Potravinarstvo Slovak Journal of Food Sciences
vol. 13, 2019, no. 1, p. 443-448
https://doi.org/10.5219/1126
Received: 25 April 2019. Accepted: 02 May 2019.
Available online: 28 June 2019 at www.potravinarstvo.com

ISSN 1337-0960 (online)

EFFECT OF PLANT GROWTH REGULATORS ON BIOCHEMICAL COMPOUNDS OF TANGERINE (*CITRUS UNSHIU* MARC.)

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ABSTRACT

We investigated the effect on tangerine of new generation plant growth regulators. The use of drugs in the period of fruit ripening has led to increased 2.0 – 3.7 times abscisic acid (AA) and 1.9 – 4.7% of Indole-acetic acid (IAA) acid in the leaves. Studies have shown that Indole-acetic acid and abscisic acid beginning of a sharp accumulation of their hormones coincides with action of stress factors and growth dormancy period. The use of the regulators had an impact not only on their content in leaves but also on fruit quality. For example, treatment Indole-acetic acid and Obstaktin led to an increase in the fruit of vitamin C. After treatments with plant growth regulators has been a significant decline in the total number of organic acids (up to 2.35% at the option of Melaphen and to 2.50% at Obstaktin, LSD ($p \leq 0.05$) = 0.06). By reducing the content in the fruits of organic acids to all variants increased the sugar-acid index. After each spraying tangerine on the treatment options plant growth regulators has been a significant increase the dry matter. Thus, the positive effect of plant growth regulators on all the quality characteristics of tangerine was shown. In the summer period, the treatment by regulators may have a protective effect, increases the content in plants the content of Indole-acetic acid. The plant growth regulators of new generation have a positive effect on quality of dwarf tangerine. Given that the plants of tangerine in the subtropical zone of Russia each summer have to drought and are losing not only in yield, fruit quality too, new regulators may exert a protective effect, because increases the content in plants is Indole-acetic acid, which activates gene expression of drought resistance.

Keywords: *Citrus unshiu* Marc.; growth regulator; spray application; dry matter; total acidity; total sugar; ascorbic acid

INTRODUCTION

Study of the effect of growth regulators on the activity of agriculture plants (in particular fruit) have been studied by many scientists. A significant contribution to the study of this problem made **Chaylakhyan (1977)**; **Sheveluha et al. (1998)**; **Gudkovskij (1999)**; **Sergeeva, Nen'ko and Kiseleva (2013)**; **Doroshenko et al. (2017)**; **Tacken (2014)**; etc. They identified a list of the most commonly used regulators, for example, on apple and pear to reduce of lost harvest fruits are often used such substances as alpha-naphthaleneacetic acid (NAA) and its potassium salt, which in parallel leads to increased fruit set, growth and ripening, as well as, to increase marketable qualities **(Agafonov and Phaustov, 1972; Edgerton, 1983; Gomez-Cadenas et al., 1996; Rath et al., 2006; Yuan and Carbaugh 2007; Doroshenko et al., 2017)**. Research of influence of regulators on fruit plants was showed not only an increase in crop yields, but also increase their resistance to adverse environmental factors **(Liholat, 1983; Zhuchenko, 2001; Chumakov, 2013; Trebichalský et al., 2016; Trebichalský et al., 2017)**. In the conditions of Krasnodar region (on the basis of Russian Research Institute of Floriculture and Subtropical Crops) was conducted to study the effect of chlor- chlorine- chloride (CCC) on plants of pear, grape and dwarf tangerine **(Gorshkov, 1976; Mikhailyuk, 1979)** and influence of micro nutrition's on dwarf tangerine and tea **(Belous, 2013; Abilfazova and Belous, 2016; Ryndin et al., 2017)**. The high responsiveness of the culture of the pear on used regulator are a reduced length growth of shoots, increased leaf formation, increased quality and break bud , increased leaf area, resulting in improved productivity. In addition, was increased drought resistance and resilience of trees pears to diseases (scab, phomopsiosis) **(Mikhailyuk, 1979)**. The effectiveness of the regulator to increase the yield of tangerine was shown. Studies have shown increase the sustainability of the culture of the vine to mildew **(Lepilov, 1985)**.

