Changes in the biochemical components of mandarin fruits after pest control in the Republic of Abkhazia

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Abstract. The study assessing biochemical components (sugars and organic acids) in mandarin fruits after pest control was carried out in matured plantings of cv. Unshiu mandarin in the Republic of Abkhazia in 2019-2020. The treated fruits contained a greater amount of soluble carbohydrates than the fruits in control. The predominant form of monosaccharides is fructose (17.78-17.85 g/l, with 11.89 g/l in the control) in the fruits from the standard treatment and in variant 6 (with a combination of Metamax, Vermitek and Karate Zelon), which is preferable, since it has the greatest sweetness (173 units), providing a sweeter taste of the fruit. According to the content of organic acids, variants 6 and 8 (four treatments with Diatomite, 6%) were distinguished, the content of citric acid on which was 11.86-11.3 mg/l. A more balanced taste of the fruit was in variants 6, 7 (four treatments with Diatomite, 3%) and 8 (sugar-acid index from 7.7 to 7.9 units). Taking into account the content of basic acids and sugars in mandarin fruits, it is possible to predict the impact of insecticides and their combinations on their basic taste properties.

1. Introduction

Citrus crops are traditionally the most harvested orchard plants in Abkhazia. Among them, the mandarin plantings (i.e. Citrus reticulata subsp. unshiu (Marcow.) D. Rivera and al.) cover more than 90% of the orchards in the republic [1,2]. Similarly to other world regions, the cultivation of citrus in Abkhazia is a complex task bearing in mind how many pests can attack citrus [1,3,4]. In the agrocenes of Abkhazia, the most harmful species are the citrus rust mite Phyllocoptruta oleivora (Ashm.), the citrus cottony scale Chloropulvinaria aurantii Ckll., the citrus whitefly Dialeurodes citri (Ashmead), the citrus leaf miner Phyllocnistis citrella Stainton, the brown marmorated stink bug Halyomorpha halys (Stål), and a recent invader, the wooly whitefly Aleurothrixus floccosus Maskell [2].

Despite the fact that a large number of low-toxic, highly effective insecticides and acaricides have appeared on the market of plant protection products in recent years, in Abkhazia, mandarin protection is done based on the traditional techniques. The following insecticides are used there: lime sulfur...
(calcium polysulfide), the Preparate 30 (vaseline oil) and Bi-58 (dimethoate). The variety of mandarin pests implies an expansion of the range of insecticides used and their new application schemes. Studies in the Russian subtropics (Sochi, Krasnodar region) showed the effectiveness of a wide range of acaricides and insecticides, belonging to various chemical classes, against citrus crop pests [5].

The taste of fruits is one of the most important qualities for the consumers. The taste characteristics depend on the content and the ratio of organic acids and sugars, which are involved in the biosynthesis of various compounds, such as vitamins, amino acids, flavor-forming volatiles, etc. [6,7]. When using various elicitors, it is important not only to define the chemical composition of fruits, but also to understand how these substances affect the components that form the organoleptic characteristics, which determines the competitiveness of products [8,9].

The purpose of this study was to improve the system of mandarin protection against arthropod pests in the humid subtropics of Abkhazia by using new, effective and environmentally friendly approaches. Thus, we evaluated the effect of different pesticide application schemes on the biochemical composition of mandarin fruits.

2. Methods and Materials

The study was carried out in 2019-2020 on matured plantings of cv. Unshiu mandarin in Gulripshi district of the Republic of Abkhazia in collaboration with the Institute of Agriculture of Academy of Sciences of Abkhazia.

The experiments assessing the effectiveness of different insecticides and acaricides were carried out according to the standard protocols [10].

Altogether, eight variants were used to estimate the effectiveness of new-generation insecticides against mandarin pests. In each case, four treatments were applied: in mid June, mid July, mid August, mid September. Here is the list of the applied variants and treatments:

