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INFLUENCE OF STIMUFUNG ON BIOLOGICALLY ACTIVE SUBSTANCES OF FRUITS OF ORANGE WASHINGTON-NAVEL

ABSTRACT

Mineral fertilizers, that contain impurities in the form of toxic elements and heavy natural radionuclides, are used in order to increase citrus crop. There is no doubt that using mineral fertilizers is associated with environmental pollution, which can be led by wrong agrotechnical measures. At the same time, it is extremely hard to conserve the fruit for a long time. So today it is very important to use organic products as fertilizers. Such is the "Stimufung"-liquid organic fertilizer for feeding plants not from roots. Stimufung is an organic substance that contains amines, amides, oligopeptides, phytohormones, minerals, micro and macro elements. It does not contain heavy metals, nitrates and other xenobiotics and is not dangerous for the environment.An influence of Stimufung on biochemical indicators of citrus fruits has been studied for the first time in this research. Due to its goal, biochemical indicators (Acidity, Common Sugar, Vitamin C, Common Phenolic Compounds, Flavonoid Glycosides) and storage ability of orange fruits after processing orange trees with Stimufung have been studied. Analysis was carried out on newly picked orange fruits, as well as on stored ones. As a result, processing orange trees with Stimufung raises its storage ability by maintaining good chemical indicators.

Keywords: Stimufung, Biyoproduct, Ecology, Washington-navel, Strogeability

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1. INTRODUCTION

Increase of agricultural productivity is impossible without using fertilizers. It should be also noted that use of mineral fertilizers received a large scale in the middle of the last century. Attention was drawn to heavy toxic elements and natural radionuclides in fertilizers, in which their concentration is often ten times higher than the same chemical and radioactive elements in the soil. At the same time, the coefficient of absorption of active substance from mineral fertilizers is too low. For nitrogen it is 35-50%, for phosphorus -20%, and for potassium -25-60%. So there is no doubt that the use of mineral fertilizers is related to environmental pollution, which may be the result of incorrect selection of agrotechnical activities. For Georgia, as for small-scale farming country, it is essential to minimize toxic chemicals in the production of agricultural goods. One of the best solutions are biological fertilizers, which are produced from vegetable raw materials, especially nanotechnologically derived products [1]. Bioproducts are topical in modern world. Several sorts of biopreparation, which are used in agriculture, have already been developed by "Biorational Technologies Research Center" (Georgia). Among them is "Stimufung" organic fertilizer of vegetable origin. "Stimufung" has received a biocertificate. The substance is produced by LLC "Lark", ready-made product is put on the market by "Biotecsi". It comes out in the form of fluid. [6]. The aim and the objectives if the research. The purpose of the research is to study an influence of Stimufung, liquid organic fertilizer for feeding not from roots, on the ecological safety and storageability of orange Washington- navel fetus.

Based on the aims of the research following objects were performed:

- The processing of orange tree (spraying) with aqueous solution of Stimufung;
- Harvesting during the technical ripeness of the fetus and storing fruits;
- Determination of chemical indicators (Acidity, Total Sugar, Vitamin C) before and after storing the harvest;
- Determination of total phenolic compounds in fetus before and after storing the harvest.
- Determination of flavonoid glycosides in fetus before and after storing the harvest;
- Determination of antioxidant activity in fetus before and after storing the harvest;
- Determination of storageability of fetus.

2. RESEARCH SIGNIFICANCE

Stimufung is of organic derivation. It contains amines, amides, oligoepeptides, phytohormones, minerals, micro and macro elements. It doesn't contain hard metals, nitrates and other xenophobics and is not a threat to the environment and to people. Amino acids and peptides are widely distributed in nature and are an integral part of organic matter. Stimufung is used in case of vegetables, potatoes, apples, grapes, cereals, sunflower, corn, lawn grasses, decorative trees and bushes, flowers (open and closed soils). Stimufung is effective for growing ecologically clean products. It improves quality of produce, assists blooming, and reduces stress caused by parasites, herbicides, climate change. Stimufung extents storageability of the produce [7]. It's the

first time when Stimufung has been tested on citrus cultures.

