Scientific Papers. Series A. Agronomy, Vol. LXVII, No. 1, 2024
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

THE EFFECT OF THE CULTURE SUBSTRATE ON THE CONTENT OF BIOACTIVE COMPOUNDS IN SOME BLACKBERRY GENOTYPES

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Abstract

Blackberries are shrubs that belong to the genus Rubus, family Rosaceae. It is a perennial plant, to have believed originated in Armenia, fast growing, cultivated mainly in Europe, Asia and North America, but its worldwide popularity is steadily increasing. Since blackberries grow naturally in extensive regions of Romania. They are cultivated only on small areas of land. The fruits of the genus Rubus are among those rich in bioactive compounds (anthocyanins, dietary fiber, vitamins, minerals and carbohydrates, so beneficial for human and animal health. The objective of the paper was to evaluate the bioactive compounds with antioxidant properties from blackberry fruits obtained from plants grown on different culture substrates, such as: manure, garden soil, forest compost, semi-fermented compost and spent mushroom substrate (SMS) applied to the soil. The studied plantation was established in the spring of 2020, and the presented results refer to the fruits harvested in 2022. The experiments were set up in the field within SCDP Băneasa - the Moara Domnească Afumați experimental farm.

Key words*: blackberry cultivation, nutritive substrates, bioactive compounds.*

INTRODUCTION

The blackberry is a shrub belonging to the genus Rubus in the family Rosaceae. The Rosaceae family is the 19th largest plant family. The genus Rubus, with 740 species, is the largest genus in this family, Rubus comprises 12 subgenera, with few domesticated species. It is a perennial plant believed to have originated in Armenia. growing wild in Europe. the Middle East. North Africa and North America. It is introduced into cultivation in America in the early 19th century and in Western Europe in the second half of the 19th century (Bălan et al., 2013) and spread and cultivated mainly in Europe. Asia and North America. but its worldwide popularity is steadily increasing (Memete et al., 2023).

In Romania. thorny wall bush grows in wild flora from low altitudes in lowland areas to over 900 m altitude in highlands (Ancu et al., 2014). Paulina Mladin is one of the researchers who studied this species in our country and among the results of her work we can mention the obtaining of the varieties 'Dar 8' and 'Dar 24'. descendents of the variety 'Darrow' (Ancu et al., 2014).

Blackberry cultivation has attracted particular attention in Romania in recent years. Thus, blackberries harvested from wild flora are of great interest to consumers, and the fruit is consumed both fresh and in the form of juices, jams, compotes, syrup or in the preparation of desserts. In 2005, the area of wild blackberries in Romania was about 2400 ha and the area cultivated with organic blackberries was only 10 ha (Strik et al., 2007). Wild blackberries produce edible fruit and are widespread throughout the world, but are mainly concentrated in the northern hemisphere.

Farmers have developed a variety of *R. fruticosus* varieties using traditional crossing procedures, which differ in firmness, shape, size, flavour, colour. Weight, yield, ripening season, nutritional content and pest resistance. Throughout history, blackberry has had a significance in medicine and has been used in many ways. Today, the demand for blackberries is increasing, this fruit is defined as functional food in medical terminology and public

awareness of this issue is still growing (Eskimez et al., 2019).
Fruits of the Rubus genus are among those rich in bioactive compounds. Numerous studies draw attention to the properties and benefits of these biochemical constituents and provide further encouragement that breeding selection can be used to increase the levels of beneficial compounds in these fruits (Cho et al., 2004; Clark & Finn, 2008; Mladin et al., 2008). Anthocyanins, ellagitannins, phenolic acids, flavonoids, vitamins, minerals as well as other compounds contribute to the high antioxidant capacity of blackberries (Diaconeasa et al., 2014; Kaume et al., 2012; Vlad et al., 2019) and also have anti-carcinogenic, anti-neurodegenerative and anti-inflammatory effect (Milosevici et al., 2012; Cuevas-Rodríguez et al., 2010; Baby et al., 2018; Vega et al., 2021). Anthocyanins are known to be powerful antioxidants that can ability to fight oxidative stress and scavenge free radicals from the body (Manganaris et al., 2014; Mikulic-Petkovsek, 2017). Blackberry fruit is rich in vitamins C, K and E, dietary fiber and low in carbohydrates. Eating vitamin C can help the body fight free radicals, (toxic compounds that naturally form in the human body when food is converted into energy) (U.S. DHHS, 2021).
The objective of this work was to evaluate bioactive compounds with antioxidant properties in mulberry fruits obtained from plants grown on different culture substrates.

MATERIALS AND METHODS

The study was carried out at the Moara Domnească Experimental Base, located NE of Bucharest (in Câmpia Vlăsiei, a subunit of the Romanian Plain), in Ilfov County, just about 17 km from Bucharest. The farm belongs to the Research and Development Station for Pomiculture (RDSFG) Băneasa.
The experimental plot was established in early spring 2020 by planting three varieties of mulberry (*Rubus fruticosus* L.): 'Dar-24', 'Triple Crown' and 'Chester', on different nutrient substrates (peat moss, semi-fermented compost. forest compost. and mixture of the 4 substrates in equal amounts), at distances of 3.0 m between rows and 1.0 m apart in each row.
The environment in which the plant grows and develops is one of the most important factors in agriculture. Substrates must be able to provide adequate water, nutrients and oxygen for the plant, as well as support for the whole plant, (Sun et al., 2004; Miller & Jones, 1995).
The better the substrate, the healthier and more vigorous the plant
Biochemical investigations were carried out at the Faculty of Biotechnologies of USAMV Bucharest, using spectrophotometric determination methods.

