and Posmyk, 2013).

GA₃ (product from ScienceLab.com, Inc. Chemical Laboratory Equipment, 14025, Smith Road, Houston, Texas77396, USA).

SA (product from Bio-World 4150 Tuller Rd., Ste 228 Dublin, OH 43017, 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).

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. Each tree received 20 L of the applied solution, in the morning hours of the day in mist form.

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 trees. A complete randomized block design was adopted in this experiment with seven treatments, where each treatment contained three replicates with one tree each.

Measurements:

Growth and yields: In early September, leaf area was measured using the formula of $0.608 \text{ constant} \times (\text{maximum leaf length} \times \text{maximum leaf breadth})$ according to Shrestha and Balakrishnan (1985). Number of shoots/one-meter branch, Number of leaves/shoot and shoot length were measured. Chlorophyll content was determined as CCI (Chlorophyll Content Index) using Chlorophyll content Meter 003109 (CCM-200 plus Opti-Sciences). At commercial harvest (colour break) in early December, yield as weight and number of fruits per tree were recorded. Canopy volume of trees was measured in early December which tree shape was considered as a one-half of a prolate spheroid (volume = $\frac{4}{6} \times \pi \times \text{height} \times \text{radius}^2$ "which $\pi = 22/7$ ") as described by Roose *et al.*, (1989). Cropping efficiency was calculated by dividing the fruit yield weight by the canopy volume according to Whitney *et al.*, (1995).

Fruit set: During the last week of June in each season, fruit set percentage was determined using the following formula:

$$\text{Fruit\%} = \frac{\text{Number of fruits setting on the shoot}}{\text{Total number of flowers per shoot}} \times 100$$

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 at 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 and sodium were determined by a flame photometer "Jenway PFP7". Magnesium, iron, manganese, zinc and 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 the National Research Centre.

Fruit quality: ten fruits were randomly sampled per each tree to determine weight, diameter and peel thickness, then from the juice to estimate total soluble solids percentage (TSS Brix %) using Carl Zeiss hand refractometer, total acidity as anhydrous citric acid % and vitamin C. The latter 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's (1955) multiple range test using a significance level of $P < 0.05$.

Results and Discussion

Data presented in Fig. 1 show that all spraying materials improved vegetative growth characteristics of 'Washington navel' orange trees compared to unsprayed trees. Spraying trees with "Gibberellin 1" gave the highest number of shoots/branch, followed by "Gibberellin 2" in the first season, while, "Salicylic acid 2" or "Gibberellin 2" were the highest in the second season, respectively. Spraying trees with "Gibberellin 1" and "Salicylic acid 2" gave the highest Number of leaves/shoot in the first season, while using "Salicylic acid 1" and "Melatonin 1" were the best in the second season. Regarding shoot length, all spraying treatments improved it especially "Melatonin 1 and 2" in the two seasons. which "Salicylic acid 2" and "Melatonin 2" gave the highest leaf area in the first season, while, no obvious difference between treatments in the second season, despite the superiority of "Gibberellin 2" and "Melatonin 1 and 2" treatments. Also, spraying "Salicylic acid 1" gave the highest chlorophyll content index in the second season, while, no clear differences between all treatments in the first season.

Photosynthesis plays an important role in plant productivity and takes place in green leaves and depends on its content of chlorophyll. Such, the low concentration of each GA and Mel show the positive effect on almost all vegetative growth characteristics followed by SA at high concentration. 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, since one melatonin molecule may scavenge up to 10 free radicals (Tan *et al.*, 2007). Its 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. Conversely, foliar application of GA₃ at 100 mg/l led to an increase in plant height, leaf area, stem diameter and

dry matter production, but no effects on the number of leaves, number of stem branches (Leite, *et al.*, 2003). Rajput and Singh (1982) reported that shoot length and leaf area were increased when 16-years-old trees of the ber cv. Banarasi Karaka were treated with GA₃ at 20 ppm. In addition, (Vazirimehr and Rigi, 2014) explained 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. In grapevine leaves, SA participates in the regulation of

