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Application of vermicompost extract to improve the phytochemical composition of berry fruits

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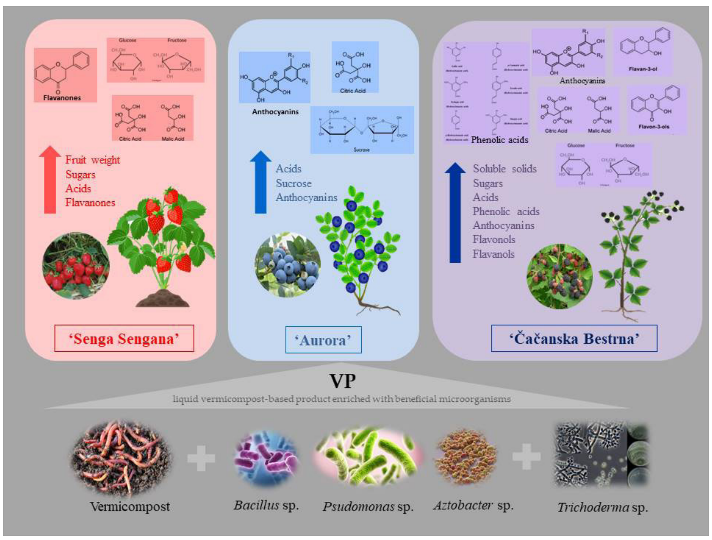
Abstract: The growing market demand for environmentally friendly products for plant nutrition has led to the development of new fertilizers, particularly liquid compost-based products such as teas, extracts, and leachates. The aim of this research was to compare the effect of chemical fertilization (CF) and its combination with liquid fertilizer based on vermicompost and beneficial microorganisms (CF + VP) on the phytochemical profile of strawberry (Senga Sengana), blackberry (Čačanska Bestrna) and blueberry (Aurora). The results showed that CF + VP positively affected fruit weight (8.8 g), soluble solids (8.9°Bx), total sugars (65.5 g/kg FW), and the sum of all phenolic groups (275.12 g/kg FW) in blackberry fruits. Significantly higher fruit weight (15.5 g), total sugars (62.4 g/kg FW), organic acids (9.6 g/kg FW), and flavanones (20.06 mg/100 g FW) content in strawberry fruits were confirmed in CF + VP. The same treatment exhibited the highest effectiveness in terms of organic acids (17.3 g/kg FW) and anthocyanins (153.26 g/kg FW) in blueberry. The application of CF + VP improved all components of fruit quality in the examined species compared to CF, and the positive impact was particularly pronounced in the phenolic composition of blackberries. Taking into account the stimulating effects on the sensory and nutritional value of the fruit of the studied berry species, the usa of VP as a supplement to CF can be regarded as a sustainable practice for enhancing growing technology aimed at obtaining biologically valuable fruits.

Key words: Vermicompost extract, biofertilization, phytochemical composition, sustainable agriculture, berry fruits

Graphical abstract



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1. Introduction

Numerous studies have consistently shown the harmful effects of chemical fertilizers on the environment through nutrient leaching and runoff (Schoebitz et al., 2016; Yadav et al., 2018; Morra et al., 2021). In response, organic fruit production has gained popularity due to its focus on sustainable intensification, which involves reduced pesticide and heavy metal residues, improved soil health, higher biodiversity, and distinct fruit flavor (Lockie et al., 2006). Compost and vermicompost are widely recognized as beneficial amendments that can enhance soil nutrient content and promote soil health, contrasting with industrialized fertilizers and raw manure (Jack and Thies, 2006). The positive impact of vermicompost on plant growth can be attributed to its nutrient composition and biologically active substances (Warman and Ang-Lopez, 2010). Vermicompost contains various plant growth regulators of microbial origin (Atiyeh et al., 2002) and significantly contributes to soil enzyme activity (Albiach et al., 2000), but in the study of its effect on red currant fruit quality, the most intense impact was observed in the 3rd year after application (Pandelea et al., 2023). However, the limited availability and challenging application of vermicompost necessitates the development of liquid vermicompost-based bioproducts. Leachates obtained from vermicomposting, which are known for their high concentration of plant nutrients, are considered beneficial and can be utilized as liquid fertilizers (Jarecki et al., 2005; Gutiérez-Miceli et al., 2007). It is important to note that these products contain plant growth-promoting microorganisms (PGPM), including bacteria and fungi, which play a crucial role in soil microbiota by promoting biological nitrogen fixation, phosphate dissolution, indole acetic acid production, and enhancing root surface area, ultimately improving plant growth (Bashan et al., 2014; Viscardi et al., 2016). García et al. (2013) have reported that liquid humus derived from vermicompost of cattle manure stimulates yield in crops such as cucumber, rice, and broccoli. Therefore, liquid vermicompost-based fertilizers enriched with beneficial microorganisms offer a solution to the challenges associated with solid vermicompost, while also positively impacting fruit quality. Currently, there is a lack of comprehensive research on the effectiveness of vermicompost leachates and similar products regarding their influence on the chemical composition of berries, including the content of primary and secondary metabolites.

At the Fruit Research Institute Čačak, a complete biotechnological procedure for a liquid biofertilizer obtained from vermicompost and enriched with selected strains of microorganisms has been developed (Pešaković et al., 2017). The newly formulated biopreparation, as highlighted by Pešaković et al. (2017), offers several advantages over the raw material (vermicompost) used for its preparation. Firstly, it contains a higher concentration of beneficial microorganisms, allowing for the use of smaller amounts of vermicompost and overcoming market limitations. The selected microorganism strains stimulate native microflora growth, enhancing nutrient content in the plant's root zone. Additionally, its liquid form increases versatility, enabling precise nutrient delivery through foliar spraying or fertigation systems. This enhances nutrient utilization efficiency for both plants and microorganisms, improving production efficiency by providing simultaneous irrigation and nutrition. The application methods reduce manual labor and justify commercial use with reduced raw material quantities. All of the mentioned factors, including the increased soil biological activity, are expected to improve fruit quality in the studied species, benefiting growers with high yields and good fruit quality. With these considerations in mind, this study aimed to assess the effects of the combined application of chemical fertilizers and a newly formulated biopreparation (a liquid biofertilizer obtained from vermicompost enriched with microorganisms) in comparison to conventional (chemical) fertilization on the phytochemical profile of strawberry, blackberry, and blueberry fruits.