3. METHOD

The object of the study was orange Washington-navel. Orange trees of equal sizes were chosen. Selected trees were processed with aqueous solution of Stimufung with concentration of 2.5ml/l, for four times. At first, trees were sprayed while blooming. The other processings were accomplished with interval of two weeks, one month and two months. Analyses were conducted with repetition of trial options for three times. Fruits were picked up in period of technical ripeness. Controlling trees were left without spraying. Chemical indicators of fruit, such as titration acid, total carbohydrate, Vitamin C were determined in juice of newly picked fruits and after storage as well; Antioxidant activity, total phenolics and flavonoid glycoside were determined in juice, skin and pulp of the fruits before and after storage. Laboratory analysis was also conducted after three months of storage. After storage the number of rotten fruits was counted in the trial and control options. HPLC of Organic Acids and Sugars The liquid chromatographic apparatus (Shimadzu LC 10Avp) consisted of an in-line degasser, pump, and controller coupled to a photo diode array detector (Shimadzu SPD 10Avp) equipped with an automatic injector (20µL injection volume) interfaced to a PC running Class VP chromatography manager software (Shimadzu, Japan). Separations were performed on a 250mm×4.6mm i.d., 5um reverse-phase Ultrasphere ODS analytical column (Beckman) operating at room temperature with a flow rate of 1mL min-1. Detection was carried out with a sensitivity of 0.1 a.u.f.s. between wavelengths of 200nm-360nm. Elution was isocratic with 0.5% aqueous meta-phosphoric acid. Components were identified by comparison of their retention times to those of authentic standards under analysis conditions and UV spectra with our in-house PDA-library. A 10 min equilibrium time was allowed between injections. The same apparatus (Shimadzu LC-10Avp) coupled to RID10A detector was used for separation of sugars, which were performed on a 150mm×4.6mm i.d., 5um reverse-phase Nucleosil NH2 analytical column (Shimadzu, Japan) at room temperature with a flow rate of 1 mL min-1. Elution was isocratic with 75% aqueous acetonitrile. Components were identified by comparison of their retention times with those of authentic standards under analysis conditions. A 10 min equilibrium time was allowed between injections.

Determination of total phenolic content: The concentration of total phenols in extracts was measured by UV spectrophotometry (UV Spectrometer UNICAM, Cambridge, UK), based on a colorimetric oxidation/reduction reaction. The oxidizing agent used was Folin-Ciocalteu reagent (AOCS, 1990). To 0.5ml of diluted extract (100mg dry extract/ ml solvent), 2.5ml of Folin-Ciocalteu reagent (diluted 10 times with water) was added and, after that (within a time interval from 0.5 to 8 min), 2 ml of Na2CO3 (75g/l) were added. The sample was incubated for 5 min at 50 8C and then cooled. For a control sample, 0.5ml of distilled water was used. The absorbance of the resulting blue-colored solutions was measured at 760 nm. Quantitative measurements were performed, based on a standard calibration curve of gallic acid in methanol. The mean (+SD) results of triplicate analyses were expressed as gallic acid equivalents (GAE) in milligrams per gram of dry-material. Determination of total flavonoid content: Total flavonoid content was determined using a method described by Sakanaka, Tachibana, and Okada (2005). Briefly, 0.25 ml of the extracts (0.625–5mg/ml) or rutin standard solution (15–