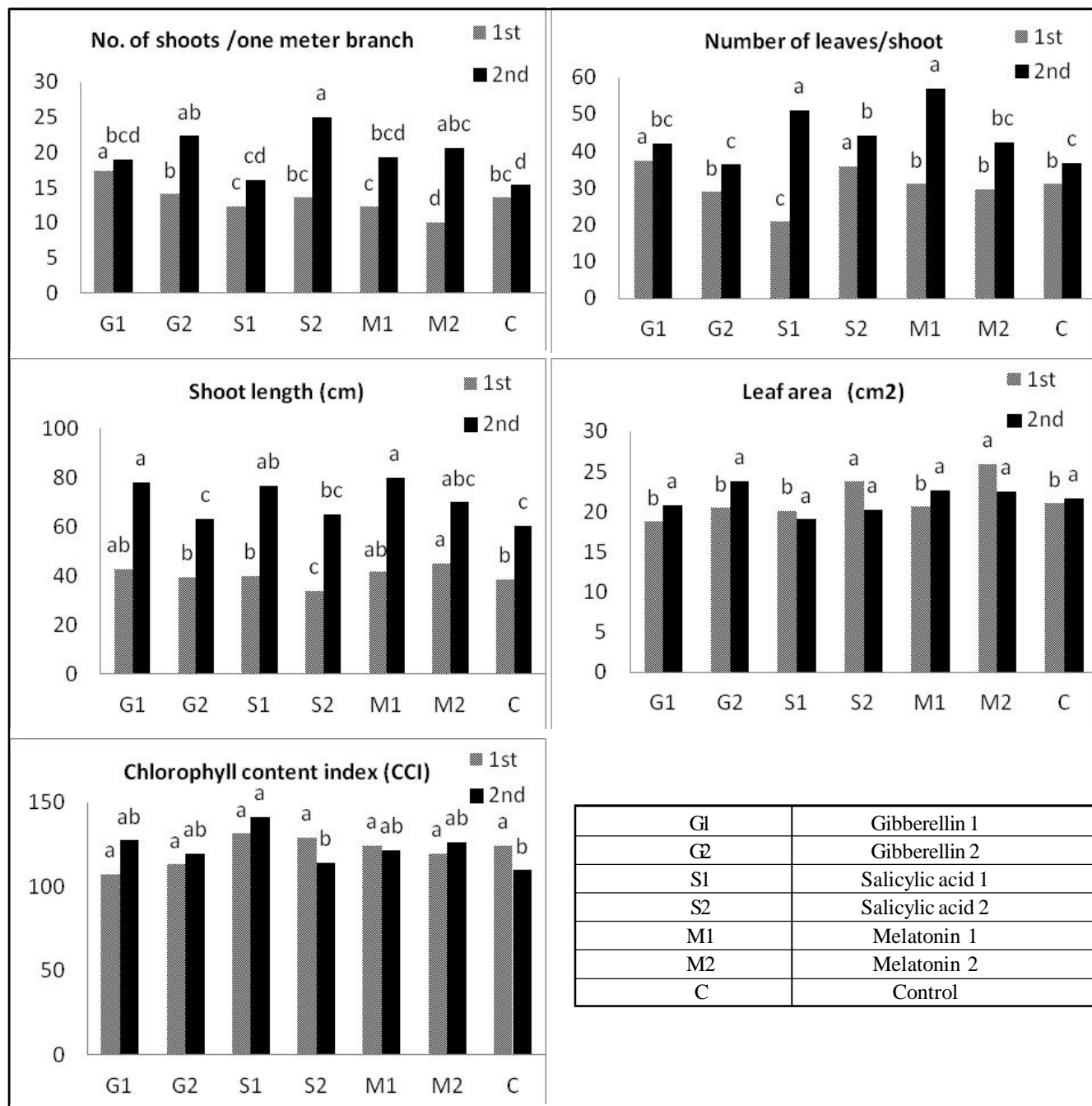


Fig. 1: Mitigation of heat stress effects using improving set treatments on vegetative growth characteristics and chlorophyll content of Washington navel orange trees during 2017 and 2018 seasons. Values followed by the same letter/s over each column didn't significantly differ at 5% level.

stomatal closure, nutrient uptake, chlorophyll and protein synthesis, inhibition of ethylene biosynthesis, transpiration, and photosynthesis (Wang *et al.*, 2010). Spraying of salicylic acid may affect some major physiological processes such as photosynthesis (Khan *et al.*, 2003). 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).

