

Article

Biochemical, Physiological and Yield Characteristics of Red Basil as Affected by Cultivar and Fertilization

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Abstract: Unconventional fertilizers can act as elicitors to encourage the synthesis of phyto-pharmaceuticals in aromatic plants. In the present research, the effects of factorial combination between two red basil cultivars, ‘Opal’ and ‘De Buzau’, and four fertilization types, biosolids, organic, microorganisms and chemical, plus an unfertilized control, were assessed on fresh and dry yield, biometrical parameters, soil plant analysis development (SPAD) and antioxidant compounds and activity. Chemical fertilization increased fresh yield compared with the control, with no difference in organic and microorganism fertilization regarding dry weight. ‘De Buzau’ enhanced the number of lateral stems and plant height, the latter being better affected by chemical and microorganisms compared to the control. Chemical fertilization showed the highest leaf dry matter, nitrate content and SPAD, whereas the control showed the lowest. Compared to the unfertilized control, biosolids increased total phenolics in ‘Opal’; microorganisms, organic and biosolids enhanced total flavonoids in ‘Opal’, with the same effect under microorganisms and organic treatments in ‘De Buzau’. Total anthocyanins showed the highest content in ‘Opal’ under organic fertilization. The highest antioxidant activity in the basil extracts was detected under microorganisms and organic applications in ‘Opal’. The present investigation results demonstrate that unconventional fertilizers increase the synthesis of antioxidants and represent a sustainable alternative to chemical fertilization for growing red basil.

Keywords: *Ocimum basilicum* L.; unconventional fertilizers; production; SPAD; nitrate; antioxidant compounds and activity

1. Introduction

Sustainable crop systems have been increasingly spreading as a consequence of the growing demand for healthy food produced by environmentally friendly practices [1–3]. Indeed, the area cultivated worldwide under low impact management has been expanding and, for example, the extreme form, represented by organic farming, has increased by 20% from 2016 to 2017, reaching 69.8 million hectares with a market value of about 90 billion euros [4].

Basil is a very popular medicinal plant, being cultivated in many geographical areas due to its bioactive principles, such as volatile oils, ascorbic acid and phenolic compounds [5–7]. It is a widely

used species in cosmetics, perfumery and in the preparation of foods, including pesto sauce and soft drinks [8,9]. Due to the several existing varieties, differing in leaf shape, color and flavor, basil has an ornamental value and is also connected to local tradition [10]. Recently, this species has been used as a companion plant in organic farming to prevent pathogen attacks [11,12].

Fertilization is a very important component of plant system management, particularly under sustainable farming [2], which implies the use of unconventional fertilizers, such as manure, compost, biosolids, microorganisms [13]. Indeed, the application of organic fertilizers may enhance soil organic matter, thus improving its physiological and chemical properties. [14] However, the latter conditions may lead to increased crop productivity, as previously reported in peas, oats and basil [15], or to temporary N-immobilization and yield reduction due to manure decomposition [16,17]. The N-form may affect plant photosynthesis and growth [18] and, in this respect, organic manures can provide essential plant nutrients and enhance crop productivity [19], but also leave a beneficial residual effect on succeeding crops [20]. In addition to yield effects, the application of unconventional fertilizers on basil crops can promote the synthesis of biological active constituents such as total phenolics [21,22].

Aromatic and medicinal plants have been increasingly spreading under sustainable farming [3], as they contain bioactive compounds that show remarkable culinary and therapeutic properties [23,24]. Notably, in addition to the beneficial impact on human health, the aforementioned bioactive substances provide plant pigmentation, pollination and protection from diverse stressors; for instance, ultraviolet radiation and herbivore attacks [25,26].

Several studies have showed that the synthesis of bioactive compounds (ascorbic acid, carotenoids, phenols, flavonoids, anthocyanins) in plants can be stimulated by external factors such as temperature [27], light [28,29], humidity, fertilization [30,31] and microorganisms [32], as these substances are produced in the secondary metabolism [25,26]. In this respect, Lobiuc et al. [29] reported that the synthesis of rosmarinic acid, one of the main phenolic acids produced in basil, increased as a result of light supply. The increased synthesis of bioactive compounds in plants is related to the physiological role of these molecules in protecting plants against ultraviolet radiation and herbivorous organisms [33].

Considering the above mentioned challenges, the main targets of this study were to assess (i) the chance of using unconventional fertilizers (biosolids, organic and microorganisms) as a sustainable alternative to chemical fertilization for the production of red basil enriched with antioxidant compounds (phenols, flavonoids, anthocyanins) and (ii) the effect of different fertilizers on fresh and dry yield, as well as biometrical and physiological characteristics.