1. control, i.e. no insecticide treatment and no water treatment;
2. standard approach: all four treatments were done with a tank mixture of Bi 58 (dimethoate, 400 g/l, Keminova A/S) (0.2%) and Preparate 30 (vaseline oil, 760 g/kg, Sober LLC, Russia) (3%);
3. the first treatment was done with Confidor extra (imidacloprid, 700 g/kg, Bayer CropScience AG) (0.05%); the second treatment – with Vertimec (abamectin, 18 g/l, Syngenta) (0.1%); the third and fourth treatments – with Karate Zeon (lambda-cyhalothrin, 50 g/l, Syngenta) (0.05%);
4. the first treatment was performed with a tank mixture of Confidor extra (imidacloprid, 700 g/kg) (0.05%) and Siliplant (0.15%); the second – with a tank mixture of Vertimec (abamectin, 18 g/l) (0.1%) and Siliplant (0.15%); the third and fourth treatments – with a tank mixture of Karate Zeon (lambda-cyhalothrin, 50 g/l) (0.05%) and Siliplant (0.15%);
5. the first treatment was done with a tank mixture of Confidor extra (imidacloprid, 700 g/kg) (0.05%) and Cytovit (0.15%); the second – with a tank mixture of Vertimec (abamectin, 18 g/l) (0.1%) and Cytovit (0.15%); the third and fourth – with a tank mixture of Karate Zeon (lambda-cyhalothrin, 50 g/l) (0.05%) and Cytovit (0.15%);
6. the first treatment was performed with a tank mixture of Metomax (Metomil 250 g/kg + bifentrin 25 g/kg, JSC ‘FM Rus’) (0.15%) and Vertimec (abamectin, 18 g/l) (0.1%); the second and third – with a tank mixture Karate Zeon (lambda-cyhalothrin, 50 g/l) (0.05%) and Vertimec (abamectin, 18 g/l) (0.1%); the fourth – with Karate Zeon (lambda-cyhalothrin, 50 g/l) (0.05%);
7. all four treatments were done with Diatomite (silicon oxide 75%) (3%);
8. all four treatments were done with Diatomite (silicon oxide 75%) (6%).

The Siliplant is a universal fertilizer with a high content of bioactive silicon (Si – 7%), potassium (K – 1%) and trace elements in chelated form (mg/l): Fe – 300; Mg – 100; Cu – 70-240; Zn – 80; Mn – 150; Co – 15; B – 90; the manufacturer is LLC Nest-M (Russia).

The Cytovit-fertilizer contains macronutrients (g/l): nitrogen – 30; phosphorus – 5; potassium – 25; and trace elements in chelated form (g/l): magnesium – 10, sulfur – 40, iron – 35, manganese – 30, boron – 8, zinc – 6, copper – 6, molybdenum – 4, cobalt – 2; the manufacturer is LLC Nest-M (Russia).
The Diatomite is a fine diatomite flour consisting of SiO$_2$ – 74.8-88.0%, Al$_2$O$_3$ – 3.3-9.7%, CaO – 0.6%, K$_2$O – 0.96%, Fe$_2$O$_3$ – 2.3-4.8%, MgO – 0.6-1.7%, Na$_2$O – 0.74%; manufactured is Russia.

Laboratory studies were carried out upon reaching the consumer maturity of the mandarin fruit. Repeatability of laboratory tests – three times.

The laboratory studies were carried out on mandarin fruits once they become ready for harvesting (i.e. be. All lab test were done in three replications.

The quantity of organic acids and sugars was estimated by using the capillary electrophoresis system ‘Kapel 105-M’ (LLC ‘Lumex-marketing’, Russia). The dry matter content in the leaves was determined through drying at a temperature of 105 °C to a constant weight; the mass fraction of soluble dry matter in the fruit was determined by refractometric method [11] using a stationary refractometer (RL-2552, Poland). The juice yield was calculated as a proportion of the fruit weight before pressing and after pressing and expressed in %; the content of ascorbic acid (AA) was determined by the iodometric method [12].

The least significant difference (LSD) test was applied to estimate the difference between analyzed data using the statistical software package STATGRAPHICS Centurion XV. The analysis of the hydrothermal conditions of the current year was carried out according to the data www.accuweather.com [13].

3. Results and discussion

As generally known, the sour taste of fruits is caused by the content of organic acids [6,9,14]. In citrus fruits, three oxocarboxylic acids, i.e. citric, malic, and acetic have the greatest effect on the fruit taste [6,7,15]. The sweetness is determined by low-molecular carbohydrates, i.e. the organic compounds that make up about 10% of fruit juices. Among carbohydrates, the monosaccharides (glucose and fructose) and the disaccharide (sucrose) have decisive taste properties. Moreover, these compounds make a different contribution to the creation of a feeling of sweetness. If we take the sweetness of sucrose to be 100%, then the sweetness of glucose is 74%, and the sweetness of fructose is 173% [15,16]. Thus, taking into account the content of basic acids and sugars in citrus fruits, it makes it possible to determine the effect of drugs on the main taste properties after treating plants with insecticides.

