

VARIATION IN HEALTH PROMOTING COMPOUNDS OF BLUEBERRY  
FRUIT ASSOCIATED WITH DIFFERENT NUTRIENT MANAGEMENT  
PRACTICES IN A SOILLESS GROWING SYSTEM

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**Abstract:** The objective of this study was to determine and compare the content of total anthocyanins (TACY), total phenolics (TPC) and total antioxidant activity (TAA) of the fruit of 'Bluecrop' highbush blueberry grown under different nutrient management practices in a soilless production system. A field study was carried out in a highbush blueberry plantation situated near Belgrade (Serbia), during the period of 2016–2017. The orchard was planted in the spring of 2016 in 50 l polypropylene pots with 5-year-old nursery plants. Each pot was filled with the mix of pine sawdust (60%), white peat (30%) and perlite (10%), and placed at a distance of 0.8 m within the row and 3.0 m between the rows (4,170 bushes ha<sup>-1</sup>). The following fertilizer treatments were evaluated: 1. organic fertilizers (Org); 2. mineral fertilizers (Min) and 3. a combination of organic and mineral fertilizers (Org-Min). Soluble NPK fertilizers were applied with irrigation water, whereas granulated mineral and pelleted organic fertilizers were mixed with the substrate. Fruit samples were collected in triplicate at the beginning of ripening, full maturity and the end of the harvest season. No significant effect of harvest time on each of the tested parameters was observed, whereas the content of TACY did not even differ under various fertilizer treatments. TPC in the fruit significantly increased in Org and Org-Min treatments (139.8 and 139.3 mg eq GA 100 g<sup>-1</sup> FW, respectively) compared to Min treatment (122.7 mg eq GA 100 g<sup>-1</sup> FW), while a considerably high TAA level was found only in berries under Org-Min treatment (0.53 mg asc g<sup>-1</sup> FW).

**Key words:** *V. corymbosum*, fertilizers, harvest time, total phenolics, total anthocyanins, antioxidant activity.

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## Introduction

Northern highbush blueberry (*Vaccinium corymbosum* L.) is presently one of the very popular commercial crops in the Republic of Serbia covering the total area of around 1,500 ha. The majority of blueberries are soil-grown, but a soilless growing system has also gained popularity in recent years. Some of the advantages of soilless culture are related to easier control of irrigation and nutrient management; manipulation with plant growth to better control of shoot length, fruit to shoot ratio, and fruit quality (Voogt et al., 2014). This production system also enables higher planting densities (4,170–5,500 plants ha<sup>-1</sup>) aiming to reach maximum production per unit area (Milivojević et al., 2017a).

The initial focus in a soilless culture system is on balanced and precise fertilizer applications which can improve the nutrient status and regulate plant development and yield of blueberries. The major concern in the application of nitrogen (N) fertilizers is N availability, which is highly related to productivity (Miller et al., 2006). It is generally accepted that blueberry plants mainly use ammonium (NH<sub>4</sub>) form of N and therefore fertilizers supplying N as ammonium (urea, ammonium sulfate) are preferable for blueberries. In a field study of Vargas and Bryla (2015) on northern highbush blueberry (*V. corymbosum* L.), all ammoniacal N and 25% to 50% of organic N were available for blueberry plants during the same year when they were applied. In the case of phosphorus (P), symptoms of deficiency are not commonly seen, and field plants seldom respond to P applications (Hanson and Hancock, 1996). Potassium (K) deficiency can be due to a number of factors: reduced root function, flooding, poor drainage, high N levels, drought and very acid soils (Retamales and Hancock, 2018). Since fruit is an important sink for K in the plant, leaf K levels are greatly influenced by fruit load. Calcium (Ca) nutrition has also recognized effects on the fruit quality (texture, firmness and ripening rate). Among microelements, iron (Fe) deficiency is common in blueberries. The most effective means to correct Fe deficiency is to adjust substrate pH within the optimal range of 4.2–5.5, which, among the other things, can be achieved using adequate fertilizers.

