

IMPACT OF FOLIAR FERTILIZATION ON THE QUALITY PARAMETERS OF BLUEBERRY FRUITS

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Abstract

*Plant nutrition is one of the determinate factors in fruit production and fruit quality. The aim of the present study is to evaluate some biochemical parameters of blueberry fruits (*Vaccinium corymbosum* L.), varieties: 'Duke', 'Blueray', 'Hannah's Choice' and 'Elliott' under the influence of foliar organic and conventional fertilizers applied, along with a control treatment without fertilization. The experiment was conducted in the years 2020-2021 in a four-year plantation in the southern region of Romania. Two organic products: Algacifo 3000 (2 L/ha) and ERT 23 Plus (1 L/ha) and one chemical product: Poly-Feed 19-19-19 + ME (10 kg/ha) were used as fertilizer treatments applied to the leaves of the plants. The experimental design was completely randomized according to the indicated treatments with three replicas. Biochemical parameters of the blueberry fruits, like organic acids, total polyphenols content, total dry matter content, total sugar and anthocyanin pigments were analyzed annually. The results indicated that foliar application with organic treatments significantly stimulated the fruit quality.*

Key words: foliar fertilization, fruit quality, biochemical characteristics, blueberry.

INTRODUCTION

The use of organic and mineral fertilizers at certain stages of plant development has proven to be an extremely important strategy for improving crop production (Mahmood et al., 2020) and fruit quality (Schoebitz et al., 2019). It is well known that nitrogen plays an important role in increasing crop yields and fruit quality. However, regular use of chemical fertilizers has a detrimental effect on the environment and agricultural production (Mostafa, 2008). Therefore, the focus of producers is on production methods with the least negative impact on the environment (Ochmian et al., 2015). In recent years, organic fruit production has gained particular interest (Neilsen et al., 2009). Fruits obtained by organic methods are considered to have a higher nutritional quality (Saba & Messina, 2003). Research confirms that the application of organic foliar fertilizers has had a positive effect on the accumulation of bioactive compounds compared to conventional fertilizers (Ochmian et al., 2015; Ciucu & Hoza, 2021).

Organic fruits and vegetables contain several polyphenols (Wojdyło et al., 2013), vitamin C (Caris-Veirat et al., 2004) and other antioxidant compounds. Wang et al. (2008); Ciucu & Hoza

(2021) found a higher content of polyphenols in blueberry fruits fertilized with organic products. However, Häkkinen & Törrönen (2000) reported a similar content of phenolic substances to some blueberry varieties grown by organic or conventional techniques. Some authors suggest that organic fertilization induces oxidative stress on plants leading to an increase in antioxidant defense mechanisms, increased production of bioactive compounds and improved antioxidant capacity of berry fruits (Frias-Moreno et al., 2021). Some authors recommend the application of organic fertilizers at the same time as chemical fertilizers to obtain high quality fruit (Kilic et al., 2021). In order to be able to conclude which of the organic or conventional practices are more effective on the biochemical quality of blueberry fruit, further research is needed. Previous studies show that blueberry fruits have a high content of phenolic substances, tannins, vitamin C and other organic acids (Huang et al., 2012; Barberis et al., 2015). Kalt et al. (2020) reported a high antioxidant capacity in these fruits. Several studies are underway to investigate the role and functions of antioxidant compounds in humans (Jideani et al., 2021). Due to the rich content of phenolic substances, blueberries have an anti-inflammatory and

antioxidant effect on the body, can prevent the occurrence of cardiovascular diseases, strokes, cancers, diabetes, hypertension, osteoporosis, etc. (Curtis et al., 2019; Si, 2020). Cranberry consumption has been associated with improved neural performance and memory (Bowtell et al., 2017; Miller et al., 2018). The cognitive benefits of eating blueberries in humans have been shown for all ages (Bell et al., 2021). Scientists have found that regular consumption of fruits rich in compounds with antioxidant and anti-inflammatory properties, such as blueberries, can delay the onset of brain aging and neurodegenerative disorders (Youdim & Joseph, 2001). The main objective of this research was to evaluate the impact of organic and chemical fertilizers on the quality of blueberry fruits, including the content of antioxidant compounds.