2. Materials and methods

2.1. Plant material and experimental design

Three berry species, strawberry (Senga Sengana), blackberry (Čačanska Bestrna), and highbush blueberry (Aurora), were used in this study. All examined species were grown at the experimental plantings of the Fruit Research Institute, Čačak, Western Serbia (latitude 43°54'N, longitude 20°22'E, 225 m altitude). The cultivation was conducted in open field conditions. The plantings were located close to each other; thus, they were in the same microclimate conditions. Considering that numerous authors who studied the effects of compost and compost-based products on the quality of different fruit species have observed large differences in quality parameters between study years and explained them by highly variable temperatures and precipitation regimes, our research was conducted in 2018 (Hargreaves, 2008; Jindo et al., 2016; Anli et al., 2020). According to weather records[1] the growing season temperature in Čačak had a mean value of 16.4 °C and the total rainfall was 410.8 mm in 2018 (Table S1). All plantings were established on alluvial soil with sandy-loam texture (51.95% sand and 48.1% loam); pH_{KCl} was 5.48, and the contents of humus, N_{TOT}, available K_2O and P_2O_5 were 3.95%, 0.20%, 27.00, and 22.95 mg/100g, respectively.

Strawberries were planted in a single-bed growing system, with 0.18 m spacing between plants and 0.50 m

[1] Republic Hydrometeorological Service of Serbia (2025). Weather records [online]. Website http://www.hidmet.gov.rs [accessed 29 May 2022].

between rows. Blackberries were planted at a spacing of 3.0 m in the row and 1.5 m within the row and trained to the three-wire trellis. Blueberries were planted in the bush training system, with spacing of 3.0 m between rows and 1.25 m between plants.

The experiment was conducted using a randomized block design with four replications. There were two treatments evaluated in the trial. The first treatment (CF + VP) involved a combination of chemical fertilizer (CF) and liquid vermicompost-based product enriched with beneficial microorganisms (VP). The second treatment (CF) consisted of single conventional fertilization, which relied on the application of chemical fertilizers. Conventional fertilization was applied as follows: 100 kg/ha N, 60 kg/ha P, and 150 kg/ha K for strawberry; 70 kg/ha N, 50 kg/ha P, and 120 kg/ha K for blackberry; 120 kg/ha N, 50 kg/ha P, and 100 kg/ha K for blueberry (Milivojević, 2022). Fertilizers were applied in accordance with the developmental stages of each studied species.

The trial was equipped with a drip irrigation system, and all plants were subjected to standard pest and disease control. The trial involved 20 bushes (five plants in four replications) for blackberry and blueberry, while for strawberry, it involved 80 plants (20 plants in four replications).

2.2. Biofertilizer preparation

The biofertilizer (specially obtained liquid extract of vermicompost of organic origin, enriched with selected strains of beneficial microorganisms) used in the study was prepared at the Fruit Research Institute Čačak (Serbia) according to Pešaković et al. (2023). The microbial consortium contained three strains of soil microorganisms belonging to bacteria of the genera *Azotobacter*, *Bacillus*, and *Pseudomonas*, and one strain of fungus from the genus *Trichoderma*. The biofertilizer had a pH of 7.67, electrical conductivity of 3.95 dS/m, total nitrogen content of 11.5 mg/kg, phosphorus content of 215.1 mg/kg, and organic matter content of 17.5%. The final concentration of the biofertilizer was adjusted to 10^9 CFU/mL.

The biofertilizer was applied three times in 2018: at the beginning of the growing period (1st), at flowering time (5%–10% flowers open) (2nd), and at the beginning of ripening (3rd) in all examined species (Table S2). The application rate was 1 L per bush for blueberry and blackberry, and 0.5 L per plant for strawberry in each treatment.

Fully ripe berries were harvested at the beginning of the second decade of May for strawberries, in the second decade of July for blackberries, and at the end of July for blueberries. Fully ripe blackberries were black, glossy, and easily picked from the branches (Karaklajić-Stajić et al., 2017). In order to obtain uniform samples, fruits of blackberries and blueberries were visually selected from the same pool of each bush at the same developmental stage. The strawberry harvest date was determined based on sensory properties (color, taste, and firmness), and the fruits were taken in the middle of the harvest period.

2.3. Determination of basic fruit quality traits

After harvest, 25 fruits of each species (n = 25) in four replicates were randomly selected (100 fruits in total) from both treatments. Fruit weight was measured using a Mettler balance (Mettler-Toledo GmbH, Greifensee, Switzerland) with ±0.01 g accuracy, and the data were expressed in grams (g). Total soluble solids content (SSC) was determined by a digital refractometer (Carl Zeiss AG, Jena, Germany) at 20 °C, and data were expressed in °Brix. After fruit weight and SSC measurements, fruit samples were immediately frozen and stored at –20 °C until chemical analyses.

2.4. Determination of sugars and organic acids

Fruits of strawberry and blackberry (5.0 g), and blueberry (3.0 g) were homogenized in double-distilled water. After 30 min at 10,000 rpm (Eppendorf Centrifuge 5810 R, Eppendorf SE, Hamburg, Germany), the samples were filtered through a 0.20 µm cellulose ester filter (Macherey-Nagel GmbH, KG, Düren, Germany) and transferred into vials for analysis using a high-performance liquid chromatography system (HPLC) (Thermo Fisher Scientific Inc., Waltham, MA, USA). Organic acid and sugar contents were analyzed using the same HPLC system, as described by Smrke et al. (2021). Briefly, sugar separations were carried out using a Rezex RCM-monosaccharide Ca^{2+} column (300 mm × 7.8 mm; Phenomenex Inc., Torrance, CA, USA), operated at 65 °C. The mobile phase was double-distilled water. The duration of the analysis was 30 min, and a refractive index (RI) detector was used. The injection volume was 20 µL, and the flow rate was maintained at 0.6 mL/min. Analyses of organic acids were performed on the same HPLC system, equipped with a UV detector set at 210 nm, using a Rezex ROA-organic acid H^+ (8%) column (300 mm × 7.8 mm; Phenomenex Inc., Torrance, CA, USA), coupled with a Chromsep guard column. The column temperature was set at 65 °C, and the elution solvent was 4 mM sulfuric acid in double-distilled water. The flow rate was 0.6 mL/min.