2. Materials and Methods

2.1. Biological Material and Experimental Site

The research was carried out on basil (*Ocimum basilicum* L.) grown in an open field in 2016 and 2017, at the experimental farm "V. Adamachi" of the University of Agricultural Sciences and Veterinary Medicine (UASVM) of Iasi, Romania (47°11'76" N, 27°33'71" E, 150 m a.s.l.), on anthropic chambic chernozem soil with the following characteristics: 62% sand, 6% silt, 32% clay; pH 7.2; EC 478 $\mu\text{S}\cdot\text{cm}^{-1}$; 2.86% organic matter; 2.8 $\text{g}\cdot\text{kg}^{-1}$ N, 32 $\text{mg}\cdot\text{kg}^{-1}$ available P (Olsen method), 218 $\text{mg}\cdot\text{kg}^{-1}$ available K (ammonium acetate method), 4.1 $\text{g}\cdot\text{kg}^{-1}$ CaCO_3 ; C/N 5.91. The meteorological conditions during the experiments are presented in Table 1.

Table 1. Mean monthly values of rainfall and temperatures at the experimental site (2016–2017).

Month	Rainfall (mm)		Average Temperature (°C)		PAR (MJ·m ⁻² ·d ⁻¹)	
	2016	2017	2016	2017	2016	2017
May	72.0	71.0	15.4	16.5	9.8	10.0
June	111.0	47.0	21.2	21.7	10.8	10.8
July	12.0	48.0	23.1	22.0	11.0	10.8
August	52.3	39.0	21.3	22.2	9.7	9.9

The sowing was carried out in mid-April in alveolar cell trays with alveoli of 31.3 cm³. Plantlets were transplanted in the field on 25 May, spaced 15 cm along the rows, which were 45 cm apart (14.8 plants per m²). The plants were harvested at the flowering stage, on 10 August.

In both research years, during the growing period, the irrigation was performed by drip method when the available water in the soil dropped to 80%. No treatments for plant protection were carried out; weed control was manually done twice.

2.2. Experimental Design

The experimental protocol was based on the factorial combination between two cultivars (Opal, (Op) and De Buzau (Bz)) and four fertilization types (biosolids, organic, microorganisms, chemical) plus an unfertilized control. a split plot design with three replicates was arranged for treatment distribution in the field and each experimental unit covered a 4.05 m² surface area including 60 plants.

The red basil cultivar ‘De Buzau’ was created at Buzau Research and Development Station for Vegetables (Romania), whereas ‘Opal’ originated from the Republic of Moldova.

The organic fertilizers were ploughed into the soil three weeks earlier than the transplantation, so as to prevent the accumulation of the ammonium that is initially released, whereas the chemical fertilizer and microorganisms were formulated just prior to transplanting; the aim was to supply the crops with 40 kg·ha⁻¹ N, based on basil nutrient requirements of 5.0 kg N, 1.7 P₂O₅ and 7.2 K₂O per ton of produce yield [34] with a projected production of 8 t·ha⁻¹, with the following details:

1. Biosolids (B): 1000 kg·ha⁻¹ were applied; it was produced at the municipal waste water treatment plant in Iasi, Romania, and had pH 6.96, 26.6% OM, 4.83% total N, 2.43% P₂O₅, 0.51% K₂O, 0.3% MgO, 0.01% Fe, 0.004% Mn, 0.002 Zn, 0.001% Cu.
2. Organic (O): 1200 kg·ha⁻¹ of the chicken manure formulate Orgevit® were supplied; it has 65% OM, 90% dry matter, pH 7, 4% N, 3% P₂O₅, 2.5% K₂O, 1% MgO, 0.02% Fe, 0.01% Mn, 0.01% B, 0.01% Zn, 0.001% Cu, 0.001% Mo.
3. Microorganisms (M): Rizotech Plus powder was placed in the soil holes made for seedling transplant (60 kg·ha⁻¹), predominantly containing the arbuscular mycorrhizal fungi (AMF) spores of *Claroideoglomus etunicatum*, *Funneliformis mosseae*, *Glomus aggregatum*, *Rhizophagus intraradices* and, in addition, fungi and bacteria species belonging to genera *Trichoderma*, *Streptomyces*, *Bacillus*, *Pseudomonas*.
4. Chemical (Ch): 350 kg·ha⁻¹ of Nutrispore® were provided. This fertilizer contains 20% of N (urea form), 20% water soluble P₂O₅, 20% water soluble K₂O, 2% water soluble MgO, 0.01% B, 0.02% Fe, 0.01% Mn and 0.003% Zn, and the same microorganisms as reported in the above microorganisms description.