MATERIALS AND METHODS

Experiment location and plant material

The study was carried out in two consecutive years 2020 and 2021 in a plantation of a private farm, located in the Argeş meadow, southern Romania (44° 54'n, 24° 52'e) using as a study material a blueberry crop with tall shrub (*Vaccinium corymbosum* L.) for four years. The plantation covers 1 ha, the planting distances being 0.85 m per row and 3 m between rows (resulting in 3860 plants/ha density). The plants were placed on billets covered with black polyethylene. Four varieties of blueberries commonly grown in Romania ('Duke', 'Blueray', 'Hannah's Choice' and 'Elliott') were used as study material. The varieties were chosen according to popularity and ripening season.

Soil Description

The plantation is located on a flat area with a brown-clay soil with a loam-clay texture in the first 60-70 cm, and in depth the texture becomes sandy. The soil was improved by adding acid peat along the rows of plants (30 t/ha). In order to lower the pH of the soil, sulfur was administered at the beginning of the vegetation.

Plant fertilization

The treatments were distributed in a completely randomized block design, with three replications, with 5 plants per plot, with a

spacing of 3 plants. Four different foliar treatments were administered, namely V1 (control variant - without fertilizer), V2 and V3 (organic variant - biostimulator and organic fertilizer) and V4 (conventional variant where chemical fertilizer was used). The experiment was bifactorial. Factor A, the highbush blueberry (*Vaccinium corymbosum* L.) had 4 levels. Factor B, the foliar fertilizer used had four fertilization treatments: V1 - control (untreated), V2 - Algacifo 3000 - brown seaweed extract *Macrocystis integrifolia* with betaine of vegetable origin (2% organic nitrogen, 10% organic carbon, 50% organic matter) (2 L/ ha), V3 - ERT 23 Plus seaweed extract (*Macrocystis integrifolia*), folic acid, glycine betaine (1.5% organic nitrogen, 11% organic carbon, 6.1% K₂O, 10% betaine) (1 L/ha) and V4 - a Poly-Feed chemical 19-19-19 + ME (10 kg/ha). The treatments were repeated four times every 10-14 days, from the formation of buds to the beginning of fruit ripening.

Fruit Biochemical Analysis

For analysis, hand-harvested fruits were used at the technical time of ripening. The following quality parameters were determined: total dry matter content, titratable acidity, vitamin C content, total sugar content and total content of polyphenols and anthocyanin pigments. The determinations were performed in three repetitions for each variety with the fertilization variants.

Total dry matter content (DM)

The total dry matter content was determined by a gravimetric method (drying 10 g of fruit tissue at 105 ° C to constant weight) according to Gergen (2004).

Total acidity

The organic acid content of blueberry fruit was determined by the titrimetric method, using 25 ml of aqueous fruit extract neutralized with a 0.1N NaOH solution in the presence of phenolphthalein as an indicator. The total acid content was expressed as malic acid (%).

Total sugar content

The total sugar content was estimated by the Fehling-Soxhlet method, 1968 (JAOAC, 1968). The principle of the method is based on the oxidation reaction between the copper in the copper alcoholate of sodium and potassium tartrate and the aldehyde and ketone grouping

of the reducing sugars. This method determines the amount of reducing sugar which reduces a certain volume of Fehling's reagent. Total sugar content was expressed as a percentage (%).

Total polyphenol content (TPH)

The TPH of the fruit was evaluated by the spectrophotocolorimetric method, with the Folin-Ciocalteu reagent (Singleton et al., 1999). The principle of the method is based on the measurement of the optical density of the obtained extract which, by complexation with the Folin-Ciocalteu reagent, absorbs in the Vis domain. Methanol (70%) was used as the solvent for the extraction of polyphenols. Gallic acid was used as standard and the results were expressed as mg GAE/kg FW.

