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Morphological, Physiological and Quality Performances of Basil Cultivars under Different Fertilization Types

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Abstract: The prospect of replacing traditional chemical fertilization with organic and microorganism-based fertilization meets the current demand for more sustainable cropping systems and healthy food. In this respect, research was carried out to evaluate the effects of the factorial combination between four basil cultivars ('Aromat de Buzau', 'Macedon', 'Cuisoare' and 'Serafim') and three types of fertilization, namely chemical fertilization (with a solid chemical fertilizer), organic fertilization (with chicken manure formulate) and microorganisms' fertilization (with microorganisms formulate), on basil yield, biochemical and physiological parameters and essential oil composition. The results showed that the biometric parameters (plant height, number of stems and leaves and leaf area) were significantly influenced by the cultivar; 'Macedon' obtained the highest values of plant height (64.7 cm) and number of stems (20.33) and leaves (618.3) and 'Serafim' the largest leaf area (4901.7 cm^2 per plant), while the type of fertilization did not affect these parameters. Regarding the biomass, the influence of the cultivar was not significant on fresh biomass but was significant on dry biomass, with 'Macedon' showing the highest value (56.4 $g{\cdot}plant^{-1}$ dry biomass). The mentioned parameters were significantly influenced by the type of fertilization, with the highest values recorded with chemical fertilization. Both the cultivar and the fertilization type significantly influenced the physiological parameters (the total content of assimilatory pigments and photosynthesis). Five phenolic compounds were quantified from leaf extracts by HPLC-MS (caffeic acid, hyperoside, isoquercitrin, rutin and quercitrin). Hyperoside was identified only in 'Macedon', while the rest of the compounds were found in all the cultivars and varied depending on the cultivar and fertilization type. Regarding the composition of the essential oil, variation was found depending on the cultivar and fertilization type. In 'Aromat de Buzau', the main compounds were methyl chavicol and β-linalool; in 'Macedon', geranial and neral; and in 'Cuisoare' and 'Serafim', β-linalool. Moreover, the PCA showed that the 'Serafim' cultivar has exclusive properties compared to the other cultivars. Our results highlight that identifying the most effective interaction between genotype and fertilization type allows to optimize yield and quality targets for sweet basil.

Keywords: *Ocimum basilicum* L.; fresh and dry yield; physiology; phenolic compounds; essential oil

1. Introduction

Sweet basil (*Ocimum basilicum* L.) is a medicinal, culinary and ornamental species of tropical origin that is characterized by a high ecological plasticity, being cultivated worldwide [1,2]. This species can be grown successfully both in open fields and in greenhouses, as well as in pots indoors [3]. Green-leafed cultivars, i.e., 'Genovese', are used in the preparation of pesto, a typical green sauce in Italian cuisine [4]. Moreover, basil can be consumed fresh as a salad or dry in the preparation of some Mediterranean dishes and drinks [5]. Due to its popularity, a number of cultivars adapted to various local conditions with different phenotypes and chemotypes have been introduced in the market [6,7]. The main phenotypic characteristics varying among the different cultivars are plant height, leaf shape and color and flower color [8]. In a general review on basil, Simon et al. [8] described the plant characteristics of basil cultivars belonging to the *Ocimum basilicum* species that are found on the North American market and showed that plant height can vary from 29 cm ('Green Ruffles') up to 65–70 cm ('Sweet Dani'). Moreover, the color of leaves can be green ('Genovese'), green-purple ('Anise') or purple ('Red Rubin Purple Leaf'), while the color of the flowers can be white ('Genovese'), pink ('Dark Opal') or bright purple ('Purple Ruffles'). Other characteristics that can vary are spread (cm), stem and spike color and the number of days to flowering [8]. Basil is rich in essential oil, and its composition determines its specific aroma and chemotype [9,10]. The most common chemotype found within the European market is considered to have the best aroma and quality due to the high content of linalool and methyl chavicol. Other chemotypes are those from 'Reunion', with a high content of methyl chavicol; from tropics, which have a high content of methyl cinnamate; and from Eastern Europe, Russia and many parts of Asia and North Africa, which have a high content of eugenol [11,12]. The content and composition of phenolic compounds and essential oil depend on the cultivar and the cultivation technology. Zheljazkov et al. [13] evaluated the essential oil content depending on the cultivar and the growing location and found that the 'Mesten' cultivar had 0.067% at Beaumont, Mississippi and 0.481% at Verona, Mississippi, while the 'German' cultivar had 0.236% at Beaumont and 0.389% at Verona. Regarding the cultivation technology, Baczek et al. [14] found that the content of linalool was higher in plants grown in an open field compared to those grown in a polytunnel. Basil is also rich in phenolic compounds such as rosmarinic acid, chicoric acid and caffeic acid, which give it bioactivities such as antioxidant, antimicrobial or insecticidal activity [15,16].

Fertilization with organic fertilizers has become more and more attractive for farmers as consumer are willing to pay premium price for organic produce. Indeed, in the EU alone, the land area under certified organic management has increased from 9.5 million hectares in 2012 to 14.7 million hectares in 2020 [17]. Microorganism-based products (i.e., arbuscular mycorrhizal fungi and plant growth-promoting rhizobacteria) can be used in organic agriculture to stimulate growth and control pests [18]. Moreover, due to the complex mechanisms of action, such as increasing the bioavailability of nutrients by solubilizing macronutrients such as phosphorus or inducing systemic resistance in plants, they can influence the synthesis of compounds with a defense role such as polyphenols [19,20]. Currently, the interest in healthy food rich in bioactive compounds has increased, and in this respect, farming management allows for improving the quality of products by increasing the content of these compounds. Organic fertilization may stimulate beneficial microorganisms and subsequently could stimulate the synthesis and accumulation of bioactive compounds [21–23], while chemical fertilization can have the opposite effect [24,25]. Moreover, in conventional cultivation systems, the use of chemical fertilizers and pesticides can cause the accumulation of some chemical residues both in soil and plant products, in contrast to certified organic crops where the use of chemical fertilizers and pesticides is prohibited [26]. Caruso et al. [21], in a comparative study, showed that in sweet pepper (*Capsicum annuum* L. ssp. *annuum*), the total content of polyphenols increased with microorganism-enriched conventional fertilization and the total flavonoid content increased with microorganism-enriched organic fertilization compared to conventional fertilization.

Due to the shortage of literature reports about the prospect of replacing traditional chemical fertilization with organic and microorganism-based fertilization types which better meet the current demands for more sustainable crop systems and healthy food, the present investigation aimed to assess the interaction effect between cultivar and fertilization type on the yield, biochemical and physiological parameters and essential oil composition of basil.