Thus, considering the above, it can be noted that the researchers made an enormous contribution to the study of influence of biologically active substances on features of functioning of agricultural plants. Currently, however, some growth regulators which used previously, or prohibited for use, or have a number of significant limitations. The nature of the impact of drugs of new generation for resistance to abiotic factors, the crop and fruit quality are not well understood, hampering their application in the practice of gardening. At the same time, the Black sea coast area (and, namely, the area of the Big Sochi) refers to the resort and adjacent to the biosphere reserve, so the use of many old regulators banned because of their danger to endemic communities. In this regard, there is an urgent need to study the mechanism of the effect on tangerine of new generation regulators with subsequent development of evidence-based recommendations for their effective use. Soil and climatic conditions of the Black sea coast of Krasnodar region is humid subtropical, however, and in these conditions systematically recorded detrimental effect on fruit plants the different climatic stress factors. For tangerine limiting abiotic stressors are cold with no snow cover, drought and elevated air temperature during summer. Thus, at the temperature of +35 °C, there is depression of vital processes of tangerine, which leads to uneven growth of the fruit, not simultaneous maturation, and deterioration of their colour and reduce palatability **(Gorshkov, 1976; Abilfazova, 2017)**.

Scientific hypothesis

The use of growth regulators has become an important component of agro-technical procedures for most of fruit plants, namely, tangerine. The plant hormones are important components in the integration of plants developmental activities. Environmental factors often exert inductive effects on metabolism and distribution of hormones within the plant. Apart from it, they also regulate expression of intrinsic genetic potential of plants. In this review, we focus on the plant growth regulators of new generation. We thinks that plant growth regulators influence on quality of dwarf tangerine - namely, increase the content of dry matter, total sugars and vitamin C in fruits.

MATERIAL AND METHODOLOGY

Field experiments were carried out on tangerine plantations (*Citrus unshiu* Marc.) in accordance with the "Program and methods of variety investigation of fruit, berry and nut crops" **(Sedov, 1999)**. The object of study – plants of the dwarf tangerine varieties 'Miagawa Wase' grafted on *Poncirus trifoliata*, planting in 1986 at the plantation Institute. As plant growth regulators used the following regulators:

1. IAA (Heteroauxin or Indole-3-acetic acid) – production control, at a concentration of 0.02%;
2. Melaphen in concentration of 1.10^{-8} – 1.10^{-9} %;
3. Obstaktin in concentration of 0.05%.

Control - treatment of water. Repeated experience is 5- samples, for a single sample is a "tree-repetition". Repeated laboratory tests are three times. Foliar treatment was carried out three times: first in the phase "the closing of the sepals" (3rd decade of May), the second by size of the fetus "walnut" (3rd decade of June), the third for 30 days before harvesting the fruits.

Heteroauxin (Indole-3-acetic acid (IAA) has many different effects, such as inducing cell elongation and cell division with all subsequent results for plant growth and development. On a larger scale, IAA serves as signalling molecule necessary for development of plant organs and coordination of growth. IAA enters the plant cell nucleus and binds to a protein complex composed of ubiquitin-activating enzyme, ubiquitin-conjugating enzyme, and a ubiquitin ligase, resulting in ubiquitination of Aux/IAA proteins with increased speed. Aux/IAA proteins bind to auxin response factor (ARF) proteins, forming a heterodimer, suppressing ARF activity. IAA inhibits the photo respiratory-dependent cell death in photorespiratory catalase mutants. This suggests a role for auxin signalling in stress tolerance. Obstaktin is phyto-regulators growth and the effect of auxin. Active substance is a potassium salt of 1-naphthylacetic acid. Melaphen is a regulator of growth and development of a new plants generation. Its distinguishing feature is the high efficiency and breadth of action at extremely low concentrations used. Application melaphen leads to significant increase in productivity and quality of the products. Laboratory analyses were made at Laboratory of biotechnology, biochemistry and plant physiology in triple repetitions: Ascorbic acid content was determined by iodometric method with 2% HCl, titrated 0.001 N solution of KIO_3; total acidity - titration with NaOH (0.1 $mol.dm^3$) in the presence of phenolphthalein indicator. The amount of sugar was determined by Bertran`s refractometric method. The method is based on the ability of sugars aldehyde group interact with Fehling's reagent and restore CuO to Cu_2O precipitated as a red solid **(Ermakov et al., 1972)**.