The sugar and acid contents were calculated based on calibration curves of the corresponding standard solutions. The results were expressed as grams per kilogram fresh weight (FW) for sugars and acids (g/kg FW), except for fumaric and shikimic acids, which were expressed as milligrams per kilogram fresh weight (mg/kg FW).

2.5. Determination of phenolic content

For the analysis of individual phenolics, berries were chopped, and 5.0 g of strawberry and blueberry, and 4.0 g of blackberry were extracted with methanol containing 5% formic acid in a cooled ultrasonic bath for 1 h.

Supernatants were centrifuged for 10 min at 10,000 rpm, filtered through 0.2 µm polyamide filters (Macherey-Nagel GmbH, KG, Düren, Germany), and transferred into vials for injection into the HPLC system coupled with a diode array detector (Thermo Fisher Scientific Inc., Waltham, MA, USA) at 280 nm, 350 nm, and 530 nm, as described by Smrke et al. (2021). The column was a Gemini C18 (150 mm × 4.4 mm, 3 µm; Phenomenex Inc., Torrance, CA, USA), coupled with a Chromsep guard column and operated at 25 °C. The elution solvents were aqueous 0.1% formic acid in double-distilled water with 3% acetonitrile (A) and 0.1% formic acid in acetonitrile with 3% double-distilled water (B). Samples were eluted using a linear gradient of 5%–20% B over the first 15 min, followed by a gradient of 20%–30% B for 5 min, an isocratic phase for 5 min, a gradient of 30%–90% B for 5 min, and another isocratic phase for 15 min before returning to the initial conditions.

The phenolic content was identified using a mass spectrometer (LTQ XL linear ion trap mass spectrometer, Thermo Fisher Scientific Inc., Waltham, MA, USA) with electrospray ionization (ESI), operating in positive (for anthocyanins) or negative (for all other phenolics) ionization mode. All mass spectrometer settings were the same as those reported by Smrke et al. (2021). Spectral data were processed using the Xcalibur software (Thermo Fisher Scientific Inc., Waltham, MA, USA). Compound identification was confirmed by comparing retention times and spectra, as well as by fragmentation patterns and comparison with literature data. The concentrations of phenolics were calculated from the peak areas of the samples and corresponding standards obtained using external standard compounds, and expressed as milligrams per 100 g fresh weight (mg/100 g FW).

2.6. Statistical analysis

The data were presented as the mean ± standard deviation of four replicates. The results were compared by one-way analysis of variance (ANOVA), and the least significant difference (LSD) test was carried out to assess significant differences between treatment means for each species (strawberry, blueberry, and blackberry) using the MSTAT-C statistical software package (Michigan State University, East Lansing, MI, USA). Differences among means at the 5% level ($p \leq 0.05$) were considered statistically significant.

3. Results

Studies related to new berry cultivars emphasize the large size of the fruit, which is significant for increasing yield, as well as for the efficiency of manual harvesting and the placement of fresh fruit. In general, the fruit weight in our study (Table 1) was lower in the control (CF) compared to the treatment with combined chemical fertilizer and biofertilizer (CF + VP). The application of combined fertilization (CF + VP) caused an increase in fruit weight by 7% (blackberry Čačanska Bestrna), 9% (blueberry Aurora), and 25% (strawberry Senga Sengana). Higher SSC in strawberries (9.8°Bx), blackberries (8.9°Bx), and blueberries (10.2°Bx) was recorded in the CF + VP treatment compared to CF, although the differences were not statistically significant, except in blackberry.

Among primary metabolites, three sugars (glucose, fructose, and sucrose) and five organic acids (citric, malic, quinic, shikimic, and fumaric) were determined in the studied berry species. Fructose was the predominant individual sugar in all examined species (54% in strawberry, 49% in blueberry, and 53% in blackberry of total sugar content) (Table 2).

The values of fructose, glucose, and total sugar content in strawberries and blackberries were significantly higher in combined treatment (CF + VP), while a reverse tendency was observed in blueberries. The sucrose content of blueberry Aurora and blackberry Čačanska Bestrna increased after the addition of biofertilizer (CF + VP), and, more importantly, these values were 1.5- and 2-fold higher, respectively, compared to those from fruits of plants grown under chemical fertilization (CF) alone.

The study revealed that citric acid was the most abundant individual organic acid (46% in strawberry, 77% in blueberry, and 49% in blackberry of total organic acids), followed by malic acid, while shikimic and fumaric acids were present in much lower amounts in the studied species (Table 3).

Values of citric and fumaric acids in strawberries, blueberries, and blackberries were significantly higher in the CF + VP treatment (Table 3). Significantly higher total acid content was found in blueberries and strawberries treated with the aforementioned biofertilizer, while blackberries also had higher total acid content, but no significant differences were observed between the treatment supplemented with biofertilizer and the nonsupplemented treatment (control).

In our research, a total of 30, 36, and 35 phenolic compounds were determined in strawberry, blackberry, and blueberry, respectively. Eight hydroxycinnamic acids, ten flavanols, two flavanones, six flavonols, and four anthocyanins were detected in strawberries (Table 4).

The flavanols were present in high amounts in both CF + VP and CF treatments and represented 40% and 41% of the total analyzed phenolic content, respectively. However, the differences between CF + VP and CF were mostly not significant. Procyanidin dimer 3 represented 29% of the total flavanols in CF + VP and 22% in CF.

The results obtained in our study indicate a considerable ratio of anthocyanins (29% in CF + VP treatment and 32% in CF) and flavanones (21% in CF + VP and 19% in CF)

Table 1. Fruit weight and soluble solids content of berries grown under different fertilization regimes.