Vitamin C content

The vitamin C (ascorbic acid) content of fruit expressed in mg/100 g FW was analyzed according to the method based on the oxidation of L-ascorbic acid to dehydroascorbic acid in an acid medium with a blue dye of 2,6-dichloroindophenol, followed by the reduction of the dye to the colorless form, which turns red at pH 4.2 (PN-A-04019: 1998).

Total anthocyanin content

The dosing of the total anthocyanin pigments from the fruit was performed by the Fuleki method (1968). The determined total anthocyanins were expressed as cyanidin 3-glucosides mg/kg FW (C3G mg/kg FW). A PG instruments T70 spectrophotometer was used.

Statistical Analysis

Statistical analysis was performed using an IBM SPSS 20 program (SPSS Inc., Chicago,

IL, USA). All results were analyzed by unidirectional analysis of variance (ANOVA) and using the Duncan Multiple Range test. The differences were considered statistically significant at $p < 0.05$.

RESULTS AND DISCUSSIONS

The present study presents the biochemical quality of the fruits of four varieties of highbush blueberry (*Vaccinium corymbosum* L.) following fertilization with organic or conventional products.

Total dry matter content

Blueberry fruits contain on average about 84-88% water, the rest is dry matter. It consists of carbohydrates, proteins, lipids, minerals, organic acids, vitamins, phenolic compounds (Kader, 2002). In essence, DM is a reflection of the quality of fruits at harvest, the most relevant components of which are starch and soluble dry matter content (Travers, 2013).

Figure 1 shows statistically assured variations between organic or conventional foliar fertilizers and untreated control over the average DM content of the fruits of the four blueberry varieties chosen for analysis. An exception to this rule is the 'Blueray' variety where a single set of homogeneous values (a) is found for all fertilization options, including the control option. In the 'Hannah's Choice' variety, there were no significant differences in the values of DM content in fruit fertilized with organic or conventional fertilizers.

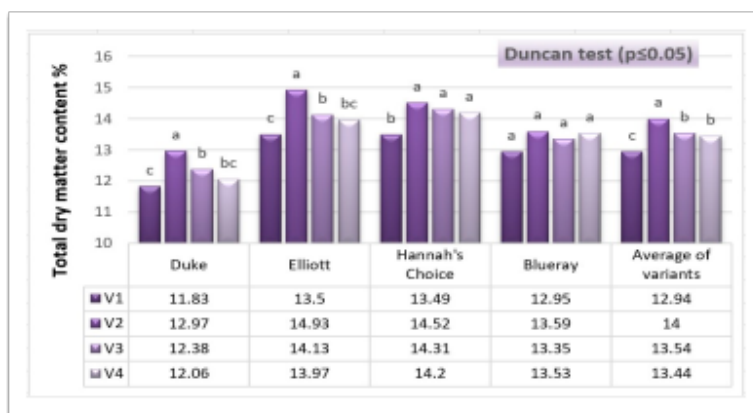


Figure 1. The influence of foliar fertilizers on the total dry matter content of fruit by variety in the years 2020-2021

The average value of DM content in fruit between 2020-2021 ranged from 11.83% ('Duke' variety, control variant - V1) to 14.93% ('Elliott' variety, variant V2 fertilized with organic fertilizer Algacifo 3000). The data of the analysis test of the Anova variance show that the total dry matter content of the fruit was very significantly influenced by the cultivar (partial eta squared = 47.9%) and the fertilization variant (19%) during the two years of study. ($p = 0.000$). The results are consistent with those in the literature. Studies have shown a variation in DM from 8.2% (Shevchuk et al., 2021) to 15.09% (Ostrowska & Ściażko, 1996).

Total titrable acidity expressed as malic acid

The content of organic acids in fruits can be influenced by growing and environmental conditions (Gündođdu, 2019). In general, the organic acid content decreased significantly following the application of foliar fertilizers (Figure 2).