Each of the two organic fertilization types (biosolids and organic) amounted to about 70% of the rate associated with the chemical fertilization (Ch). Indeed, a higher dose of chemical fertilizer has been supplied, taking into account that nitrate anion stemming from urea and ammonium oxidation is not adsorbed by soil colloids surface and, consequently, a leaching loss of a N-NO₃⁻ fraction is expected. The beneficial microorganisms application served to assess (i) the potential of beneficial microorganisms in promoting plant nutrient uptake in absence of fertilization and (ii) the effects of both

the nitrogen supplied with the chemical fertilizer composed of inorganic minerals, plus microorganisms and N applied with the organic fertilizer.

2.3. Yield and Biometrical Determinations

In order to assess basil yield, fresh plants were cut at 5 cm above the ground and weighed. Dry yield was determined by air-drying the plants in the open field, under a shading net, for 30 days. Both yield and dry weight were expressed as $\text{t}\cdot\text{ha}^{-1}$.

The number of lateral stems and plant height were determined, the latter measured by a ruler and expressed in cm.

2.4. Leaf Dry Matter

Leaf dry matter was assessed by drying the leaves in an air-forced oven at 70 °C until they reached a constant weight.

2.5. Nitrate Determination

Nitrate content was assessed on ground-dried basil leaves by ion chromatography (ICS-3000, Dionex, Sunnyvale, CA, USA) coupled to a conductivity detector, using an IonPac AG11-HC guard (4×50 mm) column and IonPac AS11-HC analytical column (4×250 mm).

2.6. SPAD Determination

Just prior to harvesting, the soil plant analysis development (SPAD) index was determined on fifteen undamaged basil leaves per experimental unit, using a non-destructive portable chlorophyll content meter (SPAD 502, Konica Minolta Sensing, Inc. Osaka, Japan), the readings being expressed as SPAD units.

2.7. Biochemical Analysis

The total phenolic content was assessed as follows: 0.1 mL of leaf extracts were mixed with Folin–Ciocalteu reagent (Merck, Kenilworth, IL, USA) and incubated for 5 min, then Na_2CO_3 7.5% was added and 90 min incubation followed. The results were calculated referring to 760 nm absorbance and, according to a calibration curve, were expressed as gallic acid equivalents per gram of dry weight [24].

The total flavonoid content was evaluated by the absorbance at 510 nm of leaf extracts reacting with 5% NaNO_2 and 10% AlCl_3 . The results were expressed, according to a calibration curve, as quercetin equivalents per gram of dry weight [29].

Anthocyanin content was determined, as described by Lee et al. [35], in 5 g of fresh leaf extracts in 95 mL of ethyl alcohol: distilled water: hydrochloric acid mixture (75:24:1). Results were expressed as mg cyanidin3-glucoside/g fresh weight.

For assessing the antioxidant activity, an aliquot of 0.1 mL leaf extract was mixed with 2.9 mL of 60 μM DPPH (2,2-diphenyl-1-picrylhydrazyl) and incubated for 180 min to allow for complete reaction. The absorbance of mixtures was read at 515 nm and the results were calculated as absorbance inhibition (in percentage) of a blank composed of DPPH and ethanol as extract solvent [29].

2.8. Statistical Analysis

The data were statistically processed by ANOVA and the mean separation was performed through Tukey's test at 0.05 probability level, using the SPSS software version 21 (IBM Corp, Armonk, NY, USA). The results were reported as means $\pm$ standard errors.

3. Results

3.1. Yield, Biometrical and Physiological Determinations

The influence of cultivar and fertilization type on fresh and dry yield, lateral stems and plant height of basil are presented in Table 2. The cultivar did not significantly affect either the fresh and dry yield or chlorophyll content, however the number of lateral stems and plant height were significantly higher in Bz than in Op. Regarding the influence of the fertilization type, Ch treatment significantly increased the fresh and dry yield, plant height and chlorophyll content. The number of lateral stems was not significantly influenced by the fertilization type.

Table 2. Fresh and dry yield, and biometrical characteristics of basil as affected by cultivar and fertilization.