The results of the study showed that all tested variants stimulate a significant synthesis of sugars in mandarin fruits (table 1). Despite the significant impact of the new generation of insecticides on the sugar content, special attention is paid to the variant with household waste treatment (standard) of plants and the variant 6, in which the predominant form of monosaccharides was fructose (17.78-17.85 g/l, at 11.89 g/l in control). This variant was most preferable, since fructose has not only the greatest sweetness and provides a sweeter taste of the fruit, but also has an antioxidant that prolongs the ‘shelf life’ of the fruit, keeping them fresh.

| Variant | Glucose, M±m | Fructose, M±m | Sucrose, M±m | Sum   |
|---------|--------------|---------------|--------------|-------|
| 1 (control) | 15.60±3.28  | 11.89±2.50   | 55.02±11.55  | 82.51 |
| 2 (standard) | 20.90±4.39  | 17.85±3.75   | 60.50±12.71  | 99.25 |
| 3       | 17.18±3.61   | 14.74±3.10   | 62.22±13.07  | 94.15 |
| 4       | 17.76±3.73   | 15.45±3.25   | 66.16±13.89  | 99.37 |
| 5       | 17.74±3.73   | 15.31±3.22   | 56.74±11.92  | 89.79 |
| 6       | 18.90±3.97   | 17.78±3.73   | 61.98±13.02  | 98.67 |
| 7       | 15.62±3.28   | 14.05±2.95   | 56.22±11.81  | 85.89 |
| 8       | 17.44±3.66   | 15.27±3.21   | 59.46±12.49  | 92.17 |
| LSD (p ≤0.05) | 1.46         | 1.32          | 3.95         | 3.34  |
The analysis of organic acids content indicated that the predominant acid in the mandarin fruit was citric acid (from 9.45 to 11.56 g/l) (table 2). At the same time, a significantly higher amount of the acids was found in variants 6, 8 and in the control, that determines the fruit balanced taste. In fact, the total acid content does not fully characterize the taste of the fruit. However, their content in fruits determines the sugar-acid index SAI (i.e. the ratio of acids and sugars), on which the harmony of taste depends.

The most balanced taste was found in the mandarin fruits where SAI was ranked between 7 and 8 (see variants 6-8 and 1 (control)) (table 2). Increasing SAI gives the mandarin fruit a sweeter, but savorless taste; in our tests, these were the variants 2-5 (table 2). Such fruits (variants 2-5) can be recommended for dietary and baby food due to low acid content.

Table 2. The content of organic acids in treated mandarin fruits, g/l.

| Variant | Malic, M±m | Citric, M±m | Acetic, M±m | Sorbic, M±m | Lactic, M±m | SAI, units |
|---------|------------|-------------|-------------|-------------|-------------|------------|
| 1 (control) | 0.46±0.05 | 11.56±1.02 | 0.07±0.001 | 0.06±0.001 | 0.06±0.001 | 6.8 |
| 2 (standard) | 0.37±0.05 | 10.48±1.02 | 0.11±0.002 | 0.07±0.001 | 0.02±0.001 | 9.0 |
| 3 | 0.53±0.06 | 9.65±1.12 | 0.09±0.002 | 0.06±0.003 | 0.04±0.002 | 9.1 |
| 4 | 0.52±0.02 | 9.50±0.98 | 0.09±0.001 | 0.07±0.002 | 0.08±0.001 | 9.7 |
| 5 | 0.50±0.02 | 9.83±0.95 | 0.09±0.001 | 0.08±0.001 | 0.03±0.001 | 8.5 |
| 6 | 0.43±0.06 | 11.86±1.02 | 0.10±0.002 | 0.07±0.001 | 0.05±0.002 | 7.9 |
| 7 | 0.54±0.03 | 10.11±1.06 | 0.08±0.001 | 0.07±0.001 | 0.05±0.001 | 7.9 |
| 8 | 0.42±0.02 | 11.30±1.08 | 0.15±0.001 | 0.07±0.001 | 0.03±0.002 | 7.7 |
| LSD (p ≤0.05) | 0.02 | 0.24 | 0.01 | NS* | 0.02 | – |

*NS – not significant.