It is important to underline that deficiency or imbalance of mineral nutrients can affect some physiological and metabolic modifications, such as lower photosynthetic activity, displacement and unusual circulation of organic compounds, as well as the accumulation of primary and secondary metabolite products (Fotirić Akšić et al., 2019). Thus, the understanding of the effects of different nutrients on the overall quality of blueberries could lead to the adoption of specific cultivation and nutrient management practices to meet certain demands of this fruit species.

Blueberries are reported to be an excellent source of bioactive compounds with high antioxidant activity and potential health benefits exhibiting anti-diabetic

properties, decreasing blood pressure and blood cholesterol, and inhibiting the development of cancer cells in the breast and colon (Calò and Marabini, 2014; Pertuzatti et al., 2016). Most researches have correlated the high antioxidant capacity with the phenolic content in the fruit, especially anthocyanins and flavanols, as well as hydroxycinnamic acids (Castrejón et al., 2008; You et al., 2011; Milivojević et al., 2012; Milivojević et al., 2016a; Milivojević et al., 2017b). However, a wider knowledge of the effect of different fertilizers on the variation of some bioactive compounds is imperative to control and manipulate preharvest changes in the blueberry fruit. Therefore, this study aimed to compare the effect of organic and mineral fertilizers on the content of total anthocyanins and total phenolics, as well as expressed antioxidant activity for the 'Bluecrop' highbush blueberry cultivar grown as a soilless culture.

## Materials and Methods

### Description of the experimental site

The field study was carried out in the 'Bluecrop' highbush blueberry plantation situated near Belgrade (44°45' N, 20°35' E, 112 m a.s.l.) during the period of 2016–2017. The climate of the region is temperate continental, with a mean annual air temperature of 10.8°C and mean annual precipitation of 650 mm. The orchard was planted in the spring of 2016 in 50 l polypropylene pots with 5-year-old nursery plants. The growing media was pine sawdust (60%), white peat (30%) and perlite (10%). Each pot, filled with the mix, was placed at a distance of 0.8 m within the row and 3.0 m between the rows (4,170 bushes ha<sup>-1</sup>).

### Experimental design and treatments

The following nutrient treatments were examined in this study: 1) organic fertilizer application (pelleted and water-soluble fertilizers); 2) mineral fertilizer application (granulated and water-soluble fertilizers); 3) combined application of organic and mineral fertilizers. Plants were fertilized with 64 kg ha<sup>-1</sup> N, 42 kg ha<sup>-1</sup> P, 52 kg ha<sup>-1</sup> K in the treatment 1 (Org); 85 kg ha<sup>-1</sup> N, 45 kg ha<sup>-1</sup> P, 64 kg ha<sup>-1</sup> K in the treatment 2 (Min), and with 72 kg ha<sup>-1</sup> N, 48 kg ha<sup>-1</sup> P, 68 kg ha<sup>-1</sup> K in the treatment 3 (Org-Min).

At the beginning of intensive vegetative growth, pelleted fertilizers (I: 6% N, 15% P, 3% K, 2% MgO, 10% CaO, 55.2% organic matters; II: 3% N, 3% P, 7% K, 2% MgO, 60% organic matters) were applied with 50 g and 30 pot<sup>-1</sup>, respectively in the treatment 1; granulated NPK (14% N; 14% P and 17% K) and ammonium sulphate (20% N; 24% S) fertilizers, both with 50 g pot<sup>-1</sup>, were applied in the treatment 2; while organic fertilizer (6% N, 15% P; 3% K; 2% MgO; 10% CaO, 55.2% organic matters) and granulated ammonium sulphate (20% N; 24% S), both