Treatment	Fresh Yield (t ha ⁻¹)	Dry Yield (t ha ⁻¹)	Lateral Stems (no. Per Plant)	Plant Height (cm)
Cultivar				
Op	8.84 ± 0.55	2.27 ± 0.14	13.24 ± 0.25	42.87 ± 0.70
Bz	9.86 ± 0.51	2.54 ± 0.13	16.78 ± 0.32	45.00 ± 0.57
	ns	ns	*	*
Fertilization type				
Ct	6.40 ± 0.41 d	1.61 ± 0.10 c	14.17 ± 0.84	41.33 ± 0.88 b
B	8.28 ± 0.46 c	2.14 ± 0.12 b	15.17 ± 0.85	42.83 ± 0.83 ab
O	10.10 ± 0.28 b	2.65 ± 0.07 a	15.94 ± 0.91	44.67 ± 0.95 ab
M	10.10 ± 0.45 b	2.64 ± 0.12 a	14.61 ± 0.93	45.17 ± 0.65 a
Ch	11.85 ± 0.32 a	2.98 ± 0.08 a	15.17 ± 0.89	45.67 ± 1.20 a

Opal (Op); De Buzau (Bz); control (Ct); biosolids (B); organic (O); microorganisms (M); chemical (Ch). Within each column, n.s. no statistically significant difference, * significant difference, values associated with different letters are significantly different according to Tukey's test at $p < 0.05$.

The leaf dry matter content (Table 3) was not affected by cultivar; however, a significant increase was obtained under the chemical fertilization compared to the unfertilized control.

Table 3. Dry matter, nitrate content and SPAD of basil leaves as affected by cultivar and fertilization.

Treatment	Leaf Dry Matter (%)	Leaf Nitrate Content (mg kg ⁻¹ f.w.)	SPAD
Cultivar			
Op	9.39 ± 0.06	294 ± 31	38.26 ± 1.27
Bz	9.37 ± 0.07	275 ± 30	36.77 ± 1.2
	ns	ns	ns
Fertilization type			
Ct	9.2 ± 0.05 b	220 ± 21 c	32.02 ± 0.57 d
B	9.32 ± 0.06 ab	313 ± 32 ab	36.23 ± 0.95 bc
O	9.47 ± 0.05 ab	278 ± 29 ac	35.18 ± 0.77 cd
M	9.37 ± 0.06 ab	254 ± 24 bc	40.27 ± 0.31 ab
Ch	9.57 ± 0.06 a	357 ± 38 a	43.87 ± 0.26 a

Opal (Op); De Buzau (Bz); control (Ct); biosolids (B); organic (O); microorganisms (M); chemical (Ch). Within each column, n.s. no statistically significant difference, * significant difference, values associated with different letters are significantly different according to Tukey's test at $p < 0.05$.

The leaf nitrate content (Table 3) was not affected by cultivar, but showed significant changes upon the fertilization treatments. Indeed, the chemical fertilization resulted in higher nitrate concentration in the basil leaves compared to the untreated control and the sole beneficial microorganism inoculation; the latter treatment, as well as the organic manure supply, did not significantly differ from the unfertilized control.

The SPAD, reflecting the chlorophyll content, was significantly affected by the interaction between cultivar and fertilization type (Figure 1): it was higher in plants cultivated under B, O, M, and Ch

fertilization, with increases of 25%, 19%, 27% and 43%, respectively, in ‘Opal’. Regarding ‘De Buzau’, significant increases were obtained under M and Ch fertilization (24% and 31%).

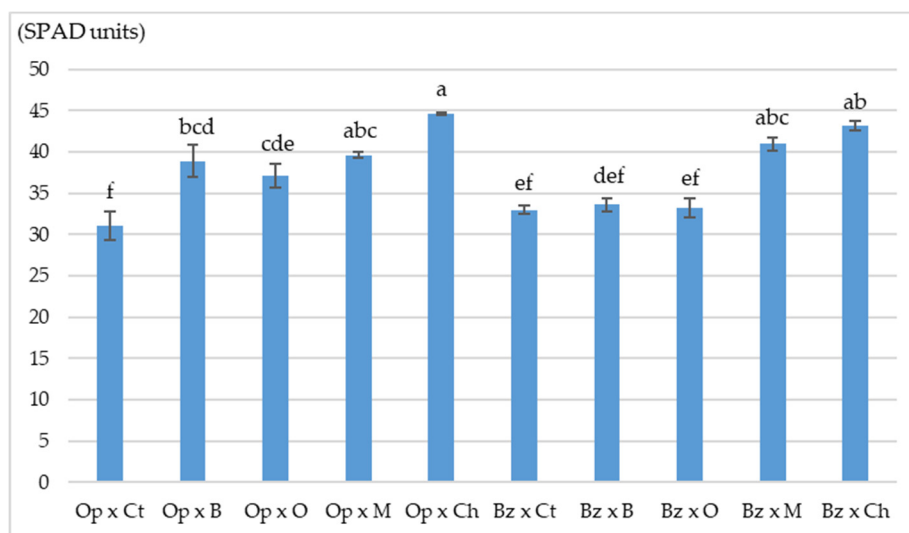


Figure 1. Chlorophyll content in basil leaves expressed as soil plant analysis development (SPAD) units, as affected by the interaction between cultivar and fertilization. Opal (Op); De Buzau (Bz); control (Ct); biosolids (B); organic (O); microorganisms (M); chemical (Ch). Values associated with different letters are significantly different according to Tukey’s test at $p < 0.05$.