Ascorbic acid (vitamin C) is also an important component of fruits as it plays an important role in nonspecific immunity and is known as an antioxidant. Many fertilizers and preparations for processing induce the expression of genes that encode the synthesis of compounds that play protective role [17, 18]. In our study, high amount of vitamin C in fruits (from 33.26 – 35.61 mg/g) was documented in the variants 2, 4, 5, 7 and 8 (figure 1).

Figure 1. The content of ascorbic acids in treated mandarin fruits (LSD (p ≤0.05=14.09). Variant 1 is control, variant 2 is standard treatment.

The synthesis of the vitamin C in a plant cell is closely related to the synthesis of polysaccharides, and as our study shows, all the insecticides used (primarily, variants with Siliplant, Cytovite and
Diatomite) significantly affect the accumulation of sugars in cells. Since the processes of biosynthesis of the vitamin C are triggered in response to a variety of endogenous and exogenous influences, the increase in ascorbic acid in fruits in these variants can be due to the activating effect of both Siliplant and CytoVite, containing chelated forms of trace elements, and diatomite flour, which includes such elements as Fe, Mg and Ca [15].

In addition to the chemical composition of fruits, their technological characteristics are of a great importance both for the consumer and for the resistance of fruits against pathogens (table 3). Thus, the thickening of the fetal integumentary tissues provides a mechanical barrier to infections. In our study, a significant thickening of the pericarp (rind) of the fruit (1.4-1.7 times higher than in control) was observed in all experimental variants. At the same time, the treatment with new-generation insecticides (in particular, in the standard treatment and on variants with Diatomite (variant 8), as well as with a combination of Metamax, Vermitek and Karate Zeon (variant 6)) led to a significant increase of soluble solids in the mass fraction. It is important, since all the substances that form the nutritional value of fruits are in dissolved form. Given that the mandarin fruits are used for the production of juice products, increasing the amount of dry matter under the influence of the insecticides seems to be a good option. For citrus fruits, high-quality juice obtained directly from the fruits should have an average of 10,0 °Bx soluble dry substances [11], which is consistent with our observations (table 3).

| Variant | weight, g | juice output, ml | soluble dry substances, °Bx |
|---------|-----------|-----------------|-----------------------------|
|         | fruit     | rind | pulp  | 88.5±10.8 | 9.5±0.4 |
| 1 (control) | 49.9±7.7 | 12.3±1.3 | 34.7±3.2 | 89.1±10.2 | 11.4±0.6 |
| 2 (standard) | 53.1±13.5 | 15.1±1.5 | 34.9±2.0 | 89.4±10.6 | 10.5±1.0 |
| 3       | 59.2±12.8 | 17.8±1.3 | 37.7±3.5 | 89.0±9.8 | 10.5±0.9 |
| 4       | 62.9±11.8 | 17.6±1.5 | 41.2±2.9 | 89.1±5.8 | 10.5±1.2 |
| 5       | 66.7±11.9 | 21.1±2.0 | 41.1±2.5 | 87.6±10.0 | 10.9±1.1 |
| 6       | 59.2±10.2 | 17.1±1.8 | 38.5±1.6 | 90.4±10.6 | 10.5±0.9 |
| 7       | 63.1±9.2 | 18.6±1.5 | 40.5±2.8 | 87.0±9.4 | 11.4±0.8 |
| 8       | 67.0±10.1 | 20.1±1.2 | 42.4±2.9 | 87.0±9.4 | 11.4±0.8 |
| LSD (p ≤0.05) | 16.2 | 3.7 | NS* | NS | 1.4 |

*NS – not significant.

4. Conclusion
We found that insecticide treatment significantly stimulates the synthesis of sugars in fruits, increasing the amount of fructose content (17.78-17.85 g/l, at 11.89 g/l in control), an antioxidant that extends ‘shelf life’ of fruits keeping them fresh.

The treatment with Diatomite (6%), as well as a combination of treatments with Metamax, Vermitek and Karate Zeon, leads to an increase in the accumulation of organic acids, that is preferable for balanced fruit taste. In the treated mandarin fruits, the content of ascorbic acid increased by 3.6-11.0% compared to the control.

When treated with insecticides, a significant (1.4-1.7 times) thickening of the pericarp (peel) was revealed; at the same time, there was a significant increase in mass fraction of soluble solids (from 10.5-11.4 °Bx; control – 9.5 °Bx).

Thus, the use of new-generation insecticides in mandarin cultivation technologies can be an effective way to protect mandarin plants for increasing fruit yield and fruit quality.

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