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).

Fig. 2, we have observed that all major elements in the leaves of all trees under the experiment were within the appropriate limits for the formation of healthy developing citrus leaf according to Werner (1992). All spraying treatments improved leaf macro elements content compared to control as shown in Fig. 2. Concerning leaf nitrogen content, spraying trees with “Gibberellin 1” and “Melatonin 1” enhanced leaves N content compared to control. Spraying with “Melatonin 1 and 2” achieved the highest leaves phosphorus content in the two seasons followed by “Gibberellin 2”. All spraying materials

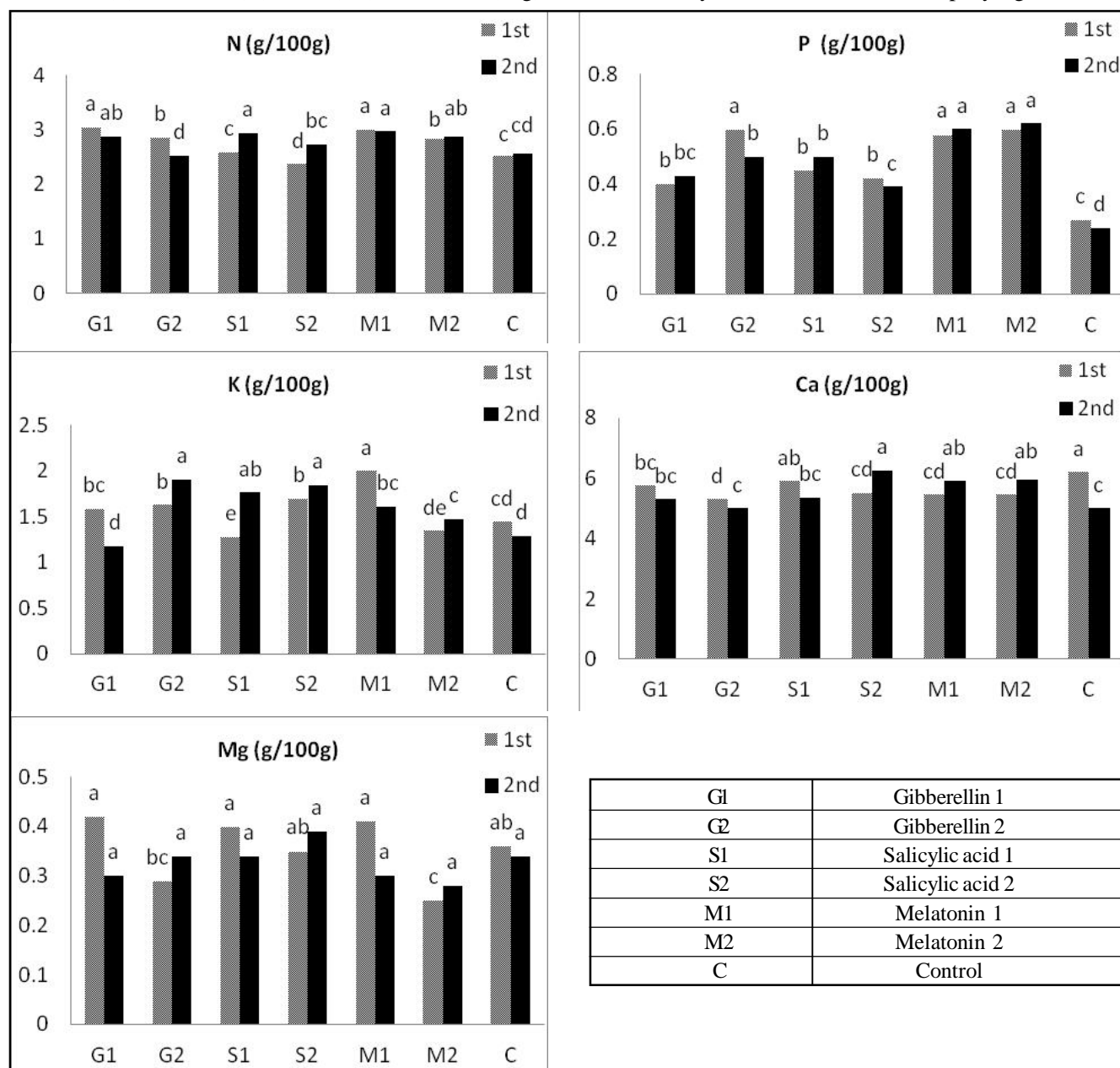


Fig. 2: Effect of improving set treatments on some minerals content of Washington navel orange leaf during 2017 and 2018 seasons.

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

improved leaf potassium content. The highest values of leaves calcium content were obtained from “Control (without spray)” and “Salicylic acid 2” in 2017 and 2018

seasons respectively, followed by “Melatonin 1 and 2” in the second season. All GA₃, Mel and SA treatments at low concentrations improved leaf magnesium content although the insignificance between treatments in the second season.

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

Results presented in Fig. 3 show that all micro elements in the leaves of all trees under the experiment were within the standard appropriate limits of the healthy citrus leaf according to Wutscher and Smith (1994). It’s discerned from Fig. 3 that all spraying materials increased leaves micro elements content, where, using “Salicylic acid 2” augmented leaves iron content in the first season, while, using “Gibberellin 2” or “Melatonin 2” were the highest in the second season. Regarding leaves manganese content, spraying with “Salicylic acid 2” was the superior in the first and second seasons. Spraying “Gibberellin 1” or “Salicylic acid 2” achieved the highest