3.2. Antioxidant Compounds and Activity

The interaction between cultivar and fertilization type was significant on the three antioxidants examined as well as on the antioxidant activity (Figures 2–5). Indeed, the total phenolics content (Figure 2) increased in ‘Opal’ plants fertilized with B by 12% compared to the control, whereas ‘De Buzau’ was not significantly affected by fertilization.

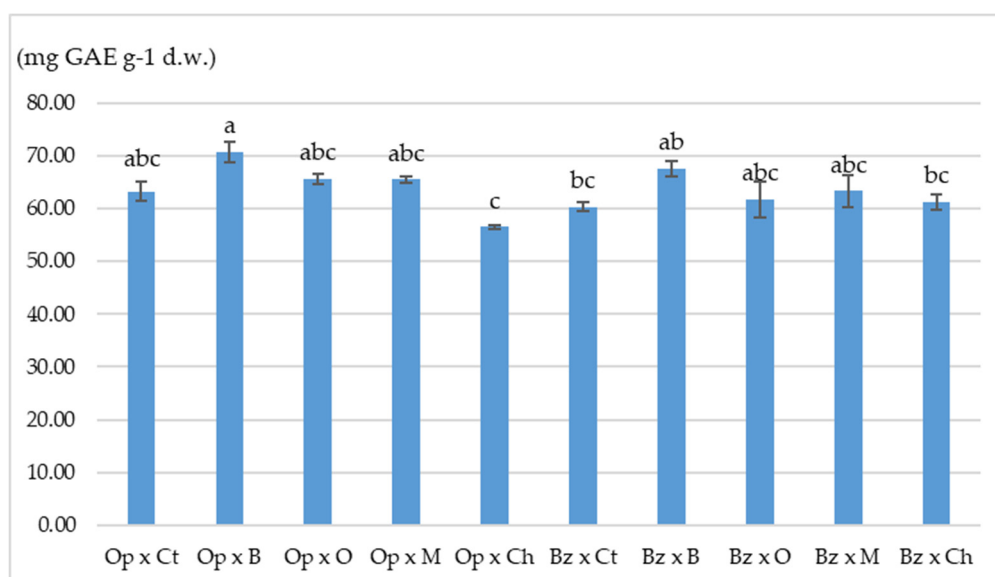


Figure 2. Total phenolics content in basil leaves, as affected by the interaction between cultivar and fertilization. Opal (Op); De Buzau (Bz); control (Ct); biosolids (B); organic (O); microorganisms (M); chemical (Ch). Values associated with different letters are significantly different according to Tukey’s test at $p < 0.05$.

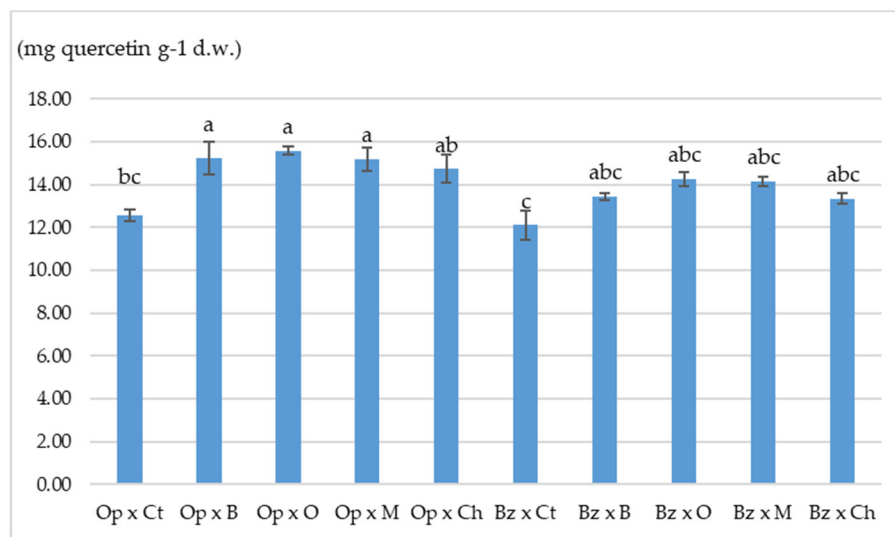


Figure 3. Total flavonoid content in basil leaves, as affected by the interaction between cultivar and fertilization. Opal (Op); De Buzau (Bz); control (Ct); biosolids (B); organic (O); microorganisms (M); chemical (Ch). Values associated with different letters are significantly different according to Tukey's test at $p < 0.05$.