Determination of total ascorbic acid content in fruit juices was performed by spectrophotometric method using potassium permanganate ($KMnO_4$) as chromogenic reagent (Zanini et al., 2018; Elgailani et al., 2017). The concentration of ascorbic acid in the samples was expressed in mg/L.

Determination of total anthocyanins content. Total anthocyanins content (TA) was carried out using the pH differential spectrophotometric method (Giusti & Wrolstad, 2000).

Determination of total phenolics
For determination of TPC, a method with Folin - Ciocalteu reagent (Sigma-Aldrich) (Singleton, 1999).
Statistical analysis
All measurements were carried out in three replicates (n = 3) and results were presented as means ± standard deviations (SD), Anova test and Duncan test were performed with SPSS software.

RESULTS AND DISCUSSIONS

The biological material consisted of three varieties of mulberry (*Rubus fruticosus* L.): 'Dar-24', 'Triple Crown' and 'Chester', from the Băneasa Pomiculture Research and Development Station - Bucharest. A brief characterization of the three blackberry cultivars and the general appearance of the fruit are presented in Table 1.

Table 1. General description of the blackberry genotypes studied

Genotip	General description	Fruit appearance
„Dar-24”	**The variety „Dar-24**”, is a thorny vine, origin Romania (ICDP-Pitesti), with a harvest period between July and August. The variety has a medium to high vigour, is frost resistant and has a good productivity. The fruit has an elongated conical shape and is black, shiny, with a sweet taste.	
“Triple Crown”	**The variety „Triple Crown**”, is a new thornless mulberry variety of American origin, bred in 1998 (Maryland, U.S.A.). It has outstanding fruit and plant qualities, high yields and good disease resistance. It is a semi-seasonal variety producing from the first decade of July to mid-August. Fruits are black, sweet, aromatic, firm with good resistance to handling and transport.	
„Chester”	**The variety „Chester**”, is one of the most frost- and disease-resistant thornless mulberry varieties. It is a semi-late ripening variety, fruit ripening is staggered from August to late autumn when frosts come. The fruit is medium sized (6 g), medium firm but very aromatic, sweet, shiny black, oval to spherical in shape, large drupes in the fruit with uniform ripening of the drupes..	

The soil at Moara Domnească is a reddish preluvosoil. Several soil profile analyses were carried out in the in-house agrochemical and biochemical laboratory to determine the soil's physico-chemical properties. The following soil characteristics were determined (by particle size analysis to determine the clay, dust and sand content of the soil): a high percentage of clay ranging from 40.55% in the upper horizon 0-40 cm to 41.63% at depths of 41-53 cm and 47.39% at depths greater than 54 cm (Table 2).

Table 2. The granulometric composition of the soil (Experimental Base Moara Domnească, 2019)

Horizon	Depth (cm)	Clay (%)	Coarse sand (%)	Fine sand (%)	Dust (%)	Texture
Ao	0-40	40.55	0.36	34.33	24.75	Clay loam
Ao/Bt	41-53	41.63	0.52	21.54	56.28	Clay loam
Bt	54-200	47.39	0.37	27.59	30.34	Clay loam
C	Over 200	36.18	0.42	32.04	32.04	Clay loam

The clay texture results in low nutrient mobility and poor soil water permeability, soil humus content is good in the first 40 cm of the profile. where most of the roots of young trees, reaching a value of 3.26%, then drops sharply to 1% in the Bt horizon profile (Table 3).

Table 3. Physical and chemical properties of the profile soil (Experimental Base Moara Domnească, 2019)

Horizons / Properties	Ao	Ao/Bt	Bt	C
Humus (%)	3.26	1.87	1.0	1.0
Soluble Ca (mg/100 g Soil)	55	32	32	30
Hydrolitic acidity (meq)	2.8	2.04	1.72	0.18
Exchangeable Bases (meq)	22.6	23.62	26.28	-
Total cation exchange capacity (meq)	28.65	28.04	30.01	-
Degree of saturation in bases (%)	78.94	84.28	87.53	-
pH	6.4	6.6	6.8	8.3
Total N (%)	0.144	0.102	0.075	0.07
Soluble P (mg/100 g soil)	50	40	40	30

The pH is slightly acidic at the soil surface (6.4), reaching alkaline in the C horizon (8.3). Other indicators such as nitrogen index (NI), hydrolytic acidity, humus, organic carbon were determined during 2020. The climate at Moara Domnească is temperate continental.

The plants were grown on different nutrient substrates (peat moss, semi-fermented compost, forest compost, and a mixture of the 4 substrates in equal amounts) (Table 4).