Statistic analysis

Statistical processing of the experimental data was carried out using the ANOVA package in STATGRAPHICS Centurion XV (version 15.1.02, StatPoint Technologies) and MS Excel 2007. Statistical analysis included univariate analysis of variance (method of comparing averages using variance analysis, *t*-test) and variance analysis (ANOVA). The significance of difference between the means of the least significant difference (LSD) results with $p < 0.05$ was considered statistically significant. All experiments were performed in triplicate and the values were expressed as mean ±*SD*. The differences between the samples were assessed using unpaired *t*-test. Correlation analysis with calculation of pair correlation coefficient, for establish the dependence of parameters on abiotic factors was used.

RESULTS AND DISCUSSION

Studies have shown that the use of a number of drugs in the period of ripening (30 days before harvest) led to an increase in 2.0 – 3.7 times abscisic acid (AA) and 1.9 – 4.7% indole-3-acetic (IAA) acid in the leaves (Figure 1). As you know, the content of AA increases in the state the growth dormancy, under the action of stress factors (for example, water deficit), during the period of abscission of fruits etc. **(Pustovoitova, Zhdanova and Zholkevich, 2004)**. Studies have shown that IAA and AA involved in signal transmission of stress and activate gene expression of drought resistance; the beginning of a sharp accumulation of these hormones coincides with the loss of plant turgor **(Levitt, 1985; Sergeeva, Nen´ko and Kiseleva, 2013; Madzhar, 2015; Doroshenko et al., 2017)**. In our case, there was a combination of two processes - the action of stress factors coincided with the growth dormancy period.

The use of the regulators had an impact not only on their content in leaves but also on fruit quality (Table 1). We have determined that ascorbic acid content in fruits on average in the variant of experience over the entire study period was 93 – 111 $mg.100g^{-1}$.

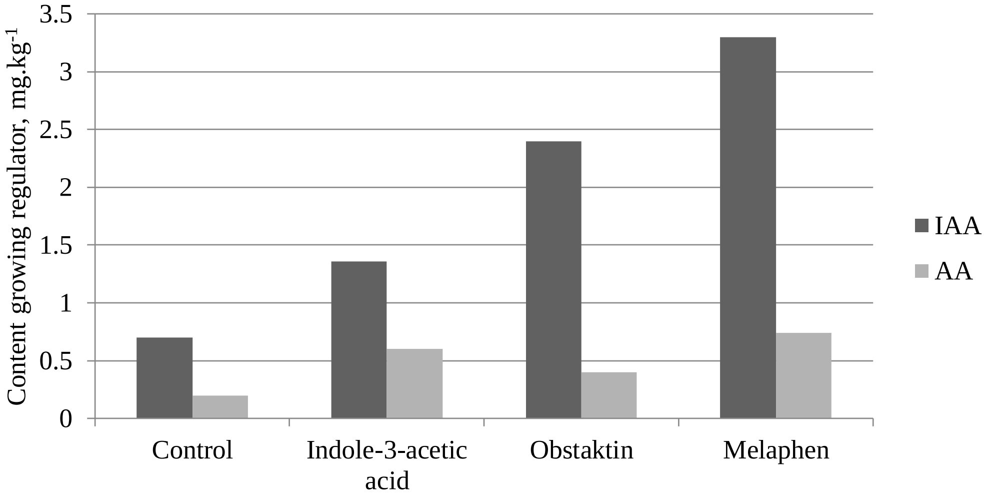

Figure 1 Influence of growth regulators on the their content in leaves, the analysis was performed 30 days after treatment.

Table 1 Dynamics of chemical composition of fruit after spraying with regulators.