2. Materials and Methods

2.1. Experimental Site

This research was carried out on sweet basil (*Ocimum basilicum* L.) in 2019 and 2020 at the experimental field of the Didactic and Experimental Station V. Adamachi within the Iasi University of Life Sciences, Romania. The soil was anthropic chambic chernozem with the following characteristics: 61% sand, 33% clay and 6% silt; pH 7.1; EC 495 $\mu S \cdot cm^{-1}$; 2.79% organic matter; 2.8 $g \cdot kg^{-1}$ N, 32 $mg \cdot kg^{-1}$ available P (Olsen method), 218 $mg \cdot kg^{-1}$ available K (ammonium acetate method) and 4.1 $g \cdot kg^{-1}$ $CaCO_3$; C/N 5.93. The main meteorological conditions during the research are presented in Table 1.

Table 1. Meteorological conditions during the study.

Month	Average Temperature (°C)		Atmospheric Humidity (%)		Rainfall (mm)	
	2019	2020	2019	2020	2019	2020
April	10.7	11.1	66	42	6.9	1.6
May	16.6	14.4	77	67	74.9	130.5
June	22.7	21.3	59	71	8.4	99.0
July	22.0	22.1	67	61	3.8	7.9
August	22.1	23.6	67	54	35.1	8.8
Average/Sum	18.8	18.5	67.2	59.0	129.1	247.8

The experiment was established in mid-April by direct sowing in polystyrene multicell trays, with 31.3 cm^3 alveoli. The seedlings were planted in mid-May in the open field, spaced 15 cm to 45 cm in row/between row spacing, resulting in a density of 14.8 plants per square meter.

During the cropping season, the following practices were performed: drip irrigation; manual weeding twice; harvesting was performed at the beginning of flowering (BBCH 61) [27], at the beginning of August. No phytosanitary treatments were necessary [28].

2.2. Experimental Design

The experimental protocol was based on the combination of two factors, and a split plot design was arranged for the treatment distribution in the field, with three repetitions: factor A was the cultivar [a_1—'Aromat de Buzau' (AB); a_2—'Macedon' (M), a_3—'Cuisoare' (C); a_4—'Serafim' (S)], assigned to the plots; factor B was the type of fertilization [b_1–chemical (Ch); b_2–organic (O); b_3–microorganisms (Mo)] assigned to the sub-plots.

The cultivars used in the experiment were developed and commercially propagated in Romania, at the Buzau Vegetable Research Development Station; three of them have already been homologated (AB, M and S), while the fourth is a genotype under test, with the aroma of clove oil (*Syzygium aromaticum* L.) (C). These cultivars have different morphological and phytochemical characteristics: Aromat de Buzau is a cultivar with green leaves and white flowers, and methyl chavicol and linalool are the main essential oil constituents; Serafim has red leaves and pink flowers, and linalool and eugenol are the main essential oil constituents; Macedon has green leaves and white flowers, and geranial and neral are the main essential oil constituents; Cuisoare has green leaves and purple flowers, and linalool and eugenol are the main essential oil constituents [18].

Regarding the applied fertilization types, the chemical one was performed with Cristaland at 200 kg·ha^{-1}, the organic one with Orgevit at 1000 kg·ha^{-1}, and the microorganisms' formulation was Micoseeds MB at 80 kg·ha^{-1}. The fertilizers were applied before planting with soil incorporation. Cristaland® is a solid chemical fertilizer containing 30% total N, of which 2% is ammoniacal N and 28% is uric N; 10% water-soluble P_2O_5; 10% water-soluble K_2O and 2% water-soluble MgO. Orgevit® is a solid ecological fertilizer with pH 7, in granular form containing 65% OM, 90% dry matter, 4% N, 3% P_2O_5, 2.5% K_2O, 1% MgO, 0.02% Fe, 0.01% Mn, 0.01% B, 0.01% Zn, 0.001% Cu and 0.001% Mo. Micoseeds MB® is a microgranulated product based on microorganisms that predominantly contains arbuscular mycorrhizal fungi (AMF), spores of *Claroideoglomus etunicatum*, *Funneliformis mosseae*, *Glomus aggregatum* and *Rhizophagus intraradices*. In addition to these spores, there are fungi and bacterial species belonging to the genera *Trichoderma*, *Streptomyces*, *Bacillus* and *Pseudomonas*.

The dose of N active substance (a.s.) per hectare from the organic fertilization with Orgevit represented approximately 70% of the dose of N a.s. per ha associated with the chemical fertilization with Cristaland, because it was taken into account that the N anion from the oxidation of urea and ammonium is not adsorbed by the surface of the soil colloids, and thus, a leaching loss of a N-NO_3^- fraction is expected. The application of beneficial microorganisms served to evaluate their potential in stimulating plant nutrient absorption in the absence of fertilization [21].

2.3. Biometric and Agroproductivity Characteristics Determination

In order to determine the biometric characteristics, the height of the plants was evaluated by measuring them with a ruler and expressing the values obtained in cm, followed by determinations regarding the number of lateral stems per plant [29].

To assess the number of leaves per plant and the leaf surface per plant (cm^2·plant^{-1}), the basil plants were harvested by cutting them 5 cm above the ground. The leaf area index (LAI) was determined using the Li-3100 Area Meter, (LICOR, inc., Lincoln, NE, USA) [30].

The amount of fresh biomass (leaves and stems), expressed in grams per plant, was determined immediately after harvesting by weighing with a Kern analytical balance, with a precision of 0.01 g. The amount of dry biomass was determined after drying the plants in a sheltered, naturally ventilated place for 30 days [31].

2.4. Physiological Parameters Determination

2.4.1. Total Chlorophyll Content Determination

The total chlorophyll content was determined with a CCM-200 plus non-destructive portable chlorophyll content meter (Opti-Sciences, ADC BioScientific Ltd., Hoddesdon, Hertfordshire, UK); the recorded values were expressed as CCI units (Chlorophyll Content Index). The measurements were taken one day before harvest. For each experimental treatment, 30 readings with 20 plants were performed. Fully developed leaves at the middle plant height were selected [32].

2.4.2. Photosynthesis Determination

Photosynthesis was determined using an LCi system (ADC Bioscientific UK Ltd., Hoddesdon, Hertfordshire, UK). A broad leaf chamber (6.4 cm^2) was used, and the measurements were performed between 9 and 11 a.m. The results were expressed as µmol m^{-2} s^{-1} [32].

2.4.3. The Color of Leaves

The color parameters of leaves (L, a and b) were assessed using a MiniScan XE Plus color meter (HunterLab, Reston, VA, USA). The value of L indicates lightness, a indicates the degree of red (+a) or green (−a) and b denotes yellow (+b) or blue (−b) color of leaves. C is the chroma [33].