Species/cultivar	Fertilization regime	Fruit weight (g)	SSC (°Bx)
Strawberry 'Senga Sengana'	CF + VP	15.5 ± 0.3 a	9.8 ± 0.9 a
	CF	12.5 ± 0.9 b	9.4 ± 0.8 a
Blueberry 'Aurora'	CF + VP	2.2 ± 0.4 a	10.2 ± 1.3 a
	CF	2.0 ± 0.2 a	10.1 ± 0.6 a
Blackberry 'Čačanska Bestrna'	CF + VP	8.8 ± 0.4 a	8.9 ± 0.8 a
	CF	7.8 ± 0.4 b	8.4 ± 1.0 b

* CF – chemical fertilizer; VP – liquid vermicompost-based product; SSC – soluble solids content. Values within columns for each species followed by the same lowercase letter are not significantly different at p ≤ 0.05 according to the LSD test.

Table 2. Content of individual and total sugars of berries grown under different fertilization regimes.

Species/cultivar	Fertilization regime	Fructose	Glucose	Sucrose	Total sugars
		(g kg^{-1} FW)			
Strawberry 'Senga Sengana'	CF + VP	33.7 ± 1.6 a	26.8 ± 1.3 a	1.8 ± 0.2 b	62.4 ± 4.3 a
	CF	23.6 ± 5.5 b	18.1 ± 4.0 b	2.0 ± 0.2 a	43.8 ± 3.5 b
Blueberry 'Aurora'	CF + VP	25.4 ± 2.8 b	22.2 ± 2.6 b	6.5 ± 0.7 a	54.1 ± 2.9 a
	CF	29.2 ± 0.9 a	24.2 ± 0.9 a	4.1 ± 0.4 b	57.6 ± 4.2 a
Blackberry 'Čačanska Bestrna'	CF + VP	34.9 ± 6.0 a	29.2 ± 4.6 a	1.5 ± 0.6 a	65.6 ± 4.4 a
	CF	22.4 ± 11.0 b	18.8 ± 8.8 b	0.6 ± 0.5 b	41.8 ± 3.4 b

* CF – chemical fertilizer; VP – liquid vermicompost-based product. Values within columns for each species followed by the same lowercase letter are not significantly different at p ≤ 0.05 according to the LSD test.

Table 3. Content of individual and total organic acids of berries grown under different fertilization regimes.

Species/cultivar	Fertilization regime	Citric acid	Malic acid	Shikimic acid	Fumaric acid	Total acids (g kg^{-1} FW)
		(g kg^{-1} FW)		(mg kg^{-1} FW)		
Strawberry 'Senga Sengana'	CF + VP	6.6 ± 0.5 a	3.0 ± 0.1 a	1.2 ± 0.5 a	1.7 ± 0.5 a	9.6 ± 2.3 a
	CF	5.2 ± 1.3 b	2.4 ± 0.5 b	0.8 ± 0.2 a	1.2 ± 0.3 b	7.7 ± 1.2 b
Blueberry 'Aurora'	CF + VP	16.2 ± 0.4 a	0.9 ± 0.1 a	3.2 ± 0.8 a	0.6 ± 0.1 a	17.3 ± 3.3 a
	CF	11.4 ± 0.2 b	1.0 ± 0.0 a	2.5 ± 0.2 a	0.2 ± 0.3 b	12.5 ± 3.0 b
Blackberry 'Čačanska Bestrna'	CF + VP	8.2 ± 1.2 a	3.2 ± 0.8 a	2.5 ± 0.7 a	0.9 ± 0.1 a	11.4 ± 0.7 a
	CF	7.0 ± 0.6 b	2.8 ± 0.2 b	3.2 ± 0.3 a	0.8 ± 0.1 b	9.9 ± 2.7 a

* CF – chemical fertilizer; VP – liquid vermicompost-based product. Values within columns for each species followed by the same lowercase letter are not significantly different at p ≤ 0.05 according to the LSD test.

Table 4. The total phenol content of strawberries grown under different fertilization regimes.

Compounds	**Strawberry Senga Sengana**	
	CF + VP	CF
1. Phenolic acids	(mg 100 g^{-1} FW)	
Hydroxycinnamic acids		
1. Caffeic acid hexoside	1.10 ± 0.27 a	1.46 ± 0.14 a
2. 5-Caffeolyquinic acid	0.72 ± 0.12 a	0.66 ± 0.09 a
3. *p*-Coumaric acid hexoside	0.72 ± 0.09 a	0.80 ± 0.16 a
4. 3-*p*-Coumaroylquinic acid	0.48 ± 0.14 a	0.24 ± 0.02 a
5. 4-*p*-Coumaroylquinic acid	0.25 ± 0.04 a	0.26 ± 0.04 a
6. 3-Feruloylquinic acid	1.24 ± 0.12 a	1.14 ± 0.12 a
7. 5-Feruloylquinic acid	0.72 ± 0.16 a	0.63 ± 0.08 a
8. Cinnamoyl hexoside	2.24 ± 0.16 a	1.63 ± 0.41 a
Total phenolic acids	7.47 ± 0.11 a	6.82 ± 0.21 a
2. Flavanols		
9. Procyanidin trimer 1	4.00 ± 0.77 a	4.27 ± 1.26 a
10. Procyanidin trimer 2	3.47 ± 0.15 b	4.84 ± 0.34 a
11. Procyanidin trimer 3	2.88 ± 0.22 a	5.89 ± 1.74 a
12. Procyanidin trimer 4	0.08 ± 0.02 a	0.07 ± 0.01 a
13. Procyanidin trimer 5	5.80 ± 0.74 a	4.43 ± 0.89 b
14. Procyanidin trimer 6	0.14 ± 0.02 a	0.18 ± 0.03 a
15. Procyanidin dimer 1	1.81 ± 0.08 b	2.53 ± 0.18 a
16. Procyanidin dimer 2	7.23 ± 0.56 a	6.51 ± 1.33 a
17. Procyanidin dimer 3	11.03 ± 0.78 a	8.33 ± 1.92 a
18. Procyanidin dimer 4	1.27 ± 0.05 b	1.68 ± 0.12 a
Total flavanols	37.71 ± 0.33 a	38.73 ± 0.65 a
3. Flavanones		
19. Apigenin acetyl hexoside	19.97 ± 1.54 a	17.31 ± 3.26 a
20. Naringenin hexoside	0.09 ± 0.02 a	0.13 ± 0.02 a
Total flavanones	20.06 ± 0.96 a	17.44 ± 1.01 b
4. Flavonols		
21. Quercetin-3-rutinoside	0.20 ± 0.03 a	0.09 ± 0.02 b
22. Quercetin-3-glucoside	0.09 ± 0.01 a	0.09 ± 0.01 a
23. Quercetin-3-glucuronide	0.24 ± 0.02 a	0.31 ± 0.05 a
24. Kaempferol coumaroyl hexoside	0.04 ± 0.01 a	0.05 ± 0.01 a
25. Kaempferol-3-glucoside	0.19 ± 0.03 a	0.15 ± 0.02 a
26. Kaempferol-acetyl hexoside	0.13 ± 0.02 a	0.14 ± 0.01 a
Total flavonols	0.89 ± 0.02 a	0.83 ± 0.02 a