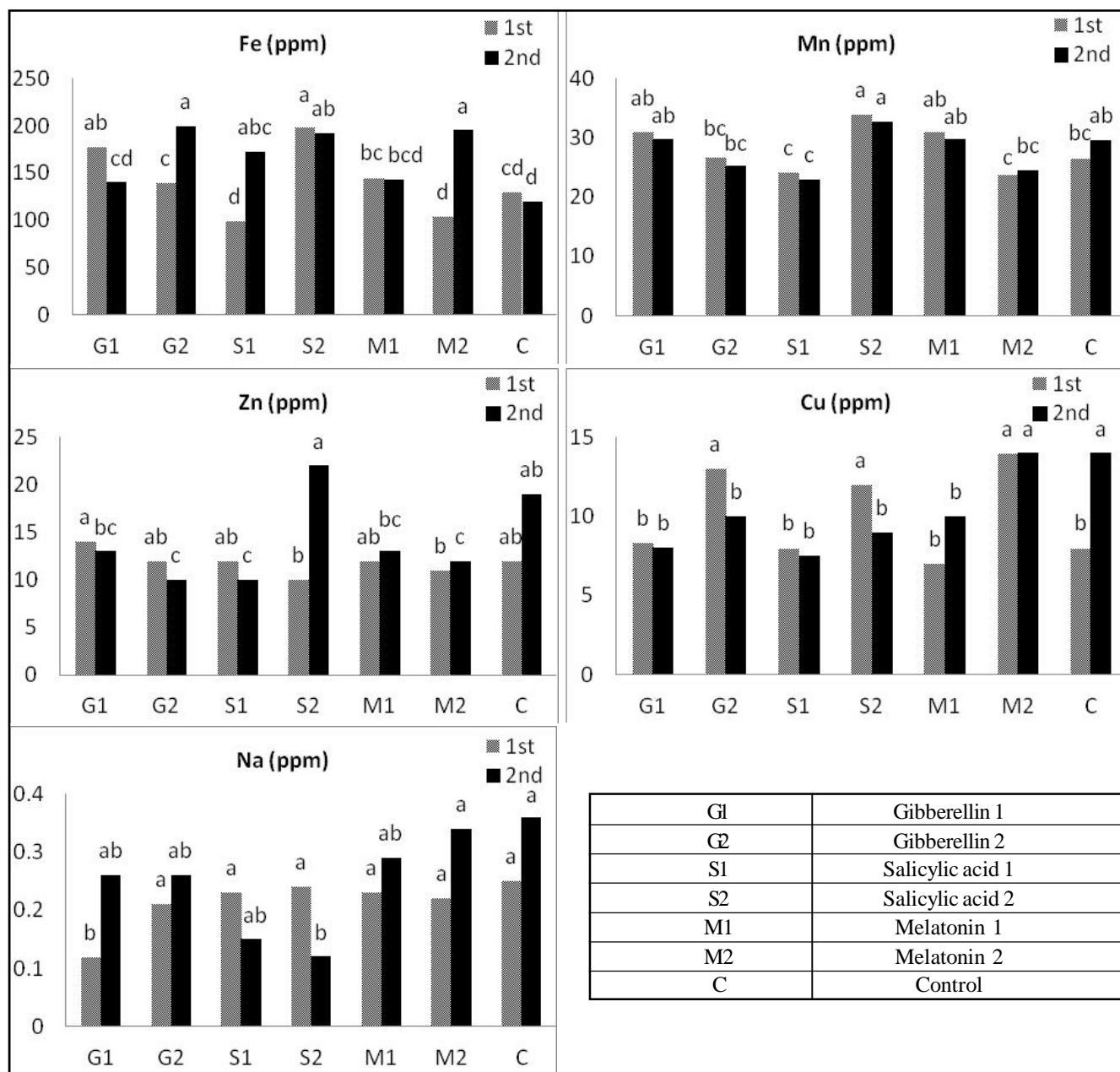


Fig. 3: Effect of improving set treatments on some minerals content of Washington navel orange leaf during 2017 and 2018 seasons.

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

values of leaf zinc content in the first and second seasons, respectively. Although the lack of differences between spraying materials in the first season, “Melatonin 2” treatment gave the highest copper values in the two seasons. Despite the low difference between treatments regarding leaf sodium content, “Melatonin 2” and “Control” treatments were the highest in this concern especially in the second season

So, spraying with Mel or GA₃ at low concentration had the high values of many elements such as, N, P, K and Mg followed by SA at high concentration which had high values of many elements such as Fe, Mn and Cu. In addition, 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, sink/source regulation, enzymatic activities, protein synthesis and photosynthetic activity as well as increasing the antioxidant capacity of plants (Tahira *et al.*, 2013).

Moreover, the 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 Jackson, 1991). Under climate change, the risk of nutrient leaching increases and soil organic content decreases by the time (Olesen and Bindi, 2002). These results are in agreement with those reported by Espinoza *et al.*, (1998).