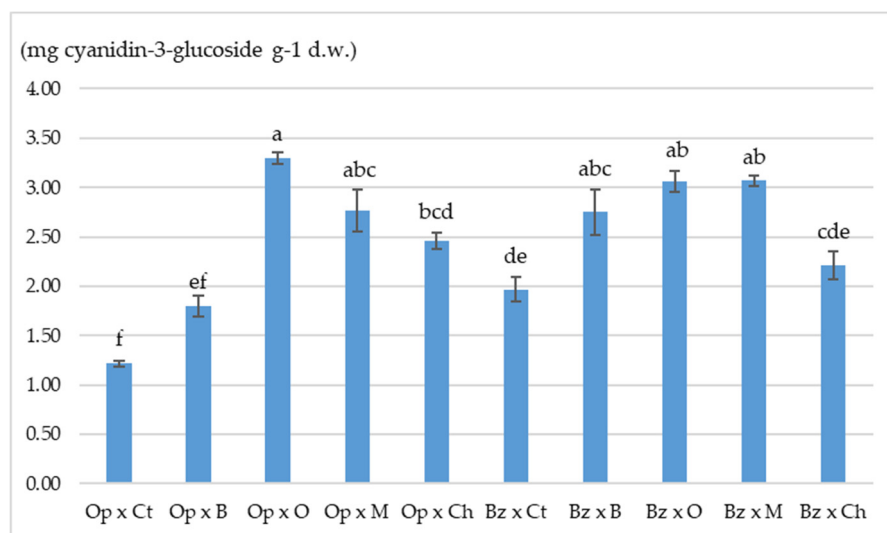


Figure 4. Total anthocyanin content in basil leaves, as affected by the interaction between cultivar and fertilization. Opal (Op); De Buzau (Bz); control (Ct); biosolids (B); organic (O); microorganisms (M); chemical (Ch). Values associated with different letters are significantly different according to Tukey's test at $p < 0.05$.

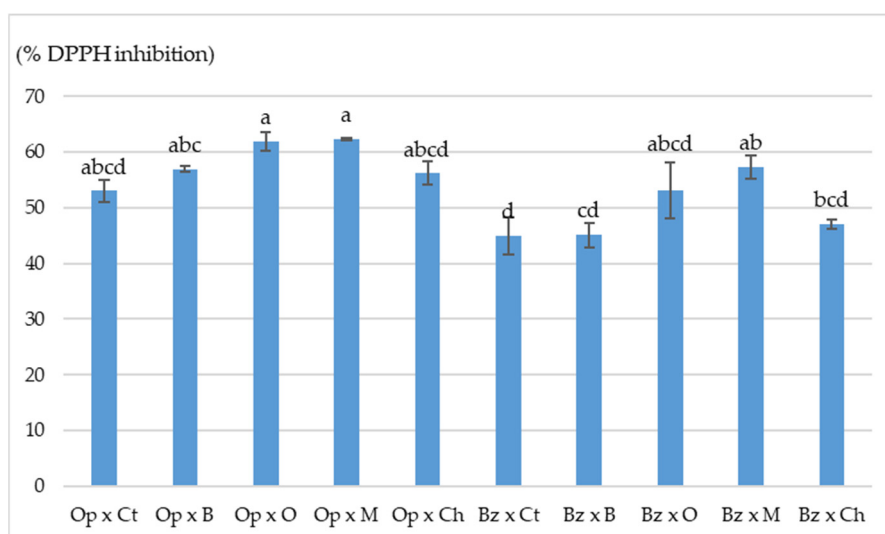


Figure 5. Antioxidant activity in basil leaves, as affected by the interaction between cultivar and fertilization. Opal (Op); De Buzau (Bz); control (Ct); biosolids (B); organic (O); microorganisms (M); chemical (Ch). Values associated with different letters are significantly different according to Tukey's test at $p < 0.05$.

The total flavonoid content (Figure 3) showed significant increases by 21%, 24% and 21% under B, O and M treatments respectively, compared to control.

The total anthocyanin content (Figure 4) showed the following variation ranges: from 1.22 to 3.30 mg g⁻¹ Cyanidin-3-glucosid equivalent (C3G) in 'Opal', with O, M and Ch attaining higher values than Ch and the unfertilized control; from 1.97 to 3.07 mg g⁻¹ C3G in 'De Buzau', with O and M resulting in higher contents compared to Ch and the untreated control, and B being slightly more than Ch.