On average, over the two years of study, the content of organic acids was significantly higher in the control variant in all studied blueberry varieties. The highest values were recorded for the 'Elliott' variety (1.28%). The data correspond to those reported by Zenkova & Pinchykova (2019).

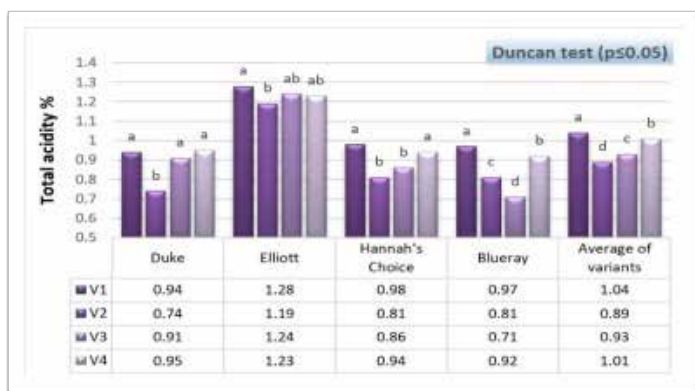


Figure 2. The influence of foliar fertilizers on the organic acid content of fruits by variety in the years 2020-2021

The titrable acidity had the lowest value in the 'Blueray' variety (0.71% - variant V3, fertilized with the biostimulant ERT 23 Plus). The average trend over the two years of the study shows a significant decrease in the titrable acidity of blueberry fruit to organic variant V2, fertilized with Algacifo 3000 fertilizer. It is largely determined by variety (partial eta squared = 82.7%). It was very significantly influenced by agronomic conditions (fertilization) by 42.8 and by meteorological conditions in 2020-2021 by 32.8% ($p = 0.000$). This has also been confirmed by Shevchuk et al. (2021).

Total sugar content

In the case of blueberries, the sugar content contributes to the determination of the organoleptic quality (Li et al., 2020). It can be influenced by maintenance technologies, environmental conditions, variety, soil conditions, the position of the fruit in the crown (Davidescu, 1999; Gündođdu, 2019). On average, during the two experimental years, the

total sugar content of the fruits of the studied blueberry varieties was significantly influenced by the growing conditions. All fertilization options (organic or conventional) had a beneficial effect on the value of this quality indicator compared to the control option (Figure 3). A variation was observed from 7.3% ('Elliott' variety, untreated variant) to 10.8% ('Hannah's Choice' variety, organic variant V3). The values obtained are in accordance with those reported in the literature (Kirina et al., 2020). The variety with the highest total fruit sugar content was 'Hannah's Choice' in all fertilization options followed by the 'Blueray' variety. The lowest values of the total sugar content of the fruit were obtained in the 'Elliott' variety. Among the fertilizers used, the greatest impact in terms of increasing the value of this quality indicator had the organic fertilizer Algacifo 3000 (fertilizer variant 2) as shown by the average effect of fertilizer variants on the total sugar content. In the

studied highbush blueberry, the tendency of sugar accumulation was symmetrical with the

dynamics of dry matter accumulation, a fact also noticed by Shevchuk et al. (2021).

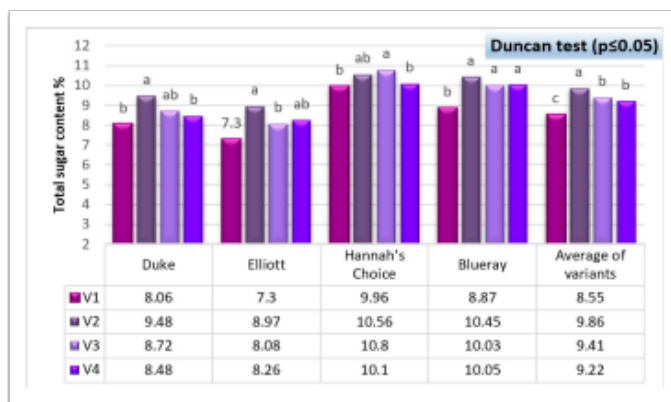


Figure 3. The influence of foliar fertilizers on the total sugar content of fruit by variety in the years 2020-2021