and Melatonin, and this resulted to improving number of fruits, fruit weight and tree yield in the two experimental seasons. The highest tree canopy was obtained when using “Gibberellin 1” treatment in the two seasons, followed by “Melatonin 2”. In addition, “Melatonin 1” treatment improved Washington navel orange crop efficiency in the first and second seasons, followed by “Melatonin 2” and “Gibberellin 2” in the second season.

So, it can be observed that the high yield resulted from GA₃ spray at low concentration was due to the high fruits number while those resulted from Mel spray at low concentration was due to the high fruit weight, this was true in the first season, whatever, in the second season, high yield resulted from GA₃ spray at low concentration or Mel spray at two concentrations was due to the high fruits number and weight, as high yield resulted from SA spray at low concentration was in second order comparing with yield unsprayed trees.

Such, increase in tree canopy is important because the largest trees usually use the most water and result in highest fruit yield (Syvertsen and Smith, 1996). These results are in agreement with those reported by (Abd El-Naby and El-Sonbaty, 2016 and Abd El-Naby *et al.*, 2020). Heat stress during the critical cell division stage will reduce final citrus size. Too much heat and light, however, can result in lack yields and poor crop growth. This is due to negative impacts of stress upon photosynthesis and crop water status. Thus, the economic yield can be increased by increasing the rate of photosynthesis.