Variant	Ascorbic acid, mg.100g^{-1}	Total sugar, %	Total acidity, %	Sugar-acid ratio, unit	Dry matter, %
1 spray application					
Control	94.92 ±0.97	10.76 ±0.39	3.03 ±0.02	3.56 ±0.12	14.30
Indole-3-acetic acid	107.30 ±1.15	10.36 ±0.29	2.79 ±0.03	3.71 ±0.07	30.30
Obstaktin	83.84 ±1.02	11.00 ±0.25	2.83 ±0.02	3.89 ±0.09	31.70
Melaphen	91.17 ±1.23	11.40 ±0.18	2.60 ±0.08	4.39 ±0.69	31.70
***LSD* (*p* ≤0.05)**	**2.07**	**0.54**	**0.08**	**0.69**	**-**
2 spray application					
Indole-3-acetic acid	103.78 ±1.02	11.19 ±0.07	2.14 ±0.03	5.24 ±0.05	29.4
Obstaktin	106.13 ±1.76	10.90 ±0.06	2.56 ±0.07	4.26 ±0.12	28.5
Melaphen	85.59 ±1.02	10.69 ±0.30	2.32 ±0.05	4.61 ±0.12	25.5
***LSD* (*p* ≤0.05)**	**2.33**	**0.47**	**0.09**	**0.5**	**-**
3 spray application					
Indole-3-acetic acid	89.12 ±1.34	11.68 ±0.11	2.35 ±0.02	4.98 ±0.05	29.8
Obstaktin	111.99 ±1.02	9.89 ±0.31	2.11 ±0.01	4.69 ±0.15	28.9
Melaphen	98.97 ±1.59	10.83 ±0.33	2.12 ±0.02	5.10 ±0.18	27.5
***LSD* (*p* ≤0.05)**	**2.92**	**0.45**	**0.04**	**0.19**	**2.65**

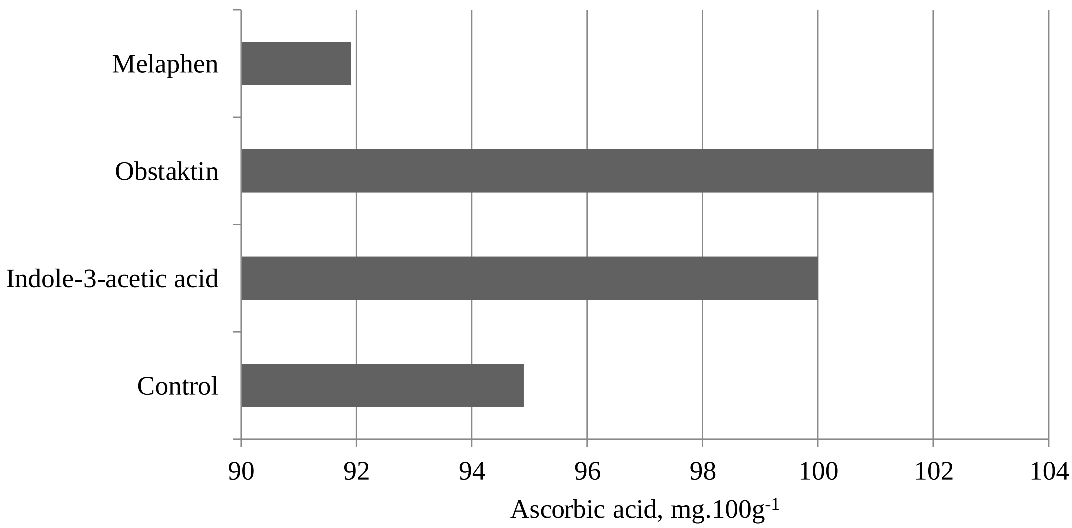

Figure 2 Content of ascorbic acid in the fruits under the treatments with regulators, LSD ($p \leq 0.05$) = 0.49.