Table 4. Variants of nutrient substrates in which blackberry varieties are planted

No.	Variants of nutrient substrates
1	Control
2	Manure
3	Mushroom Compost
4	Forest Compost
5	Semifermented Compost
6	Mixture Compost 25% of the 4 substrate variants

Biochemical determinations were performed by analyzing fresh blackberry fruit from 'Dar-24', a Romanian thorny blackberry variety, 'Triple Crown' and 'Chester', both thornless varieties. Fruits were obtained from plants grown on different culture substrates: peat moss. compost from mushroom growing substrates, forest compost, semi-fermented compost and compost made by mixing 25% of the other four substrates (peat moss, compost from mushroom growing substrates, forest compost and semi-fermented compost). The plants produced fruit on all substrates used for their culture.

Assessment of total ascorbic acid content

The evaluation of total ascorbic acid content was carried out in fruit juices by spectrophotometric method at a wavelength of 530 nm using potassium permanganate ($KMnO_4$) as chromogenic reagent. The reduction in absorbance was measured when a solution of potassium permanganate reacted with ascorbic acid solution in acidic medium.

The results for the total ascorbic acid content (g/L) of blackberry fruit are summarised in Table 5 and Figure 1. It is noted that on the control substrate all varieties of blackberry recorded significant values of vitamin C, among them 'Chester' with 2.13 ± 0.03 g/L, followed by 'Dar-24' (1.113±0.046 g/L) and 'Triple Crown' (1.073±0.74 g/L).

The Manur substrate was favourable for Vitamin C accumulation only in the case of Dar-24 fruit (1.567±0.12 g/L). On the mushroom compost substrate, the levels of vitamin C accumulated in fruits were more significant only in the case of the 'Dar-24' (1.193±0.23 g/L) and 'Chester' (1.977±0.07 g/L) blackberry varieties.

The forest compost was favourable to the plants of 'Dar-24' (1.220± 0.08 g/L), while the semi-fermented compost induced the accumulation of high levels of vitamin C in the fruits of 'Dar-24' (2.5± 0.24 g/L) and 'Chester' (2.71± 0.30 g/L). On the mixed compost. compared to the other varieties, only the blackberry fruits of the variety "Dar -24" accumulated high vitamin C values (1.273± 0.05 g/L) (Table 5 and Figure 1).

Table 5. Total ascorbic acid content (mg/L) of blackberry fruit

Descriptive Statistics

Dependent Variable: Total Ascorbic Acid (g/l)

Substrate	Variety	Mean	Std. Deviation	N
Control	DAR 24	1.11	0.05	3
	TRIPLE CROWN	1.07	0.74	3
	CHESTER	2.13	0.03	3
	AVERAGE	1.44	0.64	9
MANUR	DAR 24	1.57	0.12	3
	TRIPLE CROWN	0.32	0.14	3
	CHESTER	0.53	0.04	3
	AVERAGE	0.81	0.59	9
MUSHROOM COMPOST	DAR 24	1.19	0.23	3
	TRIPLE CROWN	0.92	0.14	3
	CHESTER	1.98	0.08	3
	AVERAGE	1.36	0.49	9
FOREST COMPOST	DAR 24	1.22	0.08	3
	TRIPLE CROWN	0.55	0.14	3
	CHESTER	0.34	0.18	3
	AVERAGE	0.70	0.42	9
MIXTURE COMPOST	DAR 24	1.27	0.05	3
	TRIPLE CROWN	0.14	0.06	3
	CHESTER	0.23	0.15	3
	AVERAGE	0.55	0.55	9
SEMIFERMENTED COMPOST	DAR 24	2.50	0.24	3
	TRIPLE CROWN	0.18	0.08	3
	CHESTER	2.71	0.30	3
	AVERAGE	1.80	1.23	9
AVERAGE	DAR 24	1.48	0.51	18
	TRIPLE CROWN	0.53	0.46	18
	CHESTER	1.32	1.02	18
	AVERAGE	1.11	0.81	54

Table 6. Duncan test for each blackberry variety

Variety - 'Dar-24'	N	Subset for alpha = 0.05		
Substrate		c	b	a
Control	3	1.11		
Mushroom Compost	3	1.19		
Forest Compost	3	1.22		
Mixture Compost	3	1.27		
Manur	3		1.57	
Semifermented Compost	3			2.50
Sig.		0.25	1.00	1.00
Means for groups in homogeneous subsets are displayed.				
a Uses Harmonic Mean Sample Size = 3.000.				

Variety - 'Triple Crown'	N	Subset for alpha = 0.05		
Substrate		b	a	
Mixture Compost	3	0.14		
Semifermented Compost	3	0.18		
Manur	3	0.32		
Forest Compost	3	0.55	0.55	
Mushroom Compost	3		0.92	
Control	3		1.07	
Sig.		0.17	0.08	
Means for groups in homogeneous subsets are displayed.				
a Uses Harmonic Mean Sample Size = 3.000.				