250mg/ml) was mixed with 1.25ml of distilled water in a test tube, followed by addition of 75ml of a 5% (w/v) sodium nitrite solution. After 6 min, 150ml of a 10% (w/v) aluminium chloride solution was added and the mixture was allowed to stand for a further 5 min before 0.5 ml of 1M NaOH was added. The mixture was made up to 2.5ml with distilled water and mixed well. The absorbance was measured immediately at 510nm. The mean (+SD) results of triplicate analyses were expressed as rutin equivalents (RE) in milligrams per gram of dry-material. Determination of DPPH radicals scavenging activity was estimated with the method used by Kato. 1mM solution of DPPH in ethanol and also 1mg/1ml extract solution in ethanol was prepared and 1.5ml of this solution was added to 1.5ml of DPPH. The absorbance was measured at 517m against the corresponding blank solution which is prepared by taking 3ml ethanol and control O.D. was prepared by taking 3ml of DPPH. The assay was performed in triplicates. Percentage inhibition of free radical DPPH was calculated based on control reading by following equation.

DPPH scavenged (%)=(Acon-Atest)/Aconx100;

A con - is the absorbance of the control reaction;

A test - is the absorbance in the presence of the sample of the extracts [4].

4. RESULTS

The taste of citrus fruit depends on the ratio between the amount of sugar and the total acidity (Brix/Acid Ratio), which determines quality of fruit [3]. Chemical indicators, such as titration acid, total carbohydrate and Vitamin C were determined in newly picked fruits of orange Washington Navel. Moreover, Brix/Acid index was calculated by division of dry substances and titration acidity. The data are given correspondingly in tables: 1, 2 and on graphic: 1, 2.

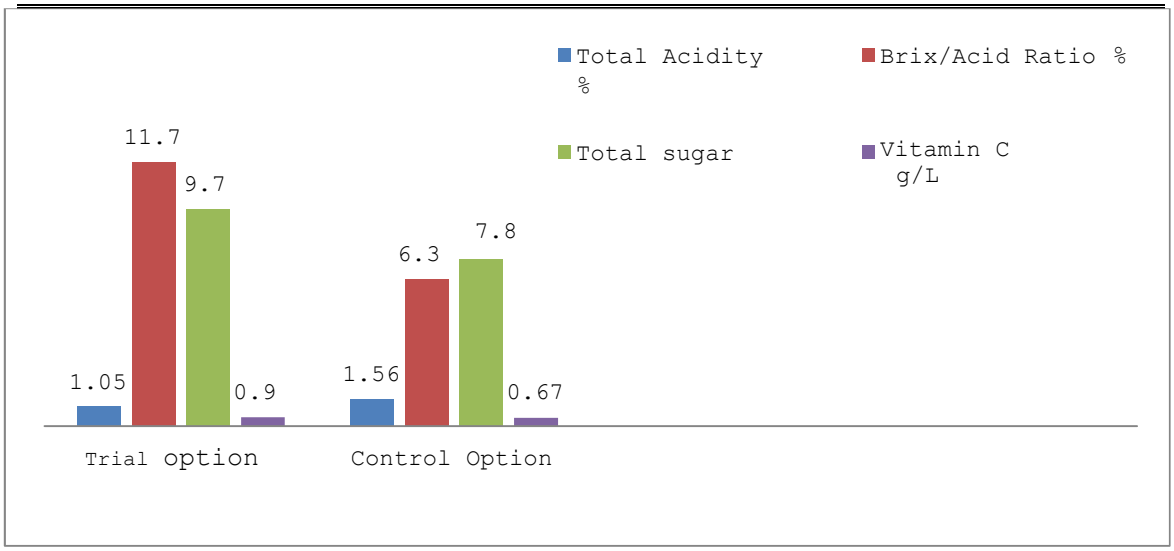
Table 1. Influence of Stimufung on chemical content of the orange Washington Navel fruit before storage

Name of the Sample	Chemical Characteristics of the Fetus (Before Storage)			
	Total Acidity (%)	Brix/Acid Ratio (%)	Total Sugar (%)	Vitamin C (g/L)
Trial Option	1.05	11.7	9.70	0.90
Control Option	1.56	6.30	7.80	0.673