A higher antioxidant activity of leaf basil extracts was recorded under M application compared to the unfertilized control in 'De Buzau' (Figure 5).

The production of bioactive compounds per soil surface unit (Table 4) did not differ between the two cultivars compared, but it was significantly affected by fertilization. In the latter respect, all the treatments resulted in higher antioxidant synthesis compared to the untreated control. However, organic and chemical fertilizers, as well as microorganism formulations, always showed the best outcomes, except for total phenols, where microorganisms did not significantly differ from biosolids.

Table 4. Antioxidant compounds in basil dry leaves per soil surface unit, as affected by cultivar and fertilization.

Treatment	Total Phenols	Flavonoids g·m ⁻²	Anthocyanins
Cultivar			
Op	3.26	0.75	0.12
Bz	3.47	0.75	0.14
	ns	ns	ns
Fertilization			
Ct	2.16 c	0.43 c	0.06 c
B	3.22 b	0.67 b	0.11 b
O	3.74 a	0.87 a	0.19 a
M	3.62 ab	0.83 a	0.16 a
Ch	4.00 a	0.95 a	0.16 a

Opal (Op); De Buzau (Bz); control (Ct); biosolids (B); organic (O); microorganisms (M); chemical (Ch). Within each column, n.s. no statistically significant difference, values associated with different letters are significantly different according to Tukey's test at $p < 0.05$.

4. Discussion

In the present research, the unconventional fertilizers (organic, microorganisms and biosolids) applied to red basil cultivars, in comparison with a chemical fertilizer, stimulated the production of plant biomass, as well as the synthesis of assimilatory pigments and phenolic compounds, though the chemical fertilization resulted in the highest fresh yield. In a previous study, Jakovljevic et al. [36] reported that the application of a chemical fertilizer overdose encouraged basil plant growth, but concurrently lowered the content of secondary compounds. Notably, the organic fertilizers (biosolids and Orgevit) showed a slower nutrient release through mineralization and, accordingly, a smaller effect on plant growth and biomass accumulation compared with chemical fertilization; consistently with our results, Bergstrand et al. [30] detected a slower nitrate availability from organic fertilizer compared to a mineral one in a pot-grown basil investigation. In particular, organic fertilizers release higher initial concentrations of ammonium which may cause toxicity [37] and, therefore, a possible impairment of basil plant growth under high nitrogen supply from organic sources [22]. In this respect, in the present research, the organic fertilizers were supplied three weeks before transplanting, in order to allow the nitrification process starting, thus preventing the initial ammonium accumulation and enhancing the total nitrate amount released to plants over the entire crop cycle. However, the low nitrification rate recorded by Bergstrand et al. [38] upon poultry manure application may have been caused by the peat-based substrate, which is sometimes characterized by a faster conversion from organic to ammonium form, compared to the subsequent nitrification [39]. In this respect, the sandy-loam soil used in our investigation has a higher air permeability than the peat substrate, thus increasing the nitrification rate [40]. However, in previous research, the optimal rate of inorganic nitrogen supply, which is easily taken up by plants and quickly assimilated, depended on the crop system: Sifola and Barbieri [41] recorded an increased production trend of basil up to 300 kg ha⁻¹ N, but yield was also optimally enhanced at lower N doses, such as 250 [22] or 160 kg ha⁻¹ [42]. In the present research, in addition to N, the fertilizers also provided the plants with necessary doses of other macroelements (P, K) and microelements (Fe, Mn, Zn), thus contributing to their optimal growth and development by stimulating primary metabolism [12,24,43]. In this respect, organic fertilizers usually provide lower P and K compared to nitrogen [44]. However, though in some cases, phosphorus supply can be lower than that which is recommended without unbalancing crop outcome [45]; in our study, P supply even exceeded basil requirements—therefore, the fraction of this element notoriously immobilized in the soil does not affect its necessary availability to plants. a similar situation to P regards the essential microelements which were supplied through both chemical and organic fertilizers, thus enhancing the content of this cations already present in the soil. As for potassium, the amount applied either with

chemical fertilizers or with the organic ones did not match the reference basil requirements, but the soil content of this element exceeds the middle-high threshold of a safe reservoir and, accordingly, no issues arose with the crops grown in our research.

The increased values of biometrical indicators (the number of lateral stems and plant height) under organic, microorganism-based or biosolids fertilization in the present investigation, may be explained by different processes that occur in the soil: the increase of soil organic matter content, phosphorus solubilization and gradual release of nutrients [46]. The stimulating effect of biosolids fertilization to plants can be attributed to the high content of organic matter (26%) and, in addition, this fertilizer is rich in microorganisms which produce enzymes such as dehydrogenase and phosphatase, contributing to the increased solubility of plant essential elements [47,48].