with 50 g pot<sup>-1</sup>, were applied in the treatment 3. During the spring, when the plants were in the vegetative (i.e. sprout) stage of production, pelleted fertilizer (10% N, 3% P, 3% K, with 72% organic matters) was mixed with substrate two times (application rate of 50 g pot<sup>-1</sup> per each) in the Org treatment, whereas in the Min and Org-Min treatments, water-soluble NPK fertilizer (20% N, 20% P and 20% K) was applied through the irrigation system 4 times a week, from the end of April to the end of May. Fertigation with water-soluble NPK fertilizer (12% N, 12% P and 36% K) was applied to the plants that had been in the cropping stage of vegetation (4 times a week, from the beginning of June to the middle July) in the Min and Org-Min treatments. At this stage of vegetation, 50 g pot<sup>-1</sup> of pelleted fertilizer (10% N, 3% P, 3% K, with 72% organic matters) was mixed with the substrate and additionally, potassium (12% K) foliar feeding was applied 2 times a week only in the Org treatment.

The trial was set up in a completely randomized design with 3 replications and 10 bushes pots<sup>-1</sup> per replication for each fertilizer treatment. A sample of 30 randomly selected fruits from all around of the bush (from each fertilizer treatment and replication) was collected at the beginning, mid and the end of the harvest season and used for analyzing the content of total anthocyanins (TACY) and phenolics (TPC), as well as total antioxidant activity (TAA). Each sample was pooled to obtain a composite sample. For the extraction of phenolics, fruits were homogenized in 80% methanol (1:3 w/v). The homogenates were centrifuged at 10,000 x g for 10 min. Three replications of supernatants were prepared for spectrophotometric analysis (Thermo Scientific Multiskan Spectrum, Vantaa, Finland). The TACY was measured with the modified pH differential absorbance method (Cheng and Breen, 1991). The absorbance was measured at 510 and 700 nm in 0.025 M potassium chloride buffer at pH 1.0 and 0.4 M sodium acetate buffer at pH 4.5. Results were expressed as micrograms of cyanidin-3-glucoside ( $\epsilon=26900\text{l}\cdot\text{mol}^{-1}\cdot\text{cm}^{-1}$ ) equivalents per 100 g of fresh weight ( $\mu\text{g g cy-3-g eq/100 g FW}$ ).

The TPC was determined according to the Folin-Ciocalteu procedure (Singleton and Rossi, 1965) using gallic acid (GA) as a standard. Results were read at 724 nm and expressed as milligrams of gallic acid equivalent per 100 g of fresh weight (mg GAE 100 g<sup>-1</sup> FW). Determination of TAA was done following the ABTS method of Arnao et al. (1999) and results were expressed as milligrams of ascorbic acid equivalent per gram of fresh weight (mg eq asc g<sup>-1</sup> FW).

#### Statistical analysis

The data obtained in the research were processed applying the Fisher model of variance analysis (ANOVA, F test) and the statistics software package STATISTICA version 8.0 (StatSoft, Inc., Tulsa, OK, USA). The analyses were

performed in three replications and the obtained values for two years were expressed as the means  $\pm$  standard error. Significant differences among the means of the treatments were determined by LSD test at a level of  $P \leq 0.05$ .