Gibberellic acid is used widely in various horticultural crops to improve fruit set and also to control cracking of pomegranate fruit (Sepahi, 1986). At lower concentrations, gibberellins increased the yield of French bean pods (Ambuko, 2001). Many investigators studied the role of GA₃ on many plants and coming to the opinion that, GA₃ caused an enhancement in plant growth and productivity of some vegetables (Pavlista *et al.*, 2012; Rathod *et al.*, 2015). Rajput and Singh (1982) reported that fruit weight and yield were increased when 16-years-old trees of the ber *cv.* Banarasi Karaka were treated with GA₃ at 20 ppm. Abd El-Naby *et al.*, (2019) revealed that GA₃ gave the highest yield of apricot. On the other hand, foliar application of salicylic acid has been shown to increase biomass and yield in a variety of plant species (Larqu e-Saavedra and Mart n-Mex, 2007; Javaheri *et al.*, 2012). These auxins are known by their ability to increase the cell size (Westwood 1993 and Davis 2004) and enhance fruit growth of Clementine (Agusti *et al.*, 1995), date palm (Shabana *et al.*, 1998 and Al-Juburi *et al.*, 2001 a & b).

It's clear from the abovementioned data in Fig. 5 that spraying Washington navel orange trees with “Gibberellin 1”, “Melatonin 1 and 2” significantly improved fruit diameter in the first and second seasons. The lowest values of peel thickness were obtained by control treatment in the two seasons and “Salicylic acid 2” in the second season. Also, “Gibberellin 1” in the first season and “Salicylic acid 2” in the second season were the superiors concerning fruit total soluble solids, followed by “control” in the two seasons. In addition, “Melatonin 2” spraying treatment enhanced fruit juice titratable acidity in the first season, while, no differences were shown between all treatments in the second season. The highest ascorbic acid content in fruit juice was obtained using “Salicylic acid 2” in the two seasons and “Melatonin 2” in the first season.

The effect of GA₃ on increasing T.S.S attributed to enhancing 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). Çolak (2018) treated jumbo blackberry with melatonin and gibberellic acid hormones and found that the number and weight (g) of fruits were most increased with the treatment of MEL + GA₃ 10 ppm (240.50 : 385.19) and MEL 10 ppm (182.38 : 280.59) and in terms of fruit size, the maximum efficiency was achieved with GA₃ 5 ppm (21.21 mm fruit length, 16.56 mm fruit width) and MEL 10 ppm (20.22 mm fruit length and 15.58 mm fruit width).

Conclusion

High temperature stress can be detrimental to plants, resulting in fruit yield reduction and fruit disorders

increment. Antioxidants may play a definite role in solving the problem of poor yielding through enhancing growth, nutritional statues, yield and fruit quality in different evergreen fruit crops such as date palms, citrus, mangoes, bananas and olives.

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. Their effect in plants result from their influence on plant metabolism. They can stimulate the synthesis of phytohormones, facilitate the uptake of nutrients from the substrate, stimulate root growth, and contribute to a higher yield and improve its quality. They are agents which increase resistance of plants to unfavorable conditions as extreme temperature,