The bifactorial analysis of the variance determined a very significant variation of the influence of the genetic characteristics determined by the variety on the total sugar content of the analyzed fruits of blueberry varieties of 43.62%, while the agronomic conditions determined their sugar content by 18.2% ($p = 0.000$). The cumulative effect of these two experimental factors was not significant.

Vitamin C content

The concentration of ascorbic acid in blueberry fruits has shown great variation in the research of scientists. Correia et al., 2016, reported a variation from 6 to 162 mg/ 100 g FW. In our

study, the highest content of vitamin C was accumulated in the fruits of all varieties in the control variant. With the richest ascorbic acid content, the 'Elliott' variety stands out (19.18 mg / 100 g FW). The lowest values were observed in the fruits of all four varieties fertilized with organic fertilizer - Algacifo 3000 (V2). The lowest value (11.65 mg/100 g FW) was obtained in the 'Hannah's Choice' variety (Figure 4). Ascorbic acid biosynthesis has been significantly influenced by culture technologies. According to the analysis test of the Anova variance, the agronomic practice had a very significant effect ($p = 0.000$) on the vitamin C content of blueberries of 57.9%.

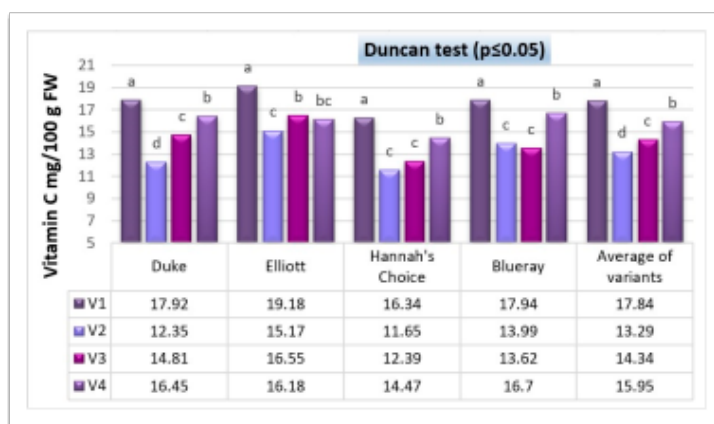


Figure 4. The influence of foliar fertilizers on the vitamin C content of fruits by variety in the years 2020-2021

Also, the genetic background of the cultivar had a very significant effect on the content of metabolized ascorbic acid (partial eta squared = 35.4%). The climatic conditions of the two years of study determined a variation on the ascorbic acid level of 18.2% ($p = 0.000$).

Total anthocyanin content

Anthocyanin pigments are part of the flavonoid class and are responsible for the blue, purple, and red colors of the fruit depending on pH. Blueberries (*Vaccinium corymbosum* L.) are distinguished by an increased anthocyanin content: malvidin 3-galactoside, delphinidin 3-arabinoside, delphinidin 3-galactoside, petunidin 3-arabinoside, petunidin 3-galactoside, malvidin 3-c glucoside, cyanidin 3-galactoside, -arabinoside, malvidin 3-glucoside, delphinidin 3-glucoside, peonidine 3-galactoside, peonidine 3-arabinoside and peonidine 3-glucoside (Mazza, & Miniati, 2018). The level of anthocyanin compounds in blueberry fruit can be significantly influenced by the time of harvest. Connor et al. (2002) found a positive influence of anthocyanin levels in fruit due to delayed harvest time. On average, over the two years of the experiment, foliar fertilizers led to a significant increase in the total anthocyanin content of blueberry fruit up to 3121.33 mg C3G/kg FW ('Hannah's Choice' variety, variant V2 - fertilized with Algacifo 3000). In all studied varieties, the content of anthocyanin pigments was significantly lower in the unfertilized control