Table 4. (Continued).

5. Anthocyanins		
27. Pelargonidin-3-glucoside	19.62 ± 3.98 a	21.83 ± 1.69 a
28. Pelargonidin-3-malonyl glucoside	4.47 ± 0.73 a	5.32 ± 0.38 a
29. Pelargonidin-3-rutinoside	1.14 ± 0.23 a	1.27 ± 0.10 a
30. Cyanidin-3-glucoside	2.31 ± 0.35 a	2.05 ± 0.34 a
Total anthocyanins	27.54 ± 1.56 a	30.47 ± 1.11 a
Total phenolics	93.67 ± 1.73 a	94.29 ± 0.60 a

* CF – chemical fertilizer; VP – liquid vermicompost-based product. Values within each row followed by the same lowercase letter are not significantly different at $p \leq 0.05$ according to the LSD test.

in the total phenol content of strawberry Senga Sengana. However, significant differences between the CF + VP and CF treatments were found only in terms of flavanone content.

The results shown in Table 5 indicate that blackberry anthocyanins contributed the most to the total phenol content (approximately 45%). Additionally, derivatives of hydroxycinnamic acids (approximately 30%) and flavanols (approximately 24%) were present in high amounts in blackberries, while flavonols contributed minimally to the overall phenol content (less than 6%). In blackberries, the most prevalent phenolic acid was 5-caffeoylquinic acid, constituting 95% of all phenolic acids as shown in Table 5. Caffeoylquinic acid comes in various forms, with mono-, di-, and tri-caffeoylquinic acids being the most abundant in fruits and vegetables.

In blueberry samples analyzed in our study, four groups of phenolic compounds were present: nine hydroxycinnamic acids, three flavanols, 15 flavonols, and eight anthocyanins (Table 6). The anthocyanins were the most abundant phenolics in both fertilization regimes (CF + VP and CF) in blueberries. Malvidin-3-galactoside was the prevailing anthocyanin (34% of total anthocyanins in the CF + VP treatment, and 41% in CF) while delphinidin-3-galactoside and petunidin-3-galactoside together represented approximately 39% (CF + VP) and 30% (CF) of all analyzed anthocyanins in blueberry. Anthocyanins made up 81% of the total phenol content in the CF + VP treatment and were significantly higher than in CF.

4. Discussion

The findings of Pešaković et al. (2018), who studied the impact of liquid vermicompost extracts (VCMo) on the productivity of Senga Sengana strawberries in an organic production system, revealed that the application of VCMo significantly increased the yield of strawberries per plant and unit area. The authors attributed this result to the presence of microorganisms in the biopreparation that provided essential nutrients to the plants, as well as increased the content of bioregulators such as indoleacetic acid or gibberellic acid. Diver (1998) also observed that compost and herbal teas could improve crop productivity by introducing soluble nutrients, useful microorganisms, and microbiological metabolites to the phyllosphere and rhizosphere. The increasing trend in terms of fruit weight in our study corresponds to the findings reported by Hassan et al. (2017) for black cherry tomatoes treated with vermicompost leachates. However, these results contradict those of Budiastuti et al. (2012), who reported that melon fruits obtained under organic or natural fertilization generally have lower weight and yield than those produced using conventional chemical fertilization. The positive effect of applying mineral fertilizers and biofertilizers on strawberry fruit quality was also confirmed in the study by Čabilovski et al. (2023), which compared vermicompost and vermicompost leachate applications with standard fertilization using mineral fertilizers (NPK) over 3 years. They found that the preplant application of vermicompost and vermicompost leachate during vegetation could not maintain the yield level achieved with mineral NPK fertilizers, but the quality of strawberry fruit could be significantly improved.

Abud-Archila et al. (2018) observed a higher value of SSC (11°Bx) in the fruit of blackberry Čačanska Bestrna under treatment with vermicompost, rock phosphate, and arbuscular mycorrhizae, which have been frequently used in organic production. Higher SSC in the same blackberry cultivar in our study was recorded under the combined application of chemical fertilizers and a vermicompost-based product. Further, Gutiérez-Miceli et al. (2007) found that the SSC of tomatoes was increased when the soil was amended with vermicompost. Accordingly, Budiastuti et al. (2012) emphasized that compost-tea treatments in melon fruit were superior with respect to some very important criteria, such as SSC. Consistently, Singh et al. (2010) reported an enhancement of SSC, SSC/acid ratio, and total sugars in strawberries coinoculated with *Azotobacter* + *Azospirillum* + *Pseudomonas*, as well as with *Azotobacter* + *Azospirillum* + arbuscular

Table 5. The total phenol content of blackberries grown under different fertilization regimes.