Variety - 'Chester'	N	Subset for alpha = 0.05			
Substrate		d	c	b	a
Mixture Compost	3	0.23			
Forest Compost	3	0.34	0.34		
Manur	3		0.53		
Mushroom Compost	3			1.98	
Control	3			2.13	
Semifermented Compost	3				2.71
Sig.		0.4	0.18	0.26	1
Means for groups in homogeneous subsets are displayed.					
a Uses Harmonic Mean Sample Size = 3.000.					

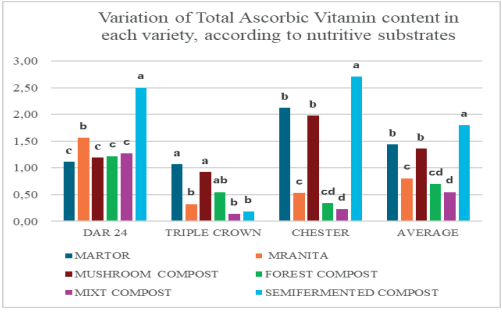

Figure 1. Variation in total ascorbic acid content (g/L) of three varieties of blackberry as a function of nutrient substrates

The data obtained from the analyses lead to the conclusion that among the three varieties tested, cv. "Dar-24" has the highest adaptability of growth and accumulation of bioactive compounds for all five culture substrates. The vitamin C content detected in the fruits of plants of the variety 'Dar-24' grown on the substrates studied ranged from 1.193±0.23 g/L to 2.5 ± 0.24 g/L. The highest vitamin C values were recorded in the fruits of plants grown on semi-fermented compost (2.5 ± 0.24 g/L) and on a mranitic substrate (1.567±0.12 g/L). In the

'Chester' wall variety, the substrates that favoured a significant accumulation of vitamin C were semi-fermented compost (2.71 ± 0.30 g/L) and compost from mushroom culture (1.977 ± 0.07 g/L). At Triple Crown there were no significant levels of vitamin C in the culture substrate variants compared to the control samples (1.073±0.74 g/L). Compared to the other substrates tested, a relatively high value of ascorbic acid close to that recorded in the control sample was obtained in the case of fruit from plants grown on mushroom compost substrate (0.923± 0.14 g/L) (Table 5 and Figure 1).

Ascorbic acid is an essential water-soluble vitamin with excellent reducing properties, well known for its high antioxidant activity due to the neutralization of free radicals and other reactive oxygen species produced by cell metabolism, which are associated with several forms of tissue damage and diseases (Skrovankova et al., 2015). Although ascorbic acid is an important antioxidant, it still provides a maximum of 10% of the total antioxidant capacity of blackberries (Landete, 2011; Li et al., 2015). Vitamin C also contributes to prolonging the shelf life of berries, including blackberries (Zia-Ul-Haq et al., 2014).

Vitamin C (ascorbic acid) has many functions for human health. It maintains nerve function, skin health, serves as a radical scavenger in the body and may contribute to immune function. Consumers are increasingly interested in buying fruit perceived to offer health values and vitamin C is well recognised as important for daily health.

Determination of total monomeric anthocyan

The results of the total anthocyanin content (mg cyanidin-3-glucoside equivalent/L) of the mulberry fruit from plants grown on different substrates are summarised in Table 7 and Figure 2. Compared to the control samples, only the fruit samples from plants of the varieties 'Dar-24' and 'Triple Crown' grown on the tested substrates recorded higher levels of anthocyanins. The 'Chester' variety had lower anthocyanin levels than the control sample.

Table 7. Total monomeric anthocyan content (mg/L) of blackberry fruit

Descriptive Statistics

Dependent Variable: Total Monomeric Anthocyan (mg/l)

Substrate	Variety	Mean	Std. Deviation	N
CONTROL	DAR 24	135.15	0.92	3
	TRIPLE CROWN	129.69	1.26	3
	CHESTER	521.45	1.07	3
	AVERAGE	262.10	194.53	9
MANUR	DAR 24	198.55	0.60	3
	TRIPLE CROWN	195.26	0.39	3
	CHESTER	376.61	1.02	3
	AVERAGE	256.81	89.87	9
MUSHROOM COMPOST	DAR 24	193.93	4.69	3
	TRIPLE CROWN	213.74	0.67	3
	CHESTER	409.24	1.71	3
	AVERAGE	272.30	103.09	9
FOREST COMPOST	DAR 24	192.09	3.28	3
	TRIPLE CROWN	243.36	3.21	3
	CHESTER	289.00	0.84	3
	AVERAGE	241.48	42.05	9
MIXTURE COMPOST	DAR 24	193.09	0.63	3
	TRIPLE CROWN	205.84	2.55	3
	CHESTER	123.01	1.26	3
	AVERAGE	173.98	38.65	9
SEMIFERMENTED COMPOST	DAR 24	230.89	0.25	3
	TRIPLE CROWN	174.45	1.84	3
	CHESTER	399.21	1.39	3
	AVERAGE	268.18	101.27	9
AVERAGE	DAR 24	190.62	29.12	18
	TRIPLE CROWN	193.72	36.38	18
	CHESTER	353.09	126.88	18
	AVERAGE	245.81	108.28	54

In the blackberry variety 'Dar-24', compared to the control sample (135.147±0.92 mg/L) the most favourable substrate for anthocyanin accumulation was semi-fermented compost

(230.89±0.25 mg/L). On the other substrates the levels of anthocyanins in fruit were relatively close, ranging from 190.43±0.43 mg/L to 198.55±0.60 mg/L.