variant (Figure 5). Among the foliar fertilizers applied, the organic fertilizer Algacifo 3000, had a stronger impact on anthocyanin pigments, in the sense of increasing their fruit content. This trend is also observed in the biosynthesis of fruit anthocyanins in variants. The exception was the 'Elliott' variety, which recorded a higher average content of anthocyanin compounds in fruit in both organic fertilizers used (variants V2 and V3), the differences between the two variants not being significant. Compared to conventional PolyFeed fertilizer, the applied organic fertilizers had a stimulating impact on the anthocyanin compounds in the fruit. The result of the analysis test of the Anova variance shows a very significant influence ($p = 0.000$) of the fertilization variant on the metabolism of anthocyanin pigments in fruits (partial eta squared = 77.5%). Also, the anthocyanin content of the fruit was very significantly influenced by the genetic background of the cultivar ($p = 0.000$) with a value of 87%. The cumulative effect of the two experimental factors on the total amount of anthocyanins in the fruit was 42.2%. And the growth conditions in the two experimental years showed a very significant influence ($p = 0.000$) on the anthocyanin pigments in the fruit (partially eta squared = 82.2%). Agronomic practices have an obvious effect on the content of anthocyanin pigments in fruits, a fact also observed by Nicola et al. (2020).

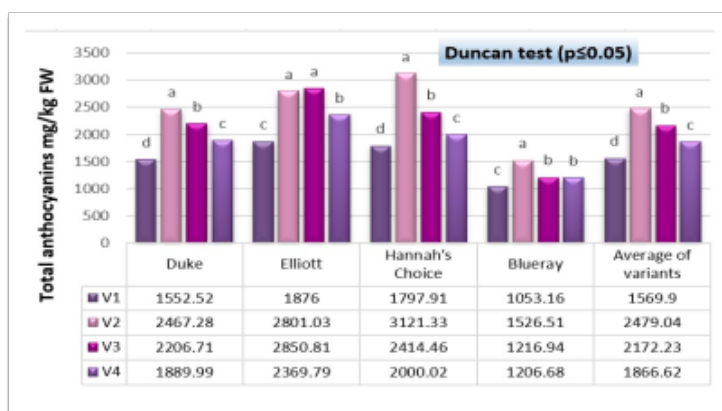


Figure 5. Influence of foliar fertilizers on the total anthocyanin content (expressed as cyanidin 3-glucosides) in fruit by variety in the years 2020-2021

Total polyphenols content

The main factor that determines the composition of blueberry polyphenols is the genetic background. However, cultivation technologies and climatic conditions can have a significant impact on the phenolic concentration of these fruits (Vittori et al., 2018). This fact was also noticed in the present study from the analysis test of the Anova variant. The data show a very significant effect ($p = 0.000$) of the genetic characteristics on the phenol levels in the fruits of the studied varieties of 89.7%. In addition, the organic or conventional agronomic practices used had a very significant influence on the phenolic content of blueberry fruit by 74.2%. And the environmental conditions between the years 2020-2021 had a statistically assured impact on the concentration of polyphenols in fruits (partially and squared = 80.3%). In the present

study, a large variation of the total polyphenol content in the fruits of the four blueberry varieties chosen for analysis was observed. 'Hannah's Choice' and 'Elliott' varieties have higher average levels of these compounds. The 'Elliott' variety showed an accumulation in the organic variant V3 fertilized with the ERT 23 Plus biostimulant of 5467.93 mg GAE/kg FW and the 'Hannah's Choice' variety obtained the highest value (5609.19 mg GAE/kg FW) in the organic variant V2 fertilized with Algacifo 3000 (Figure 6). The lowest values of the accumulated polyphenols concentration were found in the 'Blueray' variety (2148.4 mg GAE/kg FW in the control variant). Between the two applied organic fertilizers there are statistically assured differences on the average tendency of accumulation of phenolic compounds in fruits.