## Results and Discussion

The determination of TPC allows one to estimate the content of all compounds belonging to the subclass of phenolic compounds (Pertuzatti et al., 2016). Besides the genetic background as the main factor determining the phenolic composition of the blueberries, the interaction with the environmental conditions and the cultivation systems also influence the phenolic concentration (Vittori et al., 2018). In our study, the analysis of the TPC in the fruit of 'Bluecrop' highbush blueberry grown as a soilless culture showed that the effect of fertilizer treatments was more marked than the effect of harvest time (Table 1). The implementation of organic fertilizers in Org and Org-Min treatments had a stimulating impact on TPC in the fruit (139.8 and 139.3 mg eq GA 100 g<sup>-1</sup> FW, respectively). Numerous studies also confirm the fact that blueberries grown under the organic cultivation system have more polyphenols as well as other antioxidant compounds than blueberries grown under the conventional system (Wang et al., 2008; You et al., 2011; Gupta-Elera et al., 2012). Higher values of TPC were also found in strawberries treated with biofertilizers (Pešaković and Milivojević, 2014) which can be explained by highly intensive mineralizing processes in the substrate and the increased physiological functions and activity of the plant root. Wang et al. (2008) found the mean values for TPC of 319.3 and 190.3 mg eq GA 100 g<sup>-1</sup> FW in blueberries from organic and conventional cultures, respectively. According to Kim et al. (2013), the 'Bluecrop' highbush blueberry cultivar grown in Korea exhibited higher TPC (205.2 mg eq GA 100 g<sup>-1</sup> FW) compared to results in the present study. Concerning the influence of other factors, Milivojević et al. (2017b) underlined the predominant effect of the modified microclimate under a grey hail protection net, rather than the influence of the cultivation cycle on the accumulation of total phenolics in cv. 'Bluecrop'.

Among the secondary plant metabolites found in blueberries, the flavonoid subclass (anthocyanins) has received the most attention. Up to 60% of the total phenolic content in highbush blueberries is accounted for by anthocyanins, which are responsible for the blue colours in fruit and are dependent on environmental pH values (You et al., 2011; Retamales and Hancock, 2018). Pertuzatti et al. (2016) have reported that only five types of anthocyanins are found in blueberries as follows: delphinidin, malvidin, petunidin, peonidin and cyanidin. In the present study, TACY was not significantly affected by fertilizer treatment and harvest time. However, fertilizer treatment  $\times$  harvest time interaction showed a significantly higher accumulation of TACY at the mid and end of the ripening

season in Org and Org-Min treatments. In addition to the above said, a significantly higher accumulation of TACY was also observed at the beginning and mid of the ripening season in the Min treatment.

Similarly, Connor et al. (2002) found that delaying fruit harvest could have a markedly positive influence on levels of anthocyanins in blueberry fruit. The same authors reported statistically significant genotype  $\times$  environment interactions for both total anthocyanin and phenolic content; however, they did not discuss the differences between regions that may be caused by agronomic practices and climatic factors, such as differences in mineral nutrients available, ultraviolet intensity, temperature during fruit ripening, and water stress.

Table 1. Average contents of total anthocyanins (TACY) and total phenolics (TPC) in the fruit of 'Bluecrop' highbush blueberry affected by fertilizer treatment and harvest time.

Treatments	FT/HT		TACY ( $\mu\text{g cy-3-g eq g}^{-1}$ FW)	TPC (mg GAE 100 $\text{g}^{-1}$ FW)
Fertilizer treatments (FT)	Org		563.8 $\pm$ 31.5	139.8 $\pm$ 6.08a
	Min		541.0 $\pm$ 30.0	122.7 $\pm$ 4.25b
	Org-Min		577.4 $\pm$ 28.4	139.3 $\pm$ 3.46a
Harvest time (HT)	Beginning		517.7 $\pm$ 25.3	125.4 $\pm$ 5.26
	Mid		602.4 $\pm$ 25.8	138.9 $\pm$ 4.90
	End		562.1 $\pm$ 32.1	137.6 $\pm$ 4.99
FT $\times$ HT	Org	Beginning	455.6 $\pm$ 43.6d	123.0 $\pm$ 10.2
		Mid	600.9 $\pm$ 28.9ab	144.3 $\pm$ 4.22
		End	634.8 $\pm$ 9.61a	152.1 $\pm$ 9.71
	Min	Beginning	593.6 $\pm$ 13.8ab	115.2 $\pm$ 4.23
		Mid	557.7 $\pm$ 56.4abcd	125.9 $\pm$ 11.35
		End	471.7 $\pm$ 59.1cd	127.2 $\pm$ 5.15
	Org-Min	Beginning	504.0 $\pm$ 25.8cd	138.1 $\pm$ 8.66
		Mid	648.4 $\pm$ 43.5a	146.4 $\pm$ 3.77
		End	579.7 $\pm$ 43.3abc	133.4 $\pm$ 3.31
F test	FT		ns	*
	HT		ns	ns
	FT $\times$ HT		*	ns

Data are presented as the means of 2-year values and 3 replications in each year  $\pm$  standard error. Values within each column followed by the same letter are not significantly different at  $P \leq 0.05$  (LSD test). \*Significant at  $P \leq 0.05$ ; ns – not significant.