2.5. Extraction and Determination of Phenolic Compounds

The phenolic compounds (caffeic acid, hyperoside, isoquercitrin, rutin and quercitrin) were determined from 10% leaf extract in 70% ethanol by ultrasonication for 30 min at room temperature. An Agilent 1100 HPLC system by Agilent Technologies Inc., Santa Clara, CA, USA, and a Zorbax SB-C18 column were used according to the method described by Mocan et al. [34].

2.6. Extraction and Analysis of the Essential Oil Composition

The essential oil was extracted from fresh whole aboveground plant material by steam distillation for three hours and the results were expressed as %.

A GC/FID–GC/MS system (Agilent 5975C MSD coupled to Agilent 7890A GC by Agilent Technologies Inc., Santa Clara, CA, USA) was used to analyze the composition of the essential oil. The complete method was described by Teliban et al. [18], Burducea et al. [35] and Adams [36].

2.7. Statistical Analysis

The results were reported as means ± standard errors of the two-year experiment (2019 and 2020), after raw data processing by ANOVA and mean separation through the Duncan multiple range test ($p < 0.05$) using SPSS v21 software (IBM Corp, Armonk, NY, USA). The principal component analysis (PCA) was performed using OriginPro 2020 Academic by OriginLab Corporation, Northampton, MA, USA. This analysis aimed to reduce the number of variables to determine correlations and interactions between different inputs [37].

3. Results

The effect of the cultivar and the fertilization type on the biometric parameters is shown in Table 2. 'Macedon' had the highest values of plant height (+65% compared to 'Serafim'), ramifications (+56% compared to 'Aromat de Buzau') and number of leaves (+96% compared to 'Cuisoare'), while 'Serafim' displayed the highest values of leaf area (+44% compared to 'Aromat de Buzau'). The fertilization type did not induce significant differences with reference to the mentioned parameters (Table 2).

Table 2. Influence of cultivar and fertilization type on the biometric characteristics.

Treatment	Plant Height (cm)	Ramifications (No. per Plant)	No. of Leaves per Plant	Leaf Area Index (LAI) (cm_2 per Plant)
		Cultivar		
'Aromat de Buzau'	53.05 ± 1.45 a	13.22 ± 0.58 b	473.61 ± 18.70 b	3387.95 ± 174.59 b
'Macedon'	64.61 ± 4.63 a	20.55 ± 1.45 a	618.17 ± 32.69 a	3886.50 ± 167.79 b
'Cuisoare'	58.61 ± 6.00 a	14.06 ± 1.00 b	315.22 ± 8.37 c	3962.94 ± 98.77 b
'Serafim'	38.89 ± 2.00 b	14.61 ± 0.58 b	457.69 ± 34.49 b	4901.61 ± 307.60 a
		Fertilization type		
Chemical	54.63 ± 2.33	15.42 ± 0.67	471.28 ± 20.08	4319.39 ± 87.72
Organic	53.79 ± 2.33	15.67 ± 1.20	460.59 ± 41.09	4064.51 ± 353.93
Microorganisms	52.96 ± 1.76	15.75 ± 0.58	466.65 ± 38.02	3720.35 ± 196.91
	n.s.	n.s.	n.s.	n.s.

Within each column: n.s.—no statistically significant difference; values associated with the same lowercase letters are not statistically different at $p < 0.05$ according to Duncan's test.

The effect of the cultivar and the fertilization type on biomass parameters is shown in Table 3. Regarding the fresh biomass, 'Serafim' had the highest value of leaves weight (+45% compared to 'Aromat de Buzau'), and 'Macedon' had the highest weight of stems (+68% compared to 'Serafim') and of total plant (+14% compared to 'Serafim'). Regarding the dry biomass, 'Cuisoare' had the highest leaves weight (+47% compared to 'Aromat de Buzau'), and 'Macedon' had the highest weight of stems (+162% compared to 'Serafim') and of total plant (+60% compared to 'Serafim'). Chemical fertilization showed the highest significant influence on the fresh and dry weights of stems and total plant.

Table 3. Influence of cultivar and fertilization type on basil yield characteristics.

Treatment	Fresh Yield (g per Plant)			Dry Yield (g per Plant)		
	Leaves Weight	Stem Weight	Total Weight	Leaves Weight	Stem Weight	Total Weight
Cultivar						
'Aromat de Buzau'	116.17 ± 2.68 b	208.76 ± 11.03 a	324.93 ± 10.82	15.42 ± 0.19 c	31.96 ± 1.48 b	47.38 ± 1.58 b
'Macedon'	133.71 ± 5.32 b	201.15 ± 17.67 a	334.86 ± 22.78	18.43 ± 0.85 b	37.94 ± 2.68 a	56.36 ± 3.35 a
'Cuisoare'	156.59 ± 6.45 a	172.15 ± 5.83 a	328.74 ± 6.14	22.67 ± 0.63 a	25.09 ± 0.94 c	47.77 ± 0.98 b
'Serafim'	169.45 ± 9.82 a	123.85 ± 5.30 b	293.31 ± 12.67	20.62 ± 1.06 ab	14.45 ± 0.43 d	35.07 ± 1.37 c
			n.s.			
Fertilization type						
Chemical	153.87 ± 5.11	193.43 ± 10.28 a	347.30 ± 14.12 a	20.55 ± 0.74	29.49 ± 1.49 a	50.04 ± 1.97 a
Organic	141.27 ± 9.17	178.66 ± 3.61 ab	319.93 ± 5.60 ab	18.67 ± 1.00	28.40 ± 0.26 a	47.07 ± 0.84 ab
Microorganisms	136.81 ± 3.21	157.34 ± 1.92 b	294.15 ± 4.54 b	18.64 ± 0.35	24.18 ± 0.46 b	42.82 ± 0.11 b
	n.s.			n.s.		

Within each column: n.s.—no statistically significant difference; values associated with the same lowercase letters are not statistically different at $p < 0.05$ according to Duncan's test.

From the significant interaction between cultivar and fertilization type, it arose that the highest values of plant height and leaf number were recorded in 'Macedon' under the chemical fertilization, the highest ramification number was in organically fertilized 'Macedon' and the largest leaf area was in 'Serafim' supplied with organic fertilizer (Table 4).

Table 4. Interaction between cultivar and fertilization type on the biometric characteristics.

Treatment	Plant Height (cm)	Ramifications (No. per Plant)	No. of Leaves per Plant	Leaf Area Index (LAI, cm^2 Per Plant)
AB × Ch	50.83 ± 2.17 bcde	12.83 ± 0.17 d	408.67 ± 18.89 cdef	3332.17 ± 148.13 d
AB × O	48.83 ± 4.51 bcde	11.33 ± 1.74 d	480.33 ± 36.32 bcd	3258.00 ± 217.59 d
AB × Mo	59.50 ± 6.45 ab	15.50 ± 2.18 bcd	531.83 ± 77.30 abc	3573.67 ± 310.89 cd
M × Ch	69.67 ± 4.92 a	20.33 ± 1.17 ab	676.33 ± 34.51 a	4529.17 ± 9.68 abc
M × O	66.33 ± 4.21 ab	22.50 ± 1.32 a	606.00 ± 44.26 ab	3765.67 ± 275.66 cd
M × Mo	57.83 ± 5.93 abc	18.83 ± 2.73 abc	572.17 ± 59.09 abc	3364.67 ± 494.36 cd
C × Ch	60.67 ± 10.54 ab	14.50 ± 1.32 cd	353.17 ± 13.17 def	4238.83 ± 87.13 bcd
C × O	59.33 ± 2.62 ab	14.50 ± 2.00 cd	302.33 ± 28.99 ef	3855.50 ± 281.15 cd
C × Mo	55.83 ± 5.42 abcd	13.17 ± 0.33 d	290.17 ± 20.46 f	3794.50 ± 35.22 cd
S × Ch	37.33 ± 2.46 e	14.00 ± 1.89 cd	446.94 ± 34.47 bcdef	5177.38 ± 261.39 ab
S × O	40.67 ± 6.65 cde	14.33 ± 1.09 cd	453.70 ± 123.28 bcdef	5378.88 ± 909.63 a
S × Mo	38.67 ± 1.48 de	15.50 ± 1.04 bcd	472.42 ± 26.63 bcde	4148.56 ± 117.69 bcd

Within each column, values associated with the same lowercase letters are not statistically different at $p < 0.05$ according to Duncan's test. AB—'Aromat de Buzau'; M—'Macedon'; C—'Cuisoare'; S—'Serafim'; Ch—chemical; O—organic; Mo—microorganisms.

From the significant interaction between cultivar and fertilization type on fresh biomass (Table 5), it can be observed that the chemical fertilization led to the highest fresh and dry biomass of leaves in 'Cuisoare' and of stems and total plant in 'Macedon'.

The effect of cultivar and fertilization type on physiological and color parameters is shown in Table 6. The highest content of assimilatory pigments, expressed in CCI (Chlorophyll Content Index), was recorded in 'Serafim' (35.63 CCI), 161% higher than that in 'Aromat de Buzau' which had the lowest value (13.6 CCI). The highest value of photosynthesis was detected in 'Aromat de Buzau', 202% higher than that in 'Serafim'. The chemical fertilization elicited the highest content of assimilatory pigments, 10% higher than the microorganism formulation, with the latter leading to the highest value of photosynthesis, 31% higher than that with chemical fertilization. Among the color parameters (*L* lightness–darkness, *a* redness–greenness and *b* yellowness–blueness), *L* was significantly higher in 'Aromat de Buzau' and under microorganism fertilization; the highest value of *a* and the lowest value of *b* were recorded in 'Serafim', a red-leafed basil cultivar.

Table 5. Interaction between cultivar and fertilization type on basil yield characteristics.

Treatment	Fresh Yield (g per Plant) Leaves Weight	Stem Weight	Total Weight	Dry Yield (g per Plant) Leaves Weight	Stem Weight	Total Weight
AB × Ch	118.76 ± 3.96 cd	218.90 ± 10.41 ab	337.65 ± 7.85 ab	16.15 ± 0.25 de	33.62 ± 2.79 cd	49.77 ± 2.74 b
AB × O	108.72 ± 1.67 d	222.14 ± 12.12 ab	330.85 ± 13.79 abc	14.89 ± 0.46 e	35.13 ± 1.19 bc	50.01 ± 1.57 b
AB × Mo	121.05 ± 3.68 cd	185.23 ± 18.81 abcd	306.28 ± 16.16 bc	15.23 ± 0.14 e	27.13 ± 3.17 de	42.36 ± 3.07 bc
M × Ch	155.58 ± 2.86 ab	228.78 ± 20.24 a	384.36 ± 22.51 a	21.58 ± 0.91 abc	41.95 ± 1.19 a	63.52 ± 1.59 a
M × O	130.13 ± 8.53 bcd	208.96 ± 19.29 abc	339.09 ± 27.19 ab	17.90 ± 1.24 cde	40.87 ± 3.86 ab	58.77 ± 5.01 a
M × Mo	115.43 ± 16.16 d	165.69 ± 21.94 cd	281.13 ± 37.64 bc	15.80 ± 1.75 e	30.99 ± 3.26 cde	46.79 ± 4.55 b
C × Ch	163.87 ± 5.27 a	177.49 ± 1.29 bcd	341.37 ± 6.51 ab	24.63 ± 1.06 a	25.74 ± 0.90 e	50.38 ± 1.93 b
C × O	155.67 ± 12.72 ab	169.73 ± 5.38 cd	325.40 ± 7.41 abc	21.01 ± 1.07 abc	24.56 ± 1.75 e	45.58 ± 1.64 b
C × Mo	150.23 ± 8.94 abc	169.23 ± 15.34 cd	319.47 ± 22.13 abc	22.36 ± 0.59 ab	24.98 ± 1.94 e	47.34 ± 2.44 b
S × Ch	177.29 ± 16.84 a	148.54 ± 14.10 de	325.83 ± 29.22 abc	19.82 ± 1.75 bcd	16.67 ± 1.28 f	36.49 ± 2.73 c
S × O	170.55 ± 18.10 a	113.82 ± 17.77 e	284.37 ± 6.53 bc	20.87 ± 2.45 abc	13.05 ± 1.41 f	33.92 ± 1.41 c
S × Mo	160.52 ± 3.60 ab	109.21 ± 7.39 e	269.72 ± 8.97 c	21.17 ± 0.74 abc	13.62 ± 0.75 f	34.79 ± 0.79 c

Within each column, values associated with the same lowercase letters are not statistically different at $p < 0.05$ according to Duncan's test. AB—'Aromat de Buzau'; M—'Macedon'; C—'Cuisoare'; S—'Serafim'; Ch—chemical; O—organic; Mo—microorganisms.

Table 6. Influence of cultivar and fertilization type on physiological and color parameters.

Treatment	CCI	Photosynthesis µmol m^{-2} s^{-1}	*L*	*a*	*b*
Cultivar					
'Aromat de Buzau'	13.60 ± 0.06 d	2.69 ± 0.23 a	35.50 ± 0.31 a	−6.21 ± 0.09 c	13.79 ± 1.44 a
'Macedon'	22.97 ± 0.35 b	1.30 ± 0.04 c	33.83 ± 0.05 b	−5.72 ± 0.06 b	11.52 ± 0.06 a
'Cuisoare'	17.53 ± 0.33 c	1.81 ± 0.04 b	34.32 ± 0.44 b	−6.40 ± 0.12 c	12.75 ± 0.20 a
'Serafim'	35.63 ± 0.66 a	0.89 ± 0.01 d	22.83 ± 0.06 c	1.75 ± 0.08 a	−0.15 ± 0.01 b
Fertilization type					
Chemical	23.73 ± 0.03 a	1.47 ± 0.06 b	31.55 ± 0.12 ab	−4.14 ± 0.05 ab	8.95 ± 0.10
Organic	21.93 ± 0.50 b	1.61 ± 0.13 ab	31.33 ± 0.16 b	−4.03 ± 0.06 a	10.02 ± 1.11
Microorganisms	21.57 ± 0.18 b	1.93 ± 0.11 a	31.99 ± 0.16 a	−4.27 ± 0.05 b	9.46 ± 0.14
					n.s.

Within each column: n.s.—no statistically significant difference; values associated with the same lowercase letters are not statistically different at $p < 0.05$ according to Duncan's test. CCI—Chlorophyll Content Index; *L*—lightness–darkness; *a*—redness–greenness; *b*—yellowness–blueness.

From the significant interaction between cultivar and fertilization type on the content of assimilatory pigments (Table 7), it arose that the highest value was recorded in 'Serafim' under the chemical fertilization, 176% higher than that in 'Aromat de Buzau' fertilized with microorganisms, and the highest value of photosynthesis was recorded in 'Aromat de Buzau' supplied with the microorganism formulation, 357% higher compared to the chemically fertilized Serafim.

The outcome of the analysis of phenolic compounds from basil extracts based on the investigation of five compounds (caffeic acid, hyperoside, isoquercitrin, rutin and quercitrin) is presented in Table 8. Hyperoside was identified only in 'Macedon', with values between 6.62 µg·mL^{-1} for the organic fertilization and 7.87 µg·mL^{-1} for the microorganism formulation. Caffeic acid had values between 2.22 µg·mL^{-1} in the chemically fertilized 'Macedon' and 5.23 µg·mL^{-1} in the chemically fertilized 'Aromat de Buzau'. Isoquercitrin showed values between 6.52 µg·mL^{-1} ('Serafim' × chemical fertilization) and 39.49 µg·mL^{-1} ('Cuisoare' × microorganism formulation). The values of rutin ranged from 10.36 µg·mL^{-1} ('Serafim' × chemical fertilization) to 130.90 µg·mL^{-1} ('Cuisoare' × microorganism formulation). Quercitrin had values between 1.30 µg·mL^{-1} ('Serafim' × organic fertilization) and 5.79 µg·mL^{-1} ('Aromat de Buzau' × microorganism formulation).

Table 7. Interaction between cultivar and fertilization type on physiological and color parameters.

Treatment	CCI	Photosynthesis (μmol m^{-2} s^{-1})	*L*	*a*	*b*
AB × Ch	14.26 ± 0.09 e	2.68 ± 0.18 b	35.04 ± 0.44 ab	−6.20 ± 0.08 d	11.84 ± 0.35 b
AB × O	13.45 ± 0.32 e	1.96 ± 0.17 cd	35.74 ± 0.49 a	−6.19 ± 0.17 d	16.77 ± 4.02 a
AB × Mo	13.10 ± 0.44 e	3.43 ± 0.34 a	35.72 ± 0.25 a	−6.24 ± 0.07 de	12.76 ± 0.08 b
M × Ch	26.16 ± 1.41 b	0.95 ± 0.12 fg	33.95 ± 0.30 b	−5.74 ± 0.13 c	11.54 ± 0.32 b
M × O	21.14 ± 0.50 c	1.15 ± 0.36 efg	32.78 ± 0.33 c	−5.39 ± 0.02 b	10.96 ± 0.59 b
M × Mo	21.58 ± 0.27 c	1.79 ± 0.14 cd	34.78 ± 0.12 ab	−6.04 ± 0.10 cd	12.06 ± 0.14 b
C × Ch	18.39 ± 0.35 d	1.50 ± 0.01 def	33.97 ± 0.75 b	−6.28 ± 0.14 de	12.43 ± 0.31 b
C × O	17.40 ± 0.49 d	2.28 ± 0.06 bc	34.16 ± 0.31 b	−6.33 ± 0.12 de	12.69 ± 0.09 b
C × Mo	16.81 ± 0.62 d	1.64 ± 0.08 de	34.85 ± 0.53 ab	−6.60 ± 0.16 e	13.15 ± 0.36 b
S × Ch	36.16 ± 0.89 a	0.75 ± 0.07 g	23.22 ± 0.20 d	1.63 ± 0.02 a	0.01 ± 0.14 c
S × O	35.84 ± 1.81 a	1.05 ± 0.07 fg	22.64 ± 0.19 d	1.78 ± 0.08 a	−0.33 ± 0.09 c
S × Mo	34.84 ± 0.94 a	0.88 ± 0.03 g	22.63 ± 0.17 d	1.83 ± 0.17 a	−0.14 ± 0.08 c

Within each column, values associated with the same lowercase letters are not statistically different at $p < 0.05$ according to Duncan's test. CCI—Chlorophyll Content Index; *L*—lightness–darkness; *a*—redness–greenness; *b*—yellowness–blueness; AB—'Aromat de Buzau'; M—'Macedon'; C—'Cuisoare'; S—'Serafim'; Ch—chemical; O—organic; Mo—microorganisms.

Table 8. Interaction between cultivar and fertilization type on phenolic compounds (μg·mL^{-1}).

Treatment	Caffeic Acid	Hyperoside	Isoquercitrin	Rutin	Quercitrin
AB × Ch	5.23 ± 0.46 a	tr	24.39 ± 2.03 c	41.53 ± 4.82 de	3.54 ± 0.48 bc
AB × O	4.97 ± 0.39 ab	tr	28.86 ± 2.54 bc	44.95 ± 5.45 d	4.11 ± 0.23 b
AB × Mo	4.97 ± 0.67 ab	tr	28.86 ± 2.26 bc	42.12 ± 2.39 de	5.79 ± 0.49 a
M × Ch	2.22 ± 0.13 d	7.04 ± 0.06 b	35.18 ± 4.77 ab	38.71 ± 2.28 def	2.05 ± 0.14 efg
M × O	3.40 ± 0.28 c	6.62 ± 0.11 c	34.10 ± 1.91 ab	32.78 ± 0.63 f	2.05 ± 0.10 efg
M × Mo	3.41 ± 0.23 c	7.87 ± 0.09 a	37.34 ± 3.15 a	36.93 ± 0.23 ef	3.35 ± 0.39 bc
C × Ch	3.53 ± 0.17 c	tr	23.62 ± 1.61 c	95.27 ± 0.69 c	1.68 ± 0.20 fg
C × O	3.75 ± 0.43 bc	tr	34.25 ± 1.68 ab	116.06 ± 0.23 b	2.80± 0.16 cde
C × Mo	5.19 ± 0.63 a	tr	39.49 ± 4.58 a	130.90 ± 0.61 a	2.98 ± 0.17 cd
S × Ch	4.97 ± 0.28 ab	tr	6.52 ± 0.79 d	10.36 ± 0.09 h	2.05 ± 0.04 efg
S × O	4.75 ± 0.28 ab	tr	8.05 ± 0.46 d	14.51 ± 0.24 gh	1.30 ± 0.01 g
S × Mo	4.75 ± 0.09 ab	tr	10.36 ± 0.61 d	20.30 ± 0.23 g	2.42 ± 0.02 def

Within each column, values associated with the same lowercase letters are not statistically different at $p < 0.05$ according to Duncan's test; tr—traces; AB—'Aromat de Buzau'; M—'Macedon'; C—'Cuisoare'; S—'Serafim'; Ch—chemical; O—organic; Mo—microorganisms.

As shown in Figure 1, the basil cultivar 'Macedon' under microorganism treatment had the highest oil content (0.22%), though not significantly different from the chemical and organic fertilization, while the organically fertilized 'Serafim' accumulated the lowest oil amount (0.07%). Generally, the organic fertilization resulted in a lower oil content compared to the other two fertilization types.

To highlight the correlations and interactions between the experimental factors and the variables examined, a PCA was performed. The two principal components shown in the biplot graph (Figure 2) overall contributed to 72.7% of the total variability (48.48% and 24.22% for PC1 and PC2, respectively).

The extracted eigenvectors' values highlight that both the cultivars and fertilization types are based on PC1, in different ways. The cultivars 'Aromat de Buzau' and 'Macedon' have positive values, while 'Serafim' has a negative value and 'Cuisoare' is near the origin (Figure 2).

The results showed that the cultivar 'Serafim' was closely connected with the leaf area index, and 'Macedon' was connected to the other morphological variables analyzed. The leaves' weight was influenced by chemical fertilization in the cultivars 'Cuisoare' and 'Serafim' and by microorganism fertilization in 'Cuisoare'. The other yield parameters were affected by both chemical and organic fertilizations in the cultivar 'Macedon'.

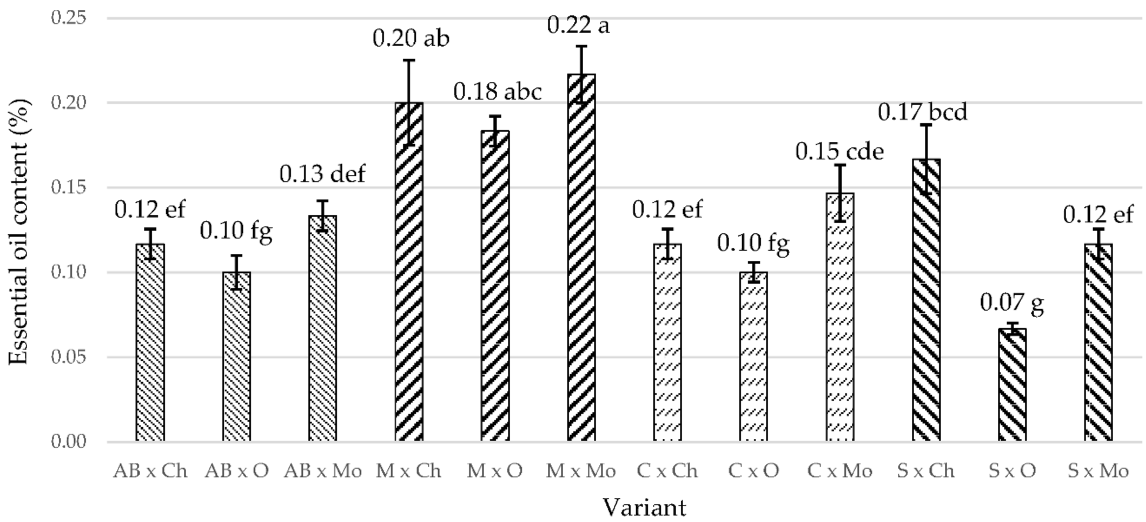

Figure 1. Interaction effects of cultivar and fertilization on essential oil content. Values associated with the same lowercase letters are not statistically different at $p < 0.05$ according to Duncan's test. AB—'Aromat de Buzau'; M—'Macedon'; C—'Cuisoare'; S—'Serafim'; Ch—chemical; O—organic; Mo—microorganisms.

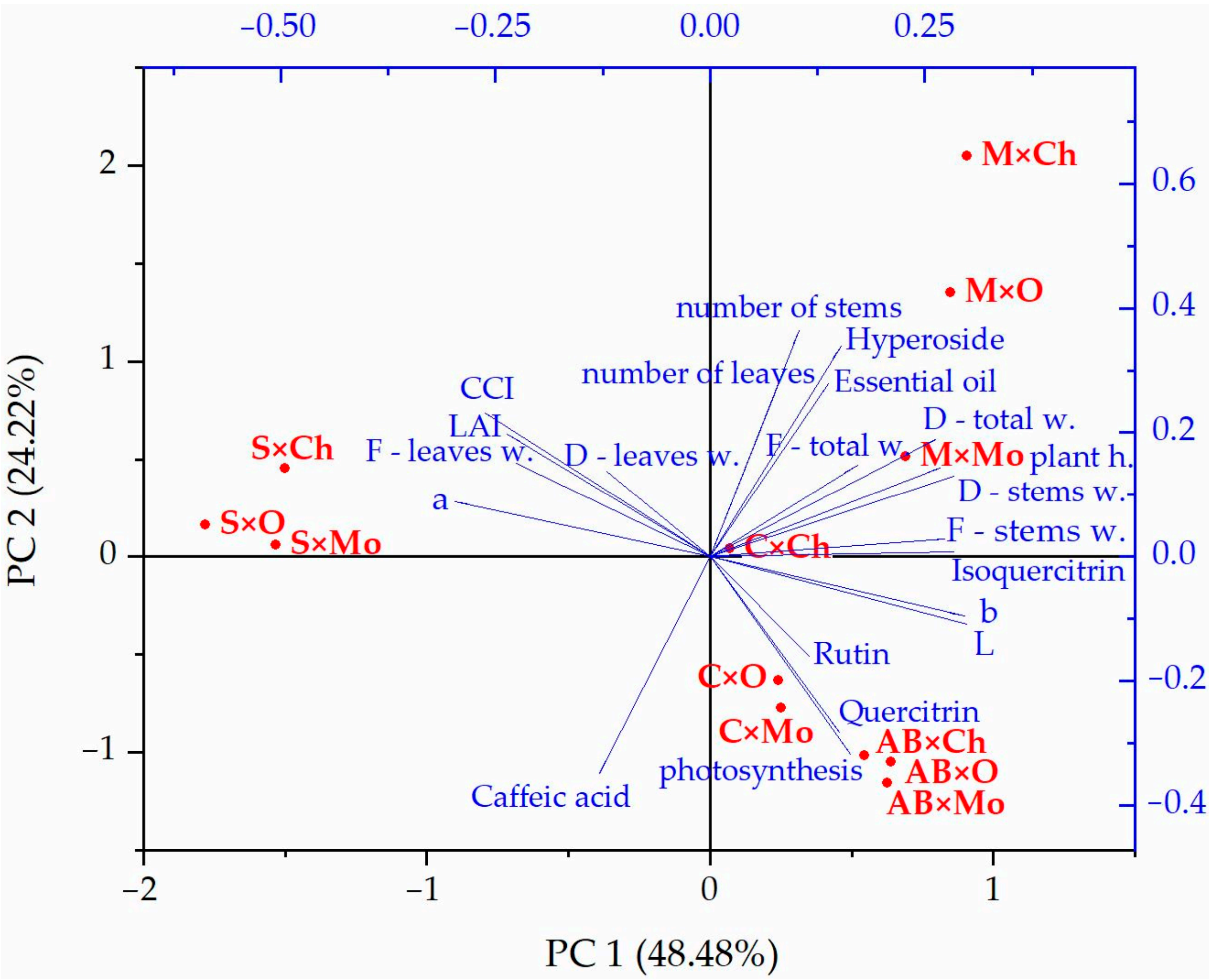

Figure 2. The 2D principal subspace for different cultivars of basil and fertilization treatments. With red color are the experimental factors (Cultivar × Fertilization type), and with blue color are the parameters and bioactive compounds analyzed at sweet basil.

The leaves of 'Serafim' depend on the Chlorophyll Content Index (CCI), especially in the red band, and 'Aromat de Buzau' is sensitive to the photosynthesis process (Figure 2).

The effects of the fertilization types on the evaluated variables were analyzed separately, and the results showed that the chemical fertilization influenced the CCI, number of leaves, plant height and all yield parameters, whereas fertilization with microorganisms increased the number of stems.

The PCA identified that phenolic compounds were specifically connected with the cultivar; hyperoside was found in 'Macedon', quercitrin in 'Aromat de Buzau' and rutin, isoquercitrin and quercitrin in 'Cuisoare'.

The statistical analysis indicated that the data obtained for all cultivars were clustered on the 2D PC diagram, which proves that the analyzed variables did not depend on the fertilization type, but only on the cultivar.

The cultivar 'Serafim' showed different characteristics compared to the other cultivars, which suggests that it is a cultivar with exclusive properties. These differences could be explained by the fact that 'Serafim' is a cultivar with purple leaves, thus having a higher pigment content due to the presence of anthocyanins compared to the cultivars with green leaves, where they were not detected. The correlation matrix between the analyzed variables of basil supports the mentioned findings, and the complete description of these variables is presented in the supplementary materials (Supplementary Table S1).

In the cultivar 'Aromat de Buzau', 30 compounds were identified in the essential oil (Table 9). Methyl-chavicol was found in the largest amount with values between 42.99% under the chemical fertilization up to 49.29% in the case of microorganism fertilization. The next compound found in large amount was β-linalool, with values between 13.07% with the chemical fertilization and 25.16% with the organic one. Other important compounds detected were β-elemene, germacrene D and epi-α-cadinol.

Table 9. Influence of fertilization on essential oil composition of 'Aromat de Buzau' (%).

No	Name	Class	RI_{calc}	RI_{lit}	Chemical	Organic	Microorganisms
1	Eucalyptol (Cineole)	Oxygenated monoterpenes	1031	1030	tr	0.25	0.21
2	cis-β-Ocimene	Monoterpene hydrocarbons	1040	1037	tr	0.24	0.11
3	β-Linalool	Oxygenated monoterpenes	1095	1096	13.07	25.16	22.84
4	Cis-thujone	Oxygenated monoterpenes	1101	1102	0.19	0.12	0.11
5	Trans-thujone	Oxygenated monoterpenes	1112	1114	tr	0.09	0.18
6	(Z)-β-Ocimene oxide	Oxygenated monoterpenes	1128	1132	tr	0.35	0.15
7	Camphor	Oxygenated monoterpenes	1141	1145	0.45	1.01	0.70
8	Methyl chavicol	Phenylpropanoids	1195	1196	42.95	47.57	49.29
9	Bornyl acetate	Oxygenated monoterpenes	1284	1285	1.36	0.70	0.58
10	Trans-linalool oxide acetate	Oxygenated monoterpenes	1287	1288	0.73	0.20	0.34
11	Neryl acetate	Oxygenated monoterpenes	1359	1361	0.27	0.14	tr
12	Geranyl acetate	Oxygenated monoterpenes	1379	1381	tr	0.11	tr
13	β-Elemene	Sesquiterpene hydrocarbons	1389	1390	7.31	3.47	2.89
14	Methyl eugenol	Phenylpropanoids	1402	1403	2.19	0.44	0.64
15	β-Caryophyllene	Sesquiterpene hydrocarbons	1417	1419	0.48	0.40	0.35
16	α-Guaiene	Sesquiterpene hydrocarbons	1436	1439	1.62	0.72	0.71
17	cis-Muurola-3,5-diene	Sesquiterpene hydrocarbons	1448	1450	0.36	tr	tr
18	trans-Muurola-3,5-diene	Sesquiterpene hydrocarbons	1451	1453	0.20	tr	tr
19	Humulene (α-Caryophyllene)	Sesquiterpene hydrocarbons	1454	1454	1.22	0.34	0.35
20	trans-Muurola-4(14),5-diene	Sesquiterpene hydrocarbons	1465	1466	0.60	0.12	0.21
21	Germacrene D	Sesquiterpene hydrocarbons	1481	1481	5.87	3.60	3.16
22	Bicyclogermacrene	Sesquiterpene hydrocarbons	1500	1501	1.81	0.48	0.57
23	α-Bulnesene	Oxygenated sesquiterpenes	1510	1509	3.26	1.38	1.10
24	γ-Cadinene	Sesquiterpene hydrocarbons	1513	1513	2.21	1.35	1.47
25	cis-Muurol-5-en-4-β-ol	Oxygenated sesquiterpenes	1551	1552	0.57	0.23	0.12
26	Elemicin	Phenylpropanoids	1555	1557	2.14	0.71	0.79
27	cis-Muurol-5-en-4-α-ol	Oxygenated sesquiterpenes	1559	1561	3.06	4.76	6.58

Table 9. *Cont.*

No	Name	Class	RI_{calc}	RI_{lit}	Chemical	Organic	Microorganisms
28	1,10-di-epi-Cubenol	Oxygenated sesquiterpenes	1618	1619	0.91	0.40	0.35
29	1-epi-Cubenol	Oxygenated sesquiterpenes	1627	1628	1.76	1.38	0.81
30	epi-α-Cadinol	Oxygenated sesquiterpenes	1638	1640	4.40	3.27	4.39
						tr ≥ 0.03	
		Monoterpene hydrocarbons			tr	0.24	0.11
		Oxygenated monoterpenes			16.06	28.13	25.10
		Phenylpropanoids			47.27	48.72	50.72
		Sesquiterpene hydrocarbons			21.67	10.48	9.72
		Oxygenated sesquiterpenes			13.96	11.43	13.35

RIcalc—calculated Kovats index; RIlit—Kovats Index by literature data [27]; tr—traces.

In the cultivar 'Macedon', 26 compounds were identified in the essential oil (Table 10). Geranial was found in the largest amount with values ranging from 26.19% in the case of chemical fertilization up to 32.20% under organic fertilization. The next compound found in large amounts was neral, with values between 20.52% with the chemical fertilization and 25.94% with the organic one. Other compounds detected in remarkable amounts were nerol, β-caryophyllene and (E)-γ-bisabolene.

Table 10. Influence of fertilization on essential oil composition of 'Macedon' (%).

No	Name	Class	RI_{calc}	RI_{lit}	Chemical	Organic	Microorganisms
1	cis-β-Ocimene	Monoterpene hydrocarbons	1041	1037	0.18	0.33	0.29
2	β-Linalool	Oxygenated monoterpenes	1095	1096	1.16	0.80	1.90
3	cis-Thujone	Oxygenated monoterpenes	1101	1102	tr	0.15	tr
4	trans-Thujone	Oxygenated monoterpenes	1112	1114	0.17	0.17	0.40
5	Camphor	Oxygenated monoterpenes	1141	1145	0.35	tr	0.29
6	(Z)-Isocitral	Oxygenated monoterpenes	1163	1164	0.94	1.09	0.91
7	(E)- Isocitral	Oxygenated monoterpenes	1179	1180	1.29	1.45	1.20
8	Methyl chavicol	Phenylpropanoids	1195	1196	0.56	0.42	1.07
9	Nerol	Oxygenated monoterpenes	1227	1229	12.19	11.27	8.86
10	Neral	Oxygenated monoterpenes	1235	1238	20.52	25.94	24.34
11	Geraniol	Oxygenated monoterpenes	1251	1252	3.18	2.86	2.31
12	Geranial	Oxygenated monoterpenes	1265	1267	26.19	32.20	29.36
13	Neryl acetate	Oxygenated monoterpenes	1359	1361	1.71	1.28	1.19
14	α-Copaene	Sesquiterpene hydrocarbons	1375	1376	0.48	0.42	0.39
15	Geranyl acetate	Oxygenated monoterpenes	1379	1381	tr	0.27	tr
16	β-Elemene	Sesquiterpene hydrocarbons	1389	1390	0.00	0.32	tr
17	Methyl eugenol	Phenylpropanoids	1403	1403	0.73	0.50	0.61
18	β-Caryophyllene	Sesquiterpene hydrocarbons	1417	1419	10.03	6.16	8.73
19	α-trans-Bergamotene	Sesquiterpene hydrocarbons	1433	1434	3.02	1.83	2.52
20	Humulene (α-Caryophyllene)	Sesquiterpene hydrocarbons	1453	1454	1.69	0.97	1.42
21	(E)-β-Farnesene	Sesquiterpene hydrocarbons	1455	1456	1.46	0.98	1.33
22	Sesquisabinene	Sesquiterpene hydrocarbons	1457	1459	0.23	0.20	tr
23	Germacrene D	Sesquiterpene hydrocarbons	1481	1481	2.20	1.35	2.18
24	(Z)-γ-Bisabolene	Sesquiterpene hydrocarbons	1514	1515	0.43	0.42	0.37
25	(E)-γ-Bisabolene	Sesquiterpene hydrocarbons	1528	1530	9.28	6.65	8.34
26	epi-α-Cadinol	Oxygenated sesquiterpenes	1638	1640	tr	0.21	tr
						tr ≥ 0.03	
		Monoterpene hydrocarbons			0.18	0.33	0.29
		Oxygenated monoterpenes			67.71	77.47	70.75
		Phenylpropanoids			1.29	0.92	1.68
		Sesquiterpene hydrocarbons			28.82	19.29	25.28
		Oxygenated sesquiterpenes			tr	0.21	tr

RIcalc—calculated Kovats index; RIlit—Kovats index by literature data [27]; tr—traces.

In the cultivar ‘Cuisoare’, 36 compounds were identified in the essential oil (Table 11). β-Linalool was found in the largest amount with values from 30.42% when applying microorganisms up to 40.17% in the case of chemical fertilization. The next important compound was epi-α-cadinol, with values between 9.92% with the chemical fertilization and 13.52% under the microorganism formulation. Other important compounds were eugenol, α-trans-bergamotene, γ-cadinene and germacrene D.

Table 11. Influence of fertilization on essential oil composition of ‘Cuisoare’ (%).

No	Name	Class	RI_{calc}	RI_{lit}	Chemical	Organic	Microorganisms
1	Sabinene	Monoterpene hydrocarbons	969	974	0.09	0.06	tr
2	Sylvestrene	Monoterpene hydrocarbons	1026	1030	0.12	0.11	0.14
3	Eucalyptol (1,8-Cineole)	Oxygenated monoterpenes	1031	1030	2.62	2.32	1.28
4	cis-β-Ocimene	Monoterpene hydrocarbons	1041	1037	0.47	0.69	0.42
5	Terpinolene	Monoterpene hydrocarbons	1086	1088	0.20	0.09	0.14
6	β-Linalool	Oxygenated monoterpenes	1095	1096	40.17	37.52	30.42
7	cis-Thujone	Oxygenated monoterpenes	1101	1102	tr	0.16	0.18
8	trans-Thujone	Oxygenated monoterpenes	1112	1114	tr	0.08	0.11
9	(Z)-β-Ocimene oxide	Oxygenated monoterpenes	1128	1132	0.63	0.58	0.32
10	Camphor	Oxygenated monoterpenes	1141	1145	0.54	0.43	0.46
11	α-Terpineol	Oxygenated monoterpenes	1188	1188	0.97	1.07	tr
12	Methyl chavicol	Phenylpropanoids	1195	