Table 2. Influence of Stimufung on chemical content of the orange Washington Navel fruit after storage

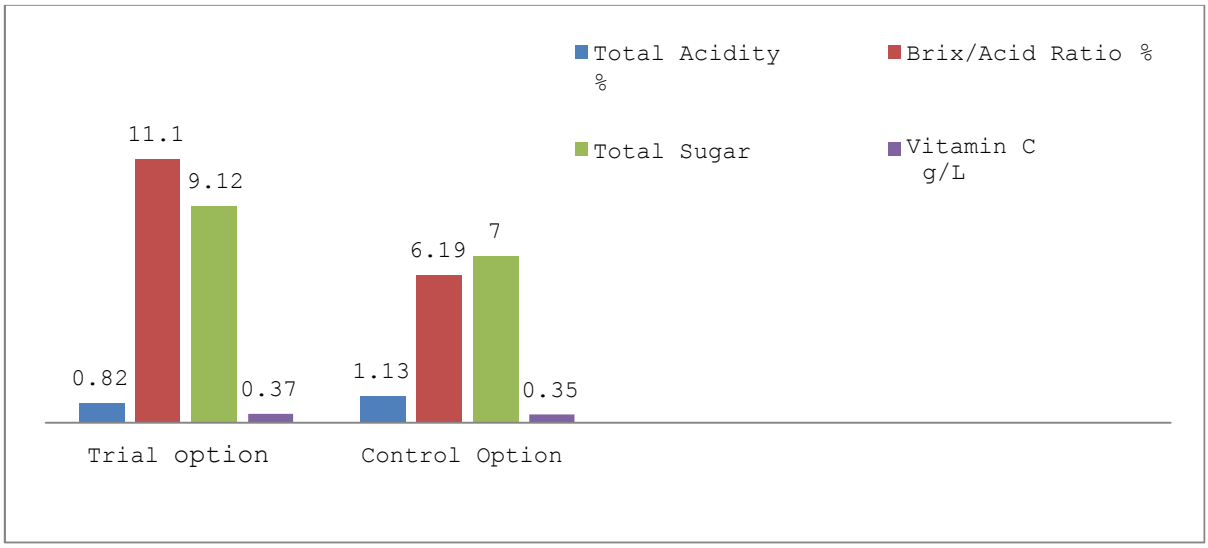
Name of the Sample	Chemical Characteristics of the Fetus (After Storage)			
	Total Acidity (%)	Brix/Acid Ratio (%)	Total Sugar (%)	Vitamin C (g/L)
Trial Option	0.82	11.1	9.12	0.372
Control Option	1.13	6.19	7.0	0.358

The results of laboratory research (graphic 1) has shown, that according to average data of trial options (processed with Stimufung) titration acid in them is 1.5 times less than in controlling ones. Total carbohydrate in trial options is 1.24 times more comparing to control options. Brix/Acid index in trial options is 1.95 times more and Vitamin C is 1.34 times more than in control options.



Graphic 1. An effect of Stimufung on chemical content of the orange Washington Navel fruit before storage

Laboratory researches in orange fruits were repeated after storage (3 months). Analysis has shown that in trial, as well as in control options, numerical values of chemical indicators decreased. However in trial option better indicators were kept (graphic 2).



Graphic 2. Influence of Stimufung on chemical content of the orange Washington Navel fruit after storage

Antioxidant activity was determined in juice, skin and pulp of the orange fetus before and after saving. The results are given in tables 3 and 4.