Milivojević et al. (2016a) also noted the variations in TACY and TPC across the harvests in the fruit of cv. 'Duke' achieving higher concentrations in the 1<sup>st</sup> and 2<sup>nd</sup> harvest, whereas no significant differences were observed in cv. 'Bluecrop'. This discrepancy may be explained by variation in solar radiation and air temperature throughout the ripening season of the mentioned cultivars as the factors known to impact phytochemical biosynthesis. Changes in TPC during

maturation of four tested cultivars were also reported by Castrejón et al. (2008), with higher concentrations of total phenolics in unripe green berries. After this stage, TPC was decreasing during color break and ripening, and then in cv. 'Bluecrop', values were maintained stable until reaching horticultural maturity.

The phytochemical content of blueberries, particularly anthocyanins and other polyphenols, is responsible for the total antioxidant activity (TAA). The contributions of TPC and TACY to expressed TAA of highbush blueberries were previously reported by Milivojević et al. (2016a, b). In the mentioned studies, a significant variation in TAA was found among the tested cultivars and growing seasons. Besides these factors, TAA can be affected by location, cultural management, maturity, and postharvest handling and storage. In our study, a considerable variation in TAA levels of cv. 'Bluecrop' was caused by applied fertilizer treatments. Significantly higher TAA was registered in the Org-Min treatment ( $0.53 \text{ mg asc g}^{-1} \text{ FW}$ ) compared to the other tested treatments (Table 2).

Table 2. Average levels of total antioxidant activity (TAA) in the fruit of 'Bluecrop' highbush blueberry affected by fertilizer treatment and harvest time.

Treatments	FT/HT	TAA (mg eq asc g <sup>-1</sup> FW)	
Fertilizer treatments (FT)	Org	$0.38 \pm 0.04\text{b}$	
	Min	$0.38 \pm 0.03\text{b}$	
	Org-Min	$0.53 \pm 0.04\text{a}$	
Harvest time (HT)	Beginning	$0.37 \pm 0.05$	
	Mid	$0.41 \pm 0.04$	
	End	$0.40 \pm 0.02$	
FT × HT	Org	Beginning	$0.24 \pm 0.06$
		Mid	$0.46 \pm 0.07$
		End	$0.42 \pm 0.03$
	Min	Beginning	$0.44 \pm 0.01$
		Mid	$0.32 \pm 0.07$
		End	$0.37 \pm 0.05$
	Org-Min	Beginning	$0.44 \pm 0.11$
		Mid	$0.46 \pm 0.08$
		End	$0.40 \pm 0.02$
F test	FT	*	
	HT	ns	
	FT × HT	ns	

Data are presented as the means of 2-year values and 3 replications in each year  $\pm$  standard error. Values within each column followed by the same letter are not significantly different at  $P \leq 0.05$  (LSD test). \*Significant at  $P \leq 0.05$ ; ns – not significant.

This result may be attributed to the TPC increment owing to the synergistic effect of organic and mineral fertilizers, although the impact of cultivation management on antioxidants is pretty controversial. On the one hand, Wang et al. (2008) found that cv. 'Bluecrop' grown organically yielded significantly higher

total phenolics, total anthocyanins and antioxidant activity (ORAC), whereas, on the other hand, Sablani et al. (2010) indicated that total anthocyanins content, phenolic content and total antioxidant activity of blueberries were not altered by the agricultural production system. Generally, the organic production system is in most cases considered a more stressful production system, due to the insufficient supply of mineral nitrogen and other nutrients, which leads to a higher accumulation of primary and secondary metabolic products (Fotirić Akšić et al., 2019).