Compounds	**Blackberry Čačanska Bestrna**	
	CF + VP	CF
1. Phenolic acids	(mg 100 g^{-1} FW)	
Hydroxycinnamic acids		
1. 5-Caffeolyquinic acid	74.94 ± 9.72 a	43.62 ± 8.53 b
2. 4-Caffeolyquinic acid	0.71 ± 0.15 a	0.91 ± 0.11 a
3. Caffeic acid hexoside 1	1.87 ± 0.24 a	1.09 ± 0.21 a
4. Caffeic acid hexoside 2	3.01 ± 0.70 a	3.60 ± 0.73 a
5. *p*-Coumaric acid hexoside 1	1.16 ± 0.15 a	0.68 ± 0.13 a
6. *p*-Coumaric acid hexoside 2	0.17 ± 0.07 a	0.03 ± 0.01 a
7. 3-*p*-Coumaroylquinic acid	0.08 ± 0.01 a	0.05 ± 0.01 a
Hydroxybenzoic acids		
8. Ellagic acid pentoside 1	0.27 ± 0.06 a	0.08 ± 0.01 b
9. Ellagic acid pentoside 2	0.19 ± 0.03 a	0.09 ± 0.01 b
10. Methylellagic acid pentoside 1	0.21 ± 0.04 a	0.09 ± 0.01 b
11. Methylellagic acid pentoside 2	0.14 ± 0.04 a	0.04 ± 0.02 a
Total phenolic acids	82.75 ± 3.21 a	50.28 ± 1.24 b
2. Flavanols		
12. Catehin	4.87 ± 1.90 a	0.95 ± 0.21 a
13. Epicatehin	4.07 ± 1.14 a	1.37 ± 0.41 a
14. Procyanidin trimer 1	11.47 ± 4.47 a	2.23 ± 0.50 b
15. Procyanidin trimer 2	8.34 ± 1.61 a	5.77 ± 0.89 a
16. Procyanidin trimer 3	8.57 ± 1.71 a	7.30 ± 1.70 a
17. Procyanidin dimer	24.12 ± 5.65 a	28.92 ± 5.88 a
Total flavanols	61.44 ± 1.58 a	46.54 ± 1.11 b
3. Flavonols		
18. Quercetin-3-rutinoside	0.33 ± 0.20 a	0.07 ± 0.01 a
19. Quercetin-3-galactoside	0.85 ± 0.22 a	0.37 ± 0.04 a
20. Quercetin-3-glucoside	0.40 ± 0.10 a	0.07 ± 0.02 b
21. Quercetin-3-xyloside	0.04 ± 0.01 a	0.07 ± 0.01 a
22. Quercetin-3-glucuronide	0.40 ± 0.11 a	0.08 ± 0.01 b
23. Quercetin-3-arabinopyranoside	0.03 ± 0.01 a	0.01 ± 0.00 b
24. Quercetin-3-arabinofuranoside	0.31 ± 0.09 a	0.09 ± 0.01 a
25. Quercetin-3-acetylhexoside	0.97 ± 0.31 a	0.31 ± 0.04 a
26. Quercetin-3-(6″-(hydroxyl-3-methylglutaroyl)-hexoside	0.26 ± 0.07 a	0.16 ± 0.02 a
27. Isorahmetin-3-glucuronide	1.13 ± 0.31 a	1.81 ± 0.22 a
28. Kaempferol-hexoside	0.09 ± 0.03 a	0.02 ± 0.00 b
Total flavonols	4.81 ± 0.18 a	3.06 ± 0.03 b
4. Anthocyanins		
29. Cyanidin-3-glucoside	102.59 ± 12.45 a	60.45 ± 12.04 b
30. Cyanidin-3-rutinoside	6.16 ± 0.75 a	3.63 ±0 .72 a
31. Cyanidin-3-arabinoside	0.62 ± 0.07 a	0.36 ± 0.07 a
32. Cyanidin-3-xyloside	4.14 ± 1.42 a	0.26 ± 0.06 b
33. Cyanidin-3-(6″-dioxalylglucoside)	7.13 ± 1.77 a	8.45 ± 1.66 a
34. Cyanidin-3-(6″-malonylglucoside)	1.50 ± 0.37 a	1.78 ± 0.35 a
35. Pelargonidin-3-glucoside	3.72 ± 0.93 a	2.65 ± 0.35 a
36. Pelargonidin-3-rutinoside	0.26 ± 0.07 a	0.19 ± 0.02 a
Total anthocyanins	126.12 ± 2.34 a	77.77 ± 1.78 b
Total phenolics	275.12 ± 1.83 a	177.65 ± 1.04 b

* CF – chemical fertilizer; VP – liquid vermicompost-based product. Values within each row followed by the same lowercase letter are not significantly different at $p \leq 0.05$ according to the LSD test.

Table 6. The total phenol content of blueberries grown under different fertilization regimes.