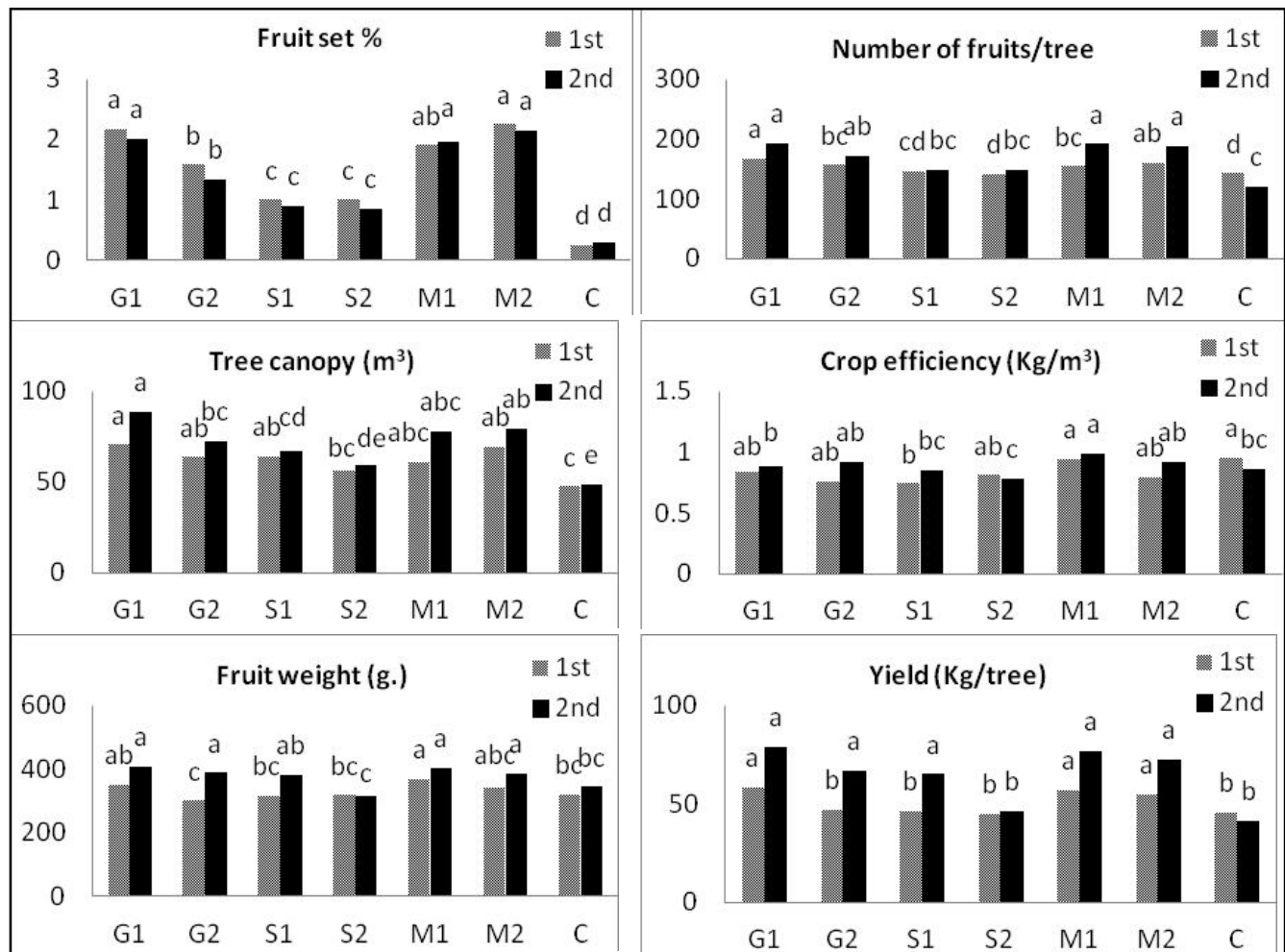


Fig. 4: Mitigation of heat stress effects by using improving set treatments on yield and its components of Washington navel orange trees during 2017 and 2018 seasons.

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

G1	Gibberellin 1
G2	Gibberellin 2
S1	Salicylic acid 1
S2	Salicylic acid 2
M1	Melatonin 1
M2	Melatonin 2
C	Control

drought, heavy metals etc. (Basak, 2008).