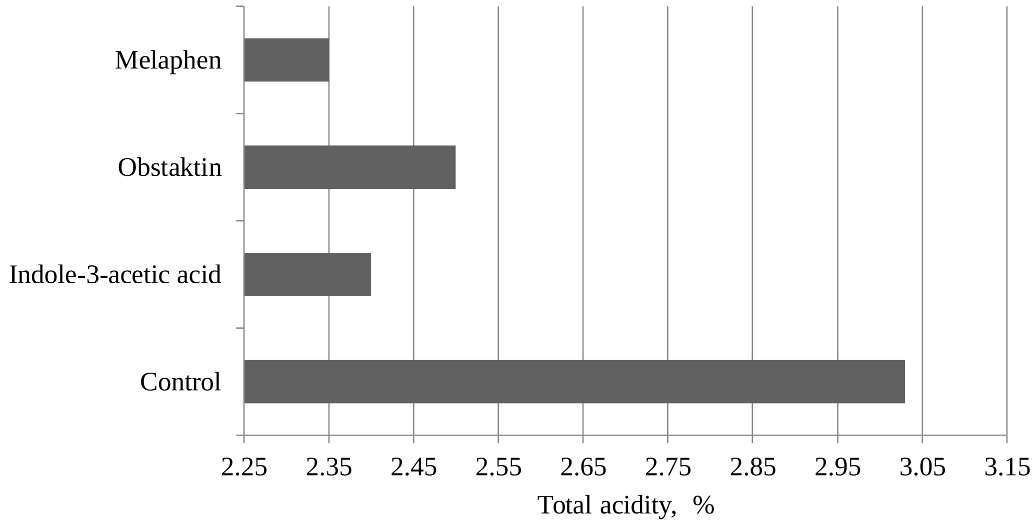

Figure 3 Content of titratable organic acid in the fruits under the treatments with regulators, LSD ($p \leq 0.05$) = 0.06.

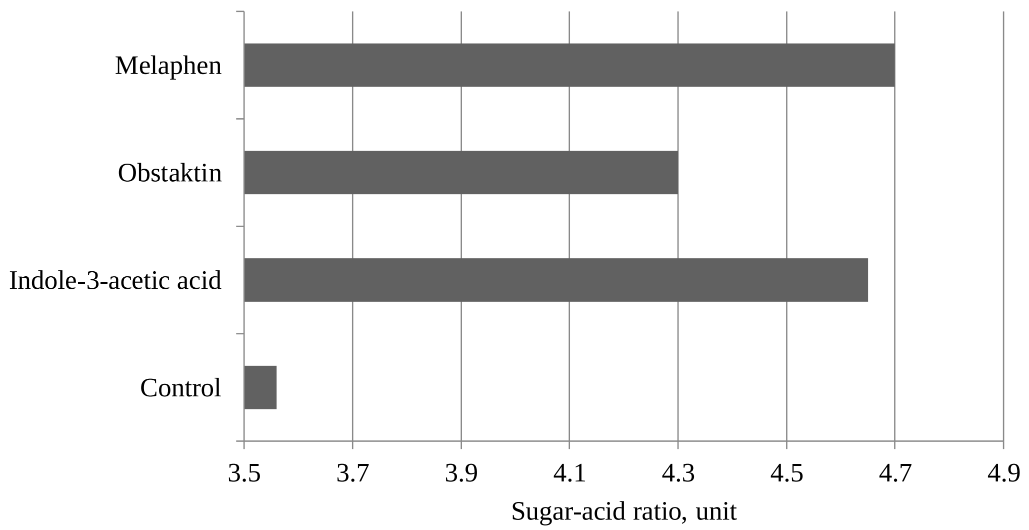

Figure 4 Sugar-acid ratio in the fruits under the treatments with regulators, LSD ($p \leq 0.05$) = 0.44.