In the variety 'Triple Crown', the anthocyanin values recorded in the fruit obtained from the substrates analysed, compared to the control (129.69±1.26 mg/L) ranged from 174.45±1.84 mg/L (V4) to 241.69±1.17 mg/L. Therefore, it can be stated that in this variety the forest compost substrate is the most favourable for the accumulation of significant levels of anthocyanins. High levels of anthocyanins in fruits were also evidenced when they were from plants grown on mushroom compost (213.74±0.66 mg/L) and mixed compost (204.84±1.68 mg/L) substrates.

At the variety 'Chester', the anthocyanin levels recorded in the fruit from the substrates analysed could not exceed the value obtained in the control sample (521.45±1.07 mg/L). However, significant anthocyanin values were recorded in fruits from the mushroom compost substrate (409.24±1.71 mg/L), followed by the semi-fermented compost substrates (399.21±1.39 mg/L) and the cranberry (376.61±1.02 mg/L). The lowest anthocyanin values were found in the fruit from mixed compost (123.01±1.26 mg/L).

The blackberry fruit is known for its intense purple color and the masked hero contributing to this beautiful color are anthocyanins (Khoo et al., 2017). Anthocyanins are water-soluble pigments that give fruits and vegetables, such as blackberries, raspberries, and purple corn, their characteristic purple, blue, and red colors (Kong et al., 2003). The colors anthocyanins produce depend on pH, light, and temperature. Colours appear reddish under more acidic conditions and turn blue if pH increases (Khoo et al., 2017).

Anthocyanins are a subset of phenolic compounds that help regulate blood pressure, reduce body inflammation, protect brain health, and improve cognitive function (Igwe et al., 2019). Many studies have shown that anthocyanins may also alleviate diabetes and provide anti-cancer and antioxidant effects (Khoo et al., 2017).

Table 8. Duncan test for each blackberry variety

Variety - 'Dar-24'	N	Subset for alpha = 0.05					
Substrate		**d**	**c**	**b**	**a**		
Control	**3**	135.15					
Forest Compost	**3**		192.09				
Mixture Compost	**3**		193.09				
Mushroom Compos	**3**		193.93				
Manur	**3**			198.55			
Semifermented Compost	**3**				230.89		
Means for groups in homogeneous subsets are displayed. a Uses Harmonic Mean Sample Size = 3.000.							
Variety – 'Triple Crown'	N	Subset for alpha = 0.05					
Substrate		f	e	d	c	b	a
Control	3	129.69					
Semifermented Compost	3		174.45				
Manur	3			195.26			
Mixture Compost	3				205.84		
Mushroom Compos	3					213.74	
Forest Compost	3						243.36
Means for groups in homogeneous subsets are displayed. a Uses Harmonic Mean Sample Size = 3.000							
Variety – 'Chester'	N	Subset for alpha = 0.05					
Substrate		f	e	d	c	b	a
Mixture Compost	3	123.01					
Forest Compost	3		289.00				
Manur	3			376.61			
Semifermented Compost	3				399.21		
Mushroom Compost	3					409.24	
Control	3						521.45
Means for groups in homogeneous subsets are displayed. a Uses Harmonic Mean Sample Size = 3.000							

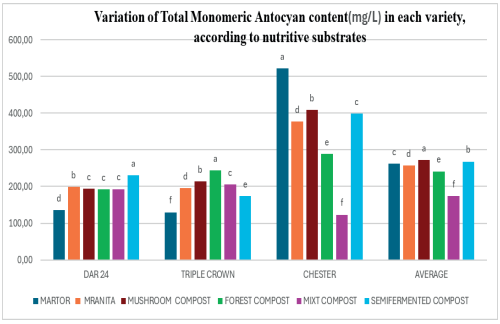

Figure 2. Variation in total monomeric antocyan content (g/L) of three varieties of blackberry as a function of nutrient substrates

Determination of total phenol content

The total phenol content (mg EGA/L) of the mulberry fruits from plants grown on different substrates was determined according to the Folin-Ciocateu method. Total phenol values were expressed as mg gallic acid equivalent (GAE)/L fresh sample and are highlighted in Table 9 and Figure 3.

It was noted that, in the control samples, the total phenol contents were higher in the thornless wall variety 'Chester' (385.49±1.22 mg EAG/L) and 'Triple Crown' (345.44±1.23 mg EAG/L) compared to the thorny wall variety 'Dar-24' (132.13±0.87 mg EAG/L).

As regards the plant culture substrates, favourable for the accumulation of a high phenol content, it was noted that in the wall variety "Dar-24" there are no significant differences between the values obtained, these being between 104.47±0.61 mg EAG/L (V4) and 120.20±0.70 mg EAG/L (V1).

The highest values were recorded for the control substrate (132.13±0.87 mg EAG/L) (Table 9 and Figure 3).