No significant effects of harvest time and fertilizer treatment  $\times$  harvest time interaction on TAA levels were observed in our study. Previous findings of Milivojević et al. (2016b) showed that TAA levels of cv. 'Bluecrop' grown in soil were increased linearly from the 1<sup>st</sup> to the 4<sup>th</sup> harvest. These discrepancies could be influenced by pre- and post-harvest factors such as environmental characteristics (temperature, humidity and solar intensity), agro-technical conditions (soil, water supply, use of fertilizers or manure), where each of them or their combination may predominantly affect biomolecule activity and availability.

### Conclusion

The changes in the content of blueberry polyphenols and antioxidant activity in cv. 'Bluecrop', occurring as a result of the application of different fertilizers in a soilless growing system, point to the fact that the most prominent effect was produced by the organic fertilizers, either applied alone (Org treatment) or in combination with mineral fertilizers (Org-Min treatment). Some inconsistent results were also found when comparing TACY and TPC in the blueberry fruit, affected by fertilizers and harvest time.

Considering the positive effect of organic fertilizers, as well as a stimulating impact of combined application of organic and mineral fertilizers on the expressed total antioxidant activity, a partial substitution of mineral by organic fertilizers may be recommended in advancing the existing technology of soilless blueberry production. Detailed research on some other bioactive compounds that also contribute to the expressed antioxidant activity, such as sugars, vitamins and enzymes should be also performed in the future.

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VARIRANJE SADRŽAJA ZDRAVSTVENO KORISNIH KOMPONENTI U  
PLODU BOROVNICE POVEZANO SA RAZLIČITIM NAČINIMA  
ISHRANE U SISTEMU GAJENJA U SUPSTRATU

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R e z i m e

Cilj ovog istraživanja bio je da se odredi i upoređi sadržaj ukupnih antocijana i ukupnih fenola, kao i antioksidativna aktivnost u plodu sorte visokožbunaste borovnice 'Bluecrop' gajene u supstratu pod uticajem različitih načina ishrane. Poljsko istraživanje je izvedeno u proizvodnom zasadu borovnice koji se nalazi u blizini Beograda (Srbija), tokom perioda 2016–2017. godine. Zasad je podignut u proleće 2016. godine sadnjom petogodišnjih biljaka u saksije od polipropilenske folije zapremine 50 l. Svaka saksija je ispunjena supstratnom smešom sastavljenom od strugotine četinarara (60%), belog treseta (30%) i perlita (10%). Primenjeno rastojanje sadnje je 0,8 m u redu i 3,0 m između redova (4.170 žbunova po ha). Ispitivana je primena sledećih đubriva: 1. organska đubriva (Org); 2. mineralna đubriva (Min) i 3. kombinovana primena organskih i mineralnih đubriva (Org-Min). Rastvorljiva NPK đubriva su primenjivana kroz sistem za navodnjavanje, dok su granulirana mineralna i peletirana organska đubriva mešana sa supstratom. Uzorci plodova su uzimani u 3 ponavljanja na početku, sredini i na kraju sezone berbe. Vreme berbe nije ispoljilo značajan uticaj na testirane parametre, dok se sadržaj ukupnih antocijana nije razlikovao čak ni pod uticajem primenjenih đubriva. Sadržaj ukupnih fenola u plodu bio je značajno povećan u Org i Org-Min tretmanima (139,8, odnosno 139,3 mg ekv. gal. kis./100 g sv.m.pl.), dok je značajno veća ukupna antioksidativna aktivnost ustanovljena u plodovima iz Org-Min tretmana (0,53 mg ask./g sv.m.pl.).

**Ključne reči:** *V. corymbosum*, đubriva, vreme berbe, ukupni fenoli, ukupni antocijani i antioksidativna aktivnost.

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