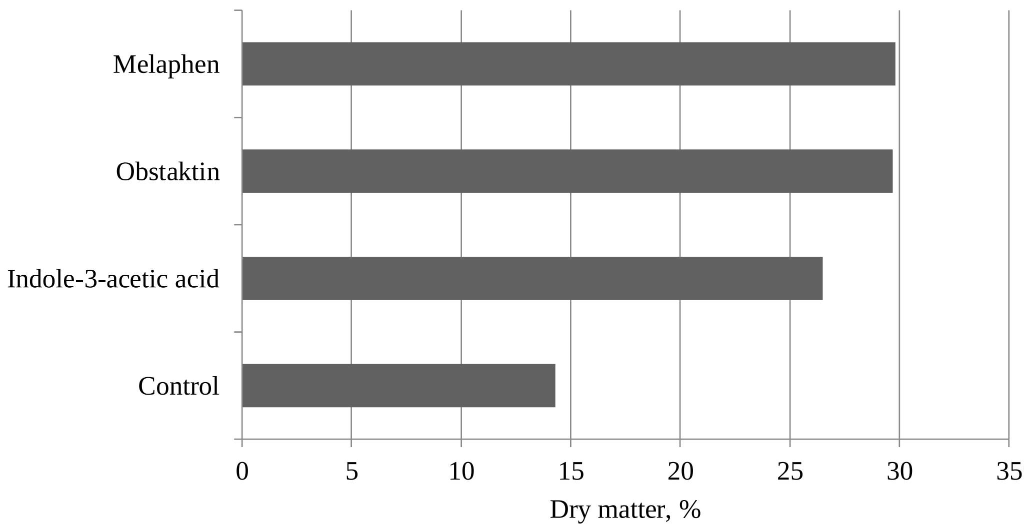

Figure 5 Content of dry matter in the fruits under the treatments with regulators, LSD ($p \leq 0.05$) = 0.65.

Moreover, an active accumulation of ascorbic acid was observed after the second and after the third spraying (Table 1). Thus, at the time of harvest fruit the variants "Indole-3-acetic acid" and "Obstaktin" were more nutritious than others (Figure 2).

The treatment of plants with growing regulators did not affect the total content of sugars, which for the entire period of studies on the variants of the experiment was in the range of 10 – 11%. Some reduction of sugars quality compared with the control was observed only after the third processing of Obstaktin (Table 1). In contrast to the sugar content, the total number of titratable organic acids in fruits has significantly changed after treatments of growing regulators (Figure 3). So, for period of studies the content of organic acids was on average 2.57%, but after each treatment there was a significant (LSD ($p \leq 0.05$) = 0.06) decline to 2.35% (Melaphen) – 2.50% (Obstaktin) compared with the control (3.03%).

The taste quality of the fruit determines not only the content of sugars and acids, but in the first place their ratio - sugar-acid index. The higher index is the better dessert quality of the fruit. In our study, this indicator ranges from 3.56 (Control) to 4.70 (Melaphen) with an average value of 4.30 units (Figure 4). As can be seen from Figure 4 all variants with spraying of phytohormones significantly exceeded the control (LSD ($p \leq 0.05$) = 0.44). Moreover, if after the first treatment, the increase in sugar-acid index was observed only at the option Melaphen (4.39 units), after the second and third treatment with phytohormones, sugar-acid index has risen on all variants relative to the control due to the reduction in the fruit organic acids (Table 1).

One of the most important indicators determining the quality of plant raw materials is the content of dry substances in the fruits. The amount of solids in the fruit's ranges from 10% to 20%. In some cases (subtropical fruits, for example, feijoa, persimmon etc.) it can reach 25% and above. In our case, the dry matter content ranged from 14.30 to control, to 29.83 – on option Melaphen, with an average value of 25.52% (Figure 5). The study showed that a significant increase of dry matter in tangerine under used hormones occurred after each spraying (Table 1).

CONCLUSION

The plant growth regulators of new generation have a positive effect on quality of dwarf tangerine - namely, increase the content of dry matter, total sugars and vitamin C in fruits. The plant growth regulators (Indole-3-acetic acid, Melaphen and Obstaktin) proved to be highly effective, as it had a positive effect on all quality characteristics of fruit. Given that the plants of tangerine in the subtropical zone of Russia each summer have to drought and are losing not only in yield, fruit quality too, new regulators may exert a protective effect, because increases the content in plants is Indole-acetic acid (IAA), which activates gene expression of drought resistance.

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