Table 9. Total phenol content (mg/L) of blackberry fruit

Descriptive Statistics

Dependent Variable: Total Phenol (mg/l)

Substrate	Variety	Mean	Std. Deviation	N
CONTROL	DAR 24	133.13	2.55	3
	TRIPLE CROWN	343.11	3.43	3
	CHESTER	385.49	1.22	3
	AVERAGE	287.24	117.06	9
MANUR	DAR 24	120.20	0.70	3
	TRIPLE CROWN	300.90	2.11	3
	CHESTER	372.66	1.82	3
	AVERAGE	264.59	112.67	9
MUSHROOM COMPOST	DAR 24	113.08	2.06	3
	TRIPLE CROWN	321.92	3.74	3
	CHESTER	67.27	0.66	3
	AVERAGE	167.42	117.58	9
FOREST COMPOST	DAR 24	104.90	0.46	3
	TRIPLE CROWN	368.53	2.45	3
	CHESTER	386.64	2.25	3
	AVERAGE	286.69	136.57	9
MIXTURE COMPOST	DAR 24	110.28	0.43	3
	TRIPLE CROWN	71.31	1.30	3
	CHESTER	157.05	0.31	3
	AVERAGE	112.88	37.18	9
SEMIFERMENTED COMPOST	DAR 24	104.47	0.61	3
	TRIPLE CROWN	339.94	0.95	3
	CHESTER	593.95	4.75	3
	AVERAGE	346.12	212.02	9
AVERAGE	DAR 24	114.34	10.29	18
	TRIPLE CROWN	290.95	103.30	18
	CHESTER	327.17	176.58	18
	AVERAGE	244.16	149.22	54

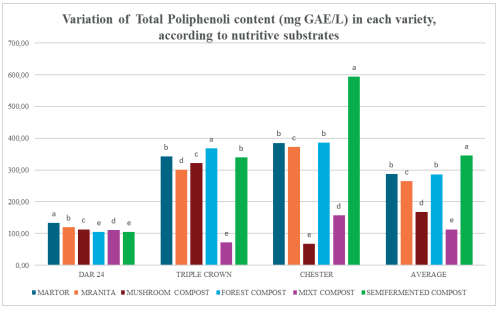

Figure 3. Variation in total phenoli content (mg GAE/L) of three varieties of blackberry as a function of nutrient substrates

In the "Triple Crown" blackberry, apart from the control substrate, the most favourable substrates for the accumulation of significant phenolic compounds were: forest compost (369.53±0.91 mg EAG/L) followed by semi-fermented compost (339.94±0.95 mg EAG/L) and mushroom compost (319.92±0.30 mg EAG/L). The lowest values were recorded in the case of blackberry plants grown on mixed compost (Table 9 and Figure 3).

In the variety 'Chester', the highest values of total phenol content were observed in the substrate variant (semi-fermented compost) with 598.28±1.08 mg EAG/L. Values comparable to those obtained on the control substrate were observed in the forest compost (385.30±0.95 mg EAG/L) and the cranberry (372.66±1.82 mg EAG/L) variants respectively. The lowest content of phenolic compounds was recorded in the samples of mixed compost substrate variant (157.05±0.31 mg EAG/L) and mushroom compost (67.27±0.66 mg EAG/L).

Phenolic compounds comprise one of the largest groups of plant metabolites and are an important part of the human diet (Krondatyuk & Pezzuto, 2004). Phenolic compounds in fruit extracts of Rubus species (e.g. raspberries. blackberries. mulberries) have been shown to have a strong ability to scavenge oxygen radical red radical species and inhibit oxidation and growth of pathogenic bacteria (Kähkönen et al., 2001). The major class of phenolic compounds in fruits of the genus Rubus are hydrolysable tannins (gallo- and ellagitannins), with anthocyanins being the second most abundant class in pigmented fruits and hydroxycinnamic acids, flavonols, flavan-3-ols and proanthocyanidins being the minor ones (Moyer et al., 2002; Maatta-Riihinen et al., 2004). This section deals mainly with those phenolic classes of pharmaceutical interest discovered in the genus Rubus in recent years.

Determination of antioxidant capacity

Fruit samples were evaluated for antioxidant activity using the DPPH method and were expressed as percentage inhibition of DPPH· radicals (RSA%) (Table 10).

Regarding the antioxidant activity of blackberries, it was found that, in general, they have a strong antioxidant capacity of more than 80% on all substrates tested. In the mulberry variety 'Dar-24', significant antioxidant activities, with quite close values, were noticed in fruits from the plant culture on substrate variants semi-fermented compost (94.72%). followed by forest compost (93.84%) and compost from mushroom culture (92.83%). respectively.

For the variety 'Triple Crown', apart from the control sample which recorded a strong antioxidant activity of 91.16%, similar values were recorded for the fruit on the V4 - semi-fermented compost variety (90.09%). For the variety 'Chester', apart from the substrate sample with the lowest value (86.16%), all other samples recorded strong antioxidant activities of over 92%. The highest antioxidant activities were noted in the blackberries of the mixed compost (96.38%) and forest compost (95.37%) variants. Following these analyses, it was noted in all three varieties tested that the substrate from which the fruit had the lowest antioxidant activity was the manur substrate (Table 10 and Figure 4).