Nitrate concentration in basil leaves was affected by the type of fertilization applied, but not by the genotype. Indeed, the chemical fertilizer presumably elicited a faster NO_3^- availability in the soil solution, which enhanced the leaf accumulation of this ion compared to both the unfertilized control and the sole microorganism inoculation. The results that stem from the present research are consistent with previous reports [49] where, compared to the unfertilized control, chemical fertilization encouraged nitrate accumulation in perennial wall rocket, radish and zucchini in different growing seasons. However, in contrast with the latter authors' findings, in our investigation, the chemical fertilizer added with beneficial microorganisms did not promote a significant nitrate increase in basil leaves compared to the organic and biosolid fertilizers. In previous research [50], the higher the amount of chemical fertilizer supplied to plants, the higher the nitrate content in different edible parts of the vegetable crops. Despite this, in the present investigation, the leaf nitrate concentrations detected upon all fertilization treatments were much lower than the thresholds recommended by the EU Regulation 1258/2011 for leafy vegetables grown in open fields, expressed in $\text{mg}\cdot\text{kg}^{-1}$ fresh weight: 3500 for spinach; 3000 (1 April to 30 September) and 4000 (31 October to 31 March) for lettuce; 6000 (1 April to 30 September) to 7000 (31 October to 31 March) for rocket.

In the present study, Ch fertilization determined the highest SPAD value, reflecting the chlorophyll content in basil plants, which may be due to the fact that chemical fertilizer makes nitrogen readily available to plants and, in this respect, previous investigations highlighted the positive correlation between soil N availability and leaf content of chlorophyll [47,51,52] which is a nitrogen-containing molecule [40]. These results are presumably connected to the highest plant biomass recorded under Ch fertilization, possibly eliciting a fast absorption and metabolism of macronutrients (N, P and K) by plants [53,54].

In the present investigation, the highest concentration of total polyphenols was recorded for the biosolid fertilization, while that of total flavonoids and total anthocyanins was better enhanced by the organic and microorganisms treatments. However, the total amount of the mentioned bioactive compounds recorded in dry basil leaves was highest under organic and chemical fertilization, as well as the microorganisms application. Consistently with our findings, previous authors [55,56] reported that the synthesis of phenolic compounds in plants depends upon environmental conditions, cultivar and farming practices; among the latter, the application of microorganisms [30] and fertilizers [57] play a significant role. However, the antioxidant synthesis can be encouraged by different types of fertilization [16] and, indeed, the increased content of polyphenols, flavonoids and anthocyanins recorded in the present study under the unconventional fertilized treatments (biosolids, organic and microorganisms), was presumably due to their stimulation effect on secondary metabolism [58]. Moreover, previous researchers reported an increased content of phenolic compounds in basil plants grown on a substrate with a low nitrogen content [59], as a consequence of the reallocation of nitrogen resources for the synthesis of secondary metabolites at the expense of protein synthesis, according to the carbon/nitrogen balance hypothesis [60]. The results of the present investigation do not diverge from the findings obtained by Jakovljevic et al. [60], who demonstrated that the synthesis of phenolic compounds in basil is linked to a low nutrient concentration in soil, which allowed basil to maintain a low level of malondialdehyde and increased activity of phenylalanine ammonium lyase.

Regarding the antioxidant activity of basil extracts detected in the current research, the best results were obtained with the application of a chemical fertilizer containing beneficial microorganisms and of with the sole application of microorganisms. This activity is essential in preventing cell membrane peroxidation and even cell death caused by large concentrations of free radicals, whereas the latter unstable compounds are beneficial in signaling biochemical processes when they are present in low concentrations [61,62]. Previous reports showed the positive correlation between the phenol content and the antioxidant activity [63].

5. Conclusions

The fresh and dry yield of both red basil cultivars ‘Opal’ and ‘De Buzau’ were positively affected by all the fertilization treatments applied in comparison with the unfertilized control, though the highest increase was achieved under the chemical treatments, which may have elicited the highest chlorophyll synthesis. The basil extracts from cultivar ‘De Buzau’ showed higher antioxidant activity upon fertilization in comparison with the control, as a consequence of the increased content of antioxidant compounds (phenols, flavonoids and anthocyanin). From the outcomes of the present research, it can be inferred that unconventional fertilization is a viable and sustainable alternative to chemical fertilizers for enhancing the synthesis of antioxidants in red basil leaves.

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