Compounds	**Blueberry Aurora**	
1. Phenolic acids	CF + VP	CF
Hydroxycinnamic acids	(mg 100 g^{-1} FW)	
1. Caffeic acid	0.01 ± 0.00 a	0.01 ± 0.00 a
2. 5-Caffeoylquinic acid 1	0.75 ± 0.41 a	1.55 ± 0.54 a
3. 5-Caffeolyquinic acid 2	0.75 ± 0.41 a	1.55 ± 0.54 a
4. *p*-Coumaric acid	2.75 ± 0.11 a	3.27 ± 0.20 a
5. 3-*p*-Coumaroylquinic acid	0.04 ± 0.00 a	0.05 ± 0.00 a
6. 5-*p*-Coumaroylquinic acid 1	0.16 ± 0.01 a	0.17 ± 0.01 a
7. 5-*p*-Coumaroylquinic acid 2	0.47 ± 0.03 a	0.50 ± 0.02 a
8. 3-Feruloyquinic acid	0.33 ± 0.01 a	0.24 ± 0.01 b
9. 5-Feruloylquinic acid	1.63 ± 0.08 a	1.64 ± 0.15 a
Total phenolic acids	6.89 ± 0.09 a	8.98 ± 0.11 a
2. Flavanols		
10. Catehin	0.71 ± 0.03 a	0.53 ± 0.03 b
11. Epicatehin	3.02 ± 0.08 a	3.79 ± 0.19 a
12. Procyanidin trimer	5.20 ± 0.15 a	5.61 ± 0.20 a
Total flavanols	8.93 ± 0.05 a	9.93 ± 0.13 a
3. Flavonols		
13. Quercetin-3-rutonoside	3.50 ± 0.20 a	3.28 ± 0.15 a
14. Quercetin-3-galactoside	3.88 ± 0.31 a	3.74 ± 0.33 a
15. Quercetin-3-glucoside	1.79 ± 0.11 a	1.47 ± 0.12 a
16. Quercetin-3-xyloside	0.88 ± 0.05 a	0.95 ± 0.05 a
17. Quercetin-3-arabinopyranoside	0.06 ± 0.01 a	0.04 ± 0.00 a
18. Quercetin-3-arabinofuranoside	0.79 ± 0.05 a	0.82 ± 0.06 a
19. Quercetin-3-rhamnoside	3.96 ± 0.23 a	3.62 ± 0.30 a
20. Quercetin-3-acetyl hexoside	0.91 ± 0.01 a	0.83 ± 0.07 a
21. Kaempferol-3-galactoside	0.08 ± 0.01 a	0.06 ± 0.01 a
22. Kaempferol-3-glucoside	0.91 ± 0.01 a	0.94 ± 0.07 a
23. Myricetin-3-hexoside 1	0.36 ± 0.05 a	0.37 ± 0.09 a
24. Myricetin-3-hexoside 2	0.85 ± 0.07 a	1.07 ± 0.07 a
25. Isorhamnetin hexoside 1	0.44 ± 0.13 a	0.57 ± 0.04 a
26. Isorhametin hexoside 2	0.04 ± 0.00 b	0.06 ± 0.01 a
27. Syringetin hexoside	0.07 ± 0.01 b	0.11 ± 0.01 a
Total flavonols	18.52 ± 0.07 a	17.93 ± 0.05 a
4. Anthocyanins		
28. Malvidin-3-galactoside	51.42 ± 4.47 a	56.00 ± 5.67 a
29. Malvidin-3-arabinoside	25.11 ± 2.27 a	23.73 ± 2.23 a
30. Delphinidin-3-galactoside	35.94 ± 3.12 a	24.93 ± 2.33 b
31. Delphinidin-3-arabinoside	10.53 ± 0.70 a	7.94 ± 0.53 b
32. Petunidin-3-galactoside	19.58 ± 2.02 a	5.79 ± 0.83 a
33. Petunidin-3-arabinoside	1.62 ± 0.17 a	1.31 ± 0.07 a
34. Cyanidin-3-galactoside	7.77 ± 0.51 a	5.86 ± 0.39 b
35. Peonidin-3-glucoside	1.29 ± 0.11 a	1.40 ± 0.14 a
Total anthocyanins	153.26 ± 1.01 a	136.96 ± 0.42 b
Total phenolics	187.60 ± 0.31 a	176.80 ± 0.18 b

* CF – chemical fertilizer; VP – liquid vermicompost-based product. Values within each row followed by the same lowercase letter are not significantly different at $p \leq 0.05$ according to the LSD test.

mycorrhizal fungi, over the recommended dose of fertilizers. The same authors explained that the increased SSC and total sugars might be due to a steady supply of nutrients by bioinoculants throughout the growing period. This increased vigor of plants and leaf area, along with higher synthesis of assimilates due to enhanced rate of photosynthesis, resulted in an increased mobility of photosynthetic products from leaves to fruits, which can positively affect the sugar content in fruits (Hudina and Štampar, 2000).

The sugar and acid content play an important role in the formation of berry flavor. The balance between the total sugars and acids is very important for consumers to accept the taste of fruits (Singh and Singh, 2006). The acid content is also useful for stabilizing ascorbic acid and anthocyanins, making it essential for fruit color and the extension of the storage life of fresh and processed fruits (Talcott, 2007). Variations in the metabolism of organic acids were recorded in many fruit species (Zheng et al., 2009), and a large number of genetic studies showed that the accumulation of organic acids (e.g., malic acid) was controlled by genes, with differences not only between species but also among cultivars (Saradhuldhat and Paull, 2007). This is confirmed by the results obtained in this work where blueberry samples contained higher levels of citric acid compared to strawberry and blackberry, but these two species, on the other hand, had significantly more malic acid. Similar to our results, Skupień and Oszmianski (2004) found a dominant presence of citric acid in the strawberry Senga Sengana grown in northwestern Poland, but the value they obtained was about two times higher than our results. On the other hand, fruits of blueberry Aurora in our study accumulated similar levels of citric acid to those obtained by Smrke et al. (2021). An increase in the acidity of the fruit can result from higher synthesis of organic acids due to the larger leaf area of plants, which shade the fruits and lower the temperature, leading to less acid consumption in the respiration process (Skupień and Oszmianski, 2004). Mikiciuk et al. (2019) failed to report any changes in fruit acidity in strawberry Rumba inoculated with liquid bioproducts containing arbuscular mycorrhizal fungi or plant growth promoting rhizobacteria. This can be explained by the variability in nutrient and microbial compositions of vermicompost leachates, which are influenced by factors such as the type of organic material used, the earthworm species, and environmental conditions during composting. For example, a study by Walia and Kaur (2024) highlighted that vermicomposts produced from different organic wastes exhibit distinct nutrient profiles and microbial communities, affecting their efficacy as fertilizers.