The total antioxidant capacity of the samples was assessed by the phosphomolybdate method (Prieto et al., 1999). The results were expressed in µg ascorbic acid equivalent/ml. In the phosphomolybdate assay, a quantitative method for assessing antioxidant capacity, all samples tested showed different degrees of activity, as shown in Table 10 and Figure 4. From the table below (Table 10) it can be seen that, in most cases, the thornless varieties 'Triple Crown' and 'Chester' have a much higher total antioxidant capacity than the thorny variety 'Dar-24'.

Table 10. Total antioxidant capacity (µg/ml) of blackberry fruit

Descriptive Statistics				
Dependent Variable: Total Antioxidant Capacity (µg/ml)				
Substrate	**Variety**	**Mean**	**Std. Deviation**	**N**
CONTROL	DAR 24	172.14	1.90	3
	TRIPLE CROWN	375.55	3.76	3
	CHESTER	332.77	4.78	3
	AVERAGE	293.49	92.93	9
MANUR	DAR 24	138.62	0.21	3
	TRIPLE CROWN	502.09	5.22	3
	CHESTER	99.57	5.42	3
	AVERAGE	246.76	192.28	9
MUSHROOM COMPOST	DAR 24	131.63	0.37	3
	TRIPLE CROWN	279.74	4.17	3
	CHESTER	526.80	2.76	3
	AVERAGE	312.72	172.91	9
FOREST COMPOST	DAR 24	155.74	0.55	3
	TRIPLE CROWN	342.51	3.89	3
	CHESTER	246.60	2.76	3
	AVERAGE	248.28	80.92	9
MIXTURE COMPOST	DAR 24	145.63	1.03	3
	TRIPLE CROWN	499.69	5.52	3
	CHESTER	130.65	0.27	3
	AVERAGE	258.66	180.91	9
SEMIFERMENTED COMPOST	DAR 24	124.28	0.21	3
	TRIPLE CROWN	321.32	1.05	3
	CHESTER	397.24	10.28	3
	AVERAGE	280.95	122.12	9
AVERAGE	DAR 24	144.67	16.29	18
	TRIPLE CROWN	386.82	88.06	18
	CHESTER	288.94	153.23	18
	AVERAGE	273.48	142.06	54

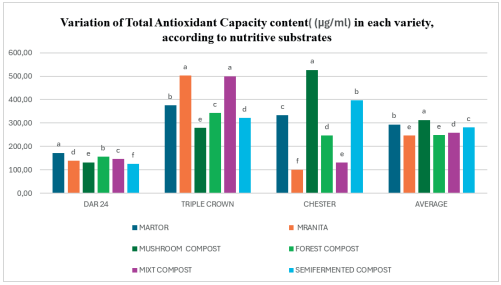

Figure 4. Variation in total antioxidant capacity content (µg/ml) of three varieties of blackberry as a function of nutrient substrates

In the variety "Dar-24" - among the variants analyzed, apart from the control variant which recorded the highest antioxidant capacity (171.47±0.98 µg EAA/ml), the substrate variants forest compost and mixed compost had the highest total antioxidant capacities (155.74±0.55 and respectively 145.63±1.03 µg EAA/ml).

In the thornless variety "Triple Crown" - among the analyzed substrate variants, blackberries from plants grown on mixed compost and on bramble substrate recorded, compared to the control, significant total antioxidant capacities (500.79±0.78 and respectively 498.76±0.55 µg EAA/ml). Regarding the fruits of the thornless "Chester" variety, the strongest total antioxidant capacity was found in the fruits of the variants mushroom compost substrate, with 525.47±0.95 µg EAA/ml, and semi-fermented compost, with a recorded value of 387.58±0.80 µg EAA/ml. On these two substrate variants, the fruits had a higher antioxidant capacity than the control substrate (335.44±0.90), but also compared to

the other substrate variants (Table 10 and Figure 4).

Veskouis et al. (2012) reported that the potential antioxidant function of a plant extract with phenolic compounds in vivo cannot be reliably correlated with the results of in vitro experiments, because they do not take into account metabolic transformations and other factors known to affect the bioavailability and biological properties of phenolic compounds (Veskouis et al., 2012).

Siriwoharn et al. (2004) investigated anthocyanins, polyphenols, and antioxidant properties of blackberry hybrids from the state of Oregon to assess the influence of cultivar and maturity and to determine variation caused by plot differences, subsampling, sample preparation, and analytical measurements. Total anthocyanin pigments increased at over-ripening for Marion blackberry and evergreen blackberry. Total phenolics did not show a pronounced change with fruit maturity and values decreased slightly from unripe to ripe fruit. Similarly, antioxidant activities, although increased with fruit ripening, did not show a marked change manifested by total anthocyanins. Wang & Lin (2000) investigated the antioxidant capacities in different genotypes and developmental stages of blackberry (*R. fruticosus*) and raspberry (*R. idaeus*) fruits and leaves. Blackberries had the highest oxygen radical uptake capacity values during the vegetative period, while raspberries had the highest activity at the mature stage. More recently, Vool et al. (2007) determined how mulching, specific harvest periods and different weather conditions in the years involved affected the biochemical content of raspberry and blackberry. Cultivation technology had a significant influence only on the soluble solids content of raspberry fruit. Harvesting time had an impact on both raspberry and blackberry fruit weight and fruit dry matter content, but soluble solids content was affected only in blackberries. Positive correlations are found between Ascorbic Acid - Anthocians, Anthocians - Poliphenols, Total Antioxidant Capacity - Anthocians (Table 11).