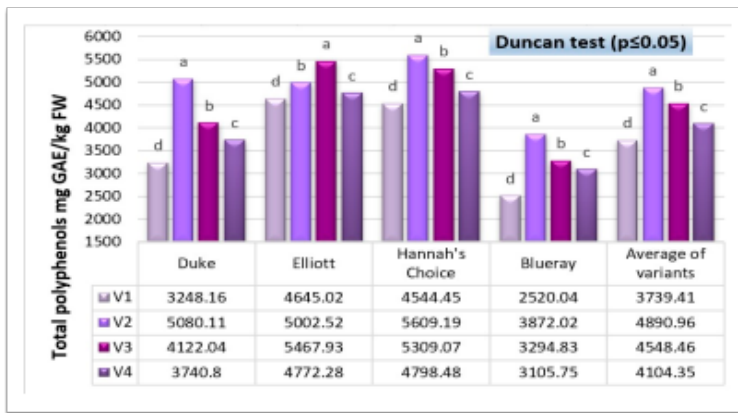


Figure 6. Influence of foliar fertilizers on the content of total fruit polyphenols by variety in the years 2020-2021

Table 1 shows the interdependence between fruit quality characteristics in the years 2020-2021. A distinctly significant correlation can be observed between the total dry matter content and the value of the total sugar content, the correlation coefficient being ($r = 0.308^{**}$). This can be explained by the fact that sugars are also included in all dry matter. From the correlation matrix a negative correlation is observed, distinctly significant between DM and the total content of polyphenols and anthocyanins ($r = 0.161^{**}$, respectively $r = 0.524^{**}$). The content of vitamin C correlates distinctly significant (negative) with the content of total polyphenols, the correlation coefficient being (r

$= - 0.289^{**}$). This means that during fruit ripening, the polyphenol content accumulates in the fruit to the detriment of the vitamin C content. A distinctly significant negative correlation was also obtained between the total sugar content and the amount of organic acids ($r = - 0.208^{**}$), the higher the total sugar content (%), the lower the organic acid content (%). A positive correlation was obtained between the total anthocyanin content and the total polyphenol content, distinctly significant ($r = 0.412^{**}$) which is normal considering that most of the phenolic compounds in blueberry fruits belong to the flavonoid class.

Table 1. Pearson correlations coefficients for the biochemical quality indicators for the studied blueberry varieties

Pearson correlations	Total dry matter %	Total acidity %	Total sugar %	Vitamin C mg/100g FW	Total anthocyanins mg/Kg FW	Total polyphenols mg GAE / Kg FW
Total dry matter %	1					
Total acidity %	0.089	1				
Total sugar %	0.306**	-0.480**	1			
Vitamin C mg/100g FW	-0.052	0.511**	-0.232**	1		
Total anthocyanins mg/Kg FW	0.524**	0.214**	0.120*	0.001	1	
Total polyphenols mg GAE / Kg FW	0.161**	0.340**	-0.056	-0.289**	0.412**	1

** . Correlation is significant at the 0.01 level (2-tailed).

* . Correlation is significant at the 0.05 level (2-tailed).

CONCLUSIONS

It has been established that the variation in the organic matter content of highbush blueberry fruit ('Duke', 'Elliott', 'Hannah's Choice' and 'Blueray' varieties) is dependent on genetic background and agronomic practices. Different cropping systems significantly affect the quality of blueberry fruit. Blueberries produced in organic culture have a significantly higher content of phytonutrients compared to those produced in conventional culture. Given the positive effect of organic fertilizers on fruit quality, a partial replacement of mineral fertilizers with organic fertilizers may be recommended.

Of the two organic fertilizers used, Algacifo 3000 fertilizer is recommended to increase the biochemical quality of the fruit, under the influence of which the highest values of phytonutrients in the fruit were obtained on average.

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