Table 3. Influence of Stimufung on chemical content of orange Washington Navel fruit before storage Note: skin and pulp dilution - 1:10

Name of The Sample	Antioxidant Activity of the Fetus AA% (Before storage)					
	Juice	Dilute	Skin	Dilute	Pulp	Dilute
Trial Option	33.0	20	49.23	20	47.13	20
Control Option	25.08	20	48.67	20	46.48	20

Table 4. Influence of Stimufung on chemical content of orange Washington Navel fruit after storage Note: skin and pulp dilution-1:1

Name of The Sample	Antioxidant Activity of the Fetus AA% (After Storage)					
	Juice	Dilute	Skin	Dilute	Pulp	Dilute
Trial Option	20.38	20	31.5	20	31.0	20
Control Option	21.01	20	27.3	20	28.2	20

As shown in the table, the rate of antioxidant activity of the newly picked (before storage) fetus in trial option is 1.32 times more comparing to the control option. In the skin and pulp the difference is insignificant, but still it's more in trial options. Table 4 shows the rate of antioxidant activity in the orange fruit after storage. As it seems, after storage antioxidant activity of the fetus reduces. The rate of decrease in trial option is smaller to a certain extent.

As a result of storing, indicators of antioxidant activity changes in the following way:

in trial option;
in the juice it reduced by 12.2;
in the skin - by 17.73;
in the pulp - by 16.13.
In control option:
in the juice by 4.07,
in the skin - by 21.37,
in the pulp - by 18.28.

Moreover, there is no actual difference between the rate of antioxidant activity of trial and control fruits after storage. However, in the skin and pulp tendency of prevalence of trial option is noticed. Phenolic compounds are not synthesized in the animal organism; they enter body by vegetable food and take part in metabolism. Different organs and tissues of plants are distinct from each other by the quantitative content of phenolic compounds. Total phenolic content was determined in the juice, skin and pulp of orange fetus before and after saving. Results are given in tables 5 and 6.

Table 5. Total Phenolic Content of The Orange Fetus Before Saving

Name of The Sample	Total Phenolic Content of The Fetus (Before Saving) Mg/100g		
	Juice	Skin	Plup
Trial Option	49.17	1425.96	764.13
Control Option	64.46	1930.50	916.30

As the table shows, total phenolic content of the juice, skin and pulp is less in trial option comparing to the control option. In particular, in trial option total phenolic content of the juice is 1.31 times, of the skin _ 1.35 times and of the pulp _ 1.2 times less than in control option. Phenolic compounds are the most common in the skin of fetus, then in the skin and the least amount is in the juice. Total phenolic compounds of the orange fetus are given in table 6.

Table 6. Total phenolic content of the orange fetus after storage

Name of The Sample	Total Phenolic Content of The Fetus (After Saving) Mg/100g		
	Juice	Skin	Dilute
Trial Option	134.38	1454.93	525.80
Control Option	72.60	1425.60	591.80

As shown in the table, total phenolic content of the juice is 1.85 times more in trial option than in controlling one. There is no actual difference in the skin. However, in the pulp total phenolic content is insignificantly more in control option. After comparing data received from the laboratory research carried out before and after storage, it can be inferred, that total phenolic content of the skin and the pulp reduces after saving in both, trial and control options. In the juice this indicator increases almost twice in trial option and insignificantly, 1.12 times, in control one. The increase in the amount of total phenoloic content of the juices in trial options can be explained by changing phenolic compounds into soluble form and their accumulation as a solution in the vacoom of the cell. Fetus of citrus contains bioflavonoids, which have particular importance for human organism. In complex with vitamin C bioflavonoids reduces herpes. In addition, bioflavonoids have a positive effect on the heart, stomach and liver. They have major role in prevention of cardiovascular and cancer diseases. They participate in antioxidant protection of the body. The human organism does not develop flavonoids, so it's necessary to make them enter the body artificially [2]. Flavonoid glycosides content was determined in orange fetus while processing with stimufung. The results are given in the table 7.