Table 11. The correlations between anthocyans, polyphenols and antioxidant properties of blackberry fruit

		VITAMIN C (g/l)	ANTHOCIANS (mg/l)	POLIPHENOLS (mg GAE/L)	TOTAL ANTIOXIDANT CAPACITY (µg/ml)
VITAMIN C (g/l)	Pearson Correlation	1.00	0.491**	0.04	-0.04
	Sig. (2-tailed)		0.00	0.76	0.79
	N	54.00	54.00	54.00	54.00
ANTHOCIANS (mg/l)	Pearson Correlation	0.491**	1.00	0.427**	0.274*
	Sig. (2-tailed)	0.00		0.00	0.05
	N	54.00	54.00	54.00	54.00
POLIPHENOLS (mg GAE/L)	Pearson Correlation	0.04	0.427**	1.00	0.24
	Sig. (2-tailed)	0.76	0.00		0.07
	N	54.00	54.00	54.00	54.00
TOTAL ANTIOXIDANT CAPACITY (µg/ml)	Pearson Correlation	-0.04	0.274*	0.24	1.00
	Sig. (2-tailed)	0.79	0.05	0.07	
	N	54.00	54.00	54.00	54.00

**Correlation is significant at the 0.01 level (2-tailed).
*Correlation is significant at the 0.05 level (2-tailed).

CONCLUSIONS

The results of the study led to the following conclusions:

- The obtained data showed that among the three varieties tested, cv. "Dar-24" has the highest growth adaptability and vitamin C accumulation for all 5 culture substrates; the highest values of vitamin C were recorded in the fruits of the plants grown on semi-fermented compost (V4) and on bramble substrate (V1); for the accumulation of

anthocyanins. the most favorable substrate for "Dar-24" was semi-fermented compost (V4);

- At "Triple Crown" no significant levels of vitamin C were recorded in the case of the culture substrate variants in comparison with the control samples; a relatively high value of ascorbic acid, close to that recorded in the control sample, was obtained in the case of fruits from plants grown on mushroom compost substrate (V2); the forest compost substrate (V3) was the most conducive to the accumulation of significant levels of anthocyans;
- In the "Chester" mulberry variety, the substrates that favored a significant accumulation of vitamin C were the semi-fermented compost (V4) and the compost from the mushroom culture (V2); for anthocyanins, the values recorded in the case of the fruits from the analyzed substrates could not exceed the value obtained in the case of the control sample; Among the 5 types of substrates, significant values of anthocyanins were recorded in the fruits on the mushroom compost substrate (V2). followed by the semi-fermented compost substrates (V4) and V1 (Manur);
- In the fruits of the "Dar-24" variety - the highest values of the total phenol content were recorded in the control substrate; in the case of the other types of substrates. there are no significant differences between the values of the concentration of phenolic compounds from fruits;
- In the "Triple Crown" mulberry variety, apart from the control substrate. the most favorable substrates for the accumulation of a significant content of phenolic compounds in the fruits were: V3 - forest compost, V4 - semi-fermented compost and V2 - crop compost of mushrooms;
- In the "Chester" variety, the highest values of the total phenol content were noted in the V4 substrate variant (semi-fermented compost);
- Regarding the antioxidant activity of blackberries, it was found that, in general, they have a strong antioxidant capacity of over 80% on all types of substrate analyzed. In most cases, the thornless varieties "Triple Crown" and "Chester" had a much higher total antioxidant capacity than the thorny variety "Dar-24".
- In the "Dar-24" mulberry variety, significant antioxidant activities were noted in the fruits from plant culture on substrate variants V4 - semi-fermented compost, followed by V3 - forest compost and respectively V2 - mushroom culture compost. In this variety, the control sample recorded the highest total antioxidant capacity, and among the substrate variants, the substrate variants V3 - forest compost and V5 - mixed compost stood out;
- In the "Triple Crown" variety, apart from the control sample, significant antioxidant activities were recorded in the case of the fruits from the V4 variant - semi-fermented compost. Blackberries from plants grown on mixed compost (V5) and on bramble substrate (V1) had a high total antioxidant capacity;
- In the variety "Chester" - the highest antioxidant activities were noted in the blackberries from the plants grown on V5 - mixed compost and V3 - forest compost, and the fruits from the V2 - substrate variants had the strongest total antioxidant capacity mushroom compost and V4 - semi-fermented compost.

The parameters analyzed in the present case could be influenced by factors, such as: ripening stage, climatic and pedological characteristics of the cultivation areas. The different climatic and agrotechnical conditions, during the growth and ripening of the fruits, can have a significant impact on the quantity and quality of the analysed compounds.

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