The main categories of phenolic compounds found in berry fruits (raspberry, blueberry, goji berry, blackcurrant, strawberry, cranberry, and blackberry) are phenolic acids, flavonoids, tannins, and stilbenes (Pap et al., 2021). When studying the benefits of the application of biostimulants (seaweed extracts), Weber et al. (2018) found no significant variations between the control treatment (with no foliar spraying of biostimulants) and the foliar application of SiO_2 and *Ascophyllum nodosum* extract in the content of flavanols, flavones, and flavonols. The same authors state that most of these compounds are regarded as defense phenolics, which are produced in plant tissue in response to different stressors (Weber et al., 2018). Therefore, biostimulants mitigate these adverse effects and reduce the plant's response by decreasing the synthesis of specific groups of secondary metabolites. Similarly, the application of arbuscular mycorrhizal fungi (AMF1 and AMF2), composts from grasses and green waste (C1 and C2, respectively), and consortia of plant growth-promoting rhizobacteria (PGPR: B1 and B2)—especially the combinations (C2 + B1, AMF2 + C1 + B2, AMF2 + C2 + B1, AMF1, and AMF2 + C2 + B2)—resulted in increased pigment contents (chlorophyll a, b, total chlorophyll, and carotenoids) compared to control plants in date palm under water stress conditions (Anli et al., 2020). It is important to emphasize the distinctly high cyanidin-3-glucoside content in blackberries compared to other identified anthocyanins in our study, especially considering that its higher antioxidant efficiency is related to the prevention of high-density lipoprotein (HDL) oxidation when compared to pelargonidin (Satué-Garcia et al., 1997). The other classes of phenolics (hydroxycinnamic acids and flavonols) accounted for a small proportion (approximately 8%–9%) of the total phenolic content.

According to Makori et al. (2021), the health benefits associated with the consumption of a variety of fruits and vegetables rich in polyphenolic compounds are, in part, attributed to the presence of caffeoylquinic acid esters in these food products. Optimizing agrotechnical practices, primarily fertilization, can be one of the effective ways to increase phenolic content (Anttonen and Karjalainen, 2006). Analysis of data related to the different groups of phenolics in blackberry fruit showed higher levels of all groups in the chemical and vermicompost-based fertilizer treatment, especially anthocyanins and phenolic acids. Similarly, comparing the effect of vermicompost and phosphate rock, Abud-Archila et al. (2018) determined more anthocyanins in blackberries produced with the vermicompost treatment. This outcome can likely be attributed to the activities of microorganisms present in the VP (*Azotobacter*, *Pseudomonas*, *Bacillus*, and *Trichoderma* genera). Numerous studies have suggested that certain species of the *Pseudomonas* genus produce various metabolites, including antibiotics and hydrogen cyanide (HCN) (Weller and Thomashow, 1993), while others generate siderophores with a strong affinity for Fe^{3+} absorption (Kloepper et al., 1980), as well as auxins (Khakipour et al., 2008). Bacteria of the *Bacillus* genus are known to produce a large number of secondary metabolites that impact their environment by both inhibiting the growth of certain harmful microorganisms and enhancing nutrient accessibility to plants (Barriuso et al., 2008). Besides, the coinoculants of PGPR and/or AMF can enhance the nutrient use efficiency of mineral fertilizers (Adesemoye et al., 2009). The quantity and number of anthocyanidins and their derivatives identified in the present study correspond to the data reported by Wang et al. (2008) for the blueberry cultivar Bluecrop. The optimal nutritional conditions in our study probably contributed to a higher accumulation of anthocyanins in the fruits of blueberries and blackberries grown in the CF + VP fertilization regime. Comparably, Schoebitz et al. (2016) found that the combined application of microbial consortium and humic substances improves blueberry plant performance, resulting in a synergistic effect when beneficial microorganisms and humic substances are introduced. The same authors indicate that a combined treatment with a microbial consortium and humic substances seems to be the most appropriate method to improve nutrient uptake, increase plant biomass, and stimulate soil microflora. Besides, phytohormones, including cytokinin, ethylene, jasmonic acid, and gibberellins, which are present in vermicompost, are important internal factors that additionally affect anthocyanin biosynthesis (Abud-Archila et al., 2018). On the other hand, no significant differences were found in the content of hydroxycinnamic acids and total flavanols between blueberry fruits in CF + VP and CF treatments. However, there are many limitations that affect the broader applicability of liquid vermicompost in production. The beneficial microorganisms present in liquid vermicompost may have a short lifespan, especially under suboptimal storage conditions (Carril et al., 2024). This necessitates timely application to ensure effectiveness.

The results of this study indicate that the application of chemical fertilizers together with biofertilizer (a liquid vermicompost-based product enriched with beneficial microorganisms) contributed to an increase in fruit weight and the content of compounds important for fruit taste, such as sugars—specifically fructose, glucose, and sucrose—compared to chemical fertilization alone in strawberries and blackberries. In contrast, the sugar content in blueberries decreased with the combined application of chemical fertilizer and biofertilizer compared to chemical fertilization alone. The combined application (CF + VP) also significantly increased organic acids compared to chemical fertilizer (CF), specifically the content of citric and fumaric acids in all the tested species.

The findings indicate that the combined use of chemical and biofertilizer had a beneficial effect on the content of all groups of phenolic compounds (phenolic acids, flavanols, flavonols, and anthocyanins) in blackberry and anthocyanins in blueberry. This combined usage also led to a significantly higher total phenolic content in both species compared to the separate use of chemical fertilizers. Additionally, it increased the flavanones content in strawberries. Nevertheless, although blackberries generally showed exceptional fruit quality with the combined use of chemical and biofertilizer, it is essential to tailor fertilization strategies to the specific requirements and characteristics of each berry species to optimize fruit quality.

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Conflicts of interest
The authors declare no conflict of interest.

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Supplementary material

Table S1. Mean monthly temperatures and rainfall during the growing season in Čačak in 2018.**

Month	Air temperature (°C)		Rainfall (mm)	
	2018	LTA*	2018	LTA*
March	6.8	6.8	52.5	49.5
April	12.9	11.5	52.4	52.3
May	13.7	16.8	118.3	75.7
June	20.2	20.0	73.6	89.4
July	22.5	21.5	42.5	69.1
August	22.1	21.2	61.5	44.3
Mean of growing season	16.4	16.3	410.8	443.7

* Long-term average (54-year average, i.e. 1965–2018 period).
**Data on meteorological conditions during the trial were obtained from the Republic Hydrometeorological Service of Serbia.[1]

Table S2. Overview of biofertilizer application during the investigation.

Species/cultivar	First application	Second application	Third application
Blackberry 'Čačanska Bestrna'	1 May 2018	30 May 2018	10 July 2018
Blueberry 'Aurora'	11 April 2018	10 June 2018	15 August 2018
Strawberry 'Senga Sengana'	15 March 2018	15 April 2018	20 May 2018