Table 7. Flavonoid glycosides content in the orange fetus before saving

Name of The Sample	Flavonoid Glycosides Content in The Fetus (Before Saving) mg/100g		
	Juice	Skin	Dilute
Trial Option	0.05	2.98	0.36
Control Option	0.06	3.15	0.43

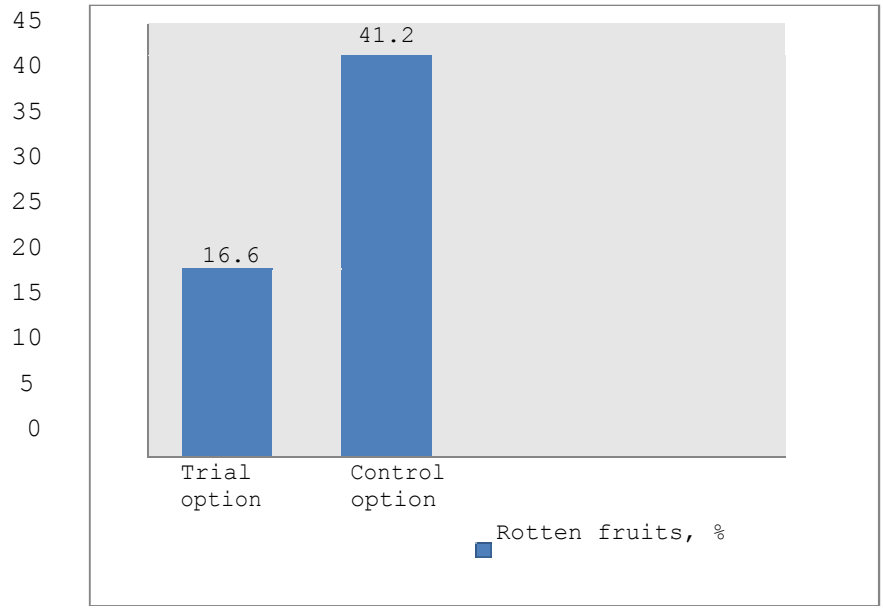
As it can be seen from the table, quantitative difference in flavonoid glycosides content in the juice, skin and pulp is insignificant between trial and control options. Flavonoid glycosides content was determined in the skin, juice and pulp of the fetus after storage as well. The results are given in table 8.

Table 8. Flavonoid glycosides content in the fetus after storage

Name of The Sample	Flavonoid Glycosides Content in The Fetus (After Saving) mg/100g		
	Juice	Skin	Dilute
Trial Option	0.16	2.78	0.74
Control Option	0.14	2.93	0.72

As it is inferred from the table, flavonoid glycosides content in trial and control options are slightly different. As a result of comparison between 7^{th} and 8^{th} tables it appeared that flavonoid glycosides content in the skin of fetus slightly decreases, whereas in the pulp and juice it increases after saving in both options. Fruits of orange Washington navel were stored in wooden boxes in the equal conditions. An amount of rotten fruits in trial and control options were counted after three months of storing. Results are given in graphic 3.

As it can be inferred from the graphic, in options processed by Stimufung average amount of rotten fetus is 16.6%, whereas in control option it is 41.2%. Storageability has grown 2.48 times. This is the best indicator of preservation of citrus cultures.



Graphic 3. An influence of Stimufung on storegeability of orange Washington Navel

5. CONCLUSION AND RECOMMENDATION

Some conclusions are presented below:

- Processing orange Washington Navel trees with Stimufung has a major impact on the quality indicators of the fetus: titration acidity decreases, amount of total carbohydrate and Vitamin C increases. As a result good taste characteristics of the fetus are formed.
- Usage of Stimufing solution significantly increases storageability of orange fruits.
- Stimufung is effective for processing orange trees to get ecologically clean fetus.
- There is no significant difference between rates of antioxidant activity in trial and control options.
- The substantial difference between experimental and controllable varieties of orange fruits in the quantity of common phenolic compounds is not observed until the fetus is stored;
- After storage in trial option the rate of total phenols in the fetus juice increased more than in control option.
- Quantitative variability of flavonoid glycosides in control and trial options after and before saving remained the same.
- Received results let us test Stimufung on other citrus cultures.

NOTE

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