Such, the low concentration of each GA₃ and Mel show positive effects on almost all vegetative growth characteristics followed by SA at high concentration. So, the spray with Mel or GA₃ at low concentration had the high values of many elements such as N, P, K and Mg followed by SA at high concentration which it had high values of many elements such as Fe, Mn and Cu. So, it can be observed that the high yield resulted from GA₃ spray at low concentration was due to the high fruits number while those resulted from Mel spray at low concentration was due to the high fruit weight, this was

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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*

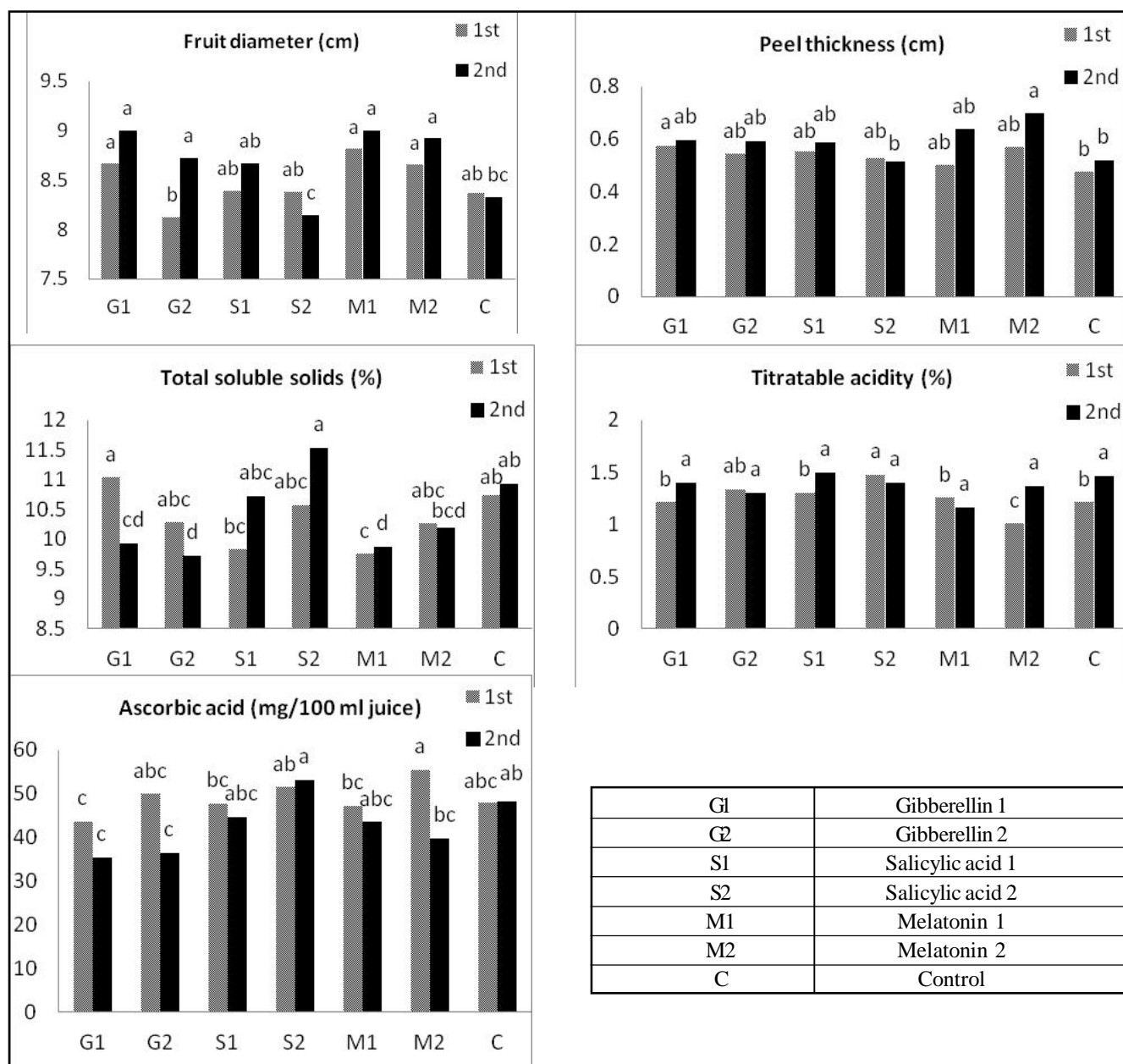


Fig. 5: Mitigation of heat stress effects by using improving set treatments on fruit quality characteristics of Washington navel orange during 2017 and 2018 seasons.

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

al., 2010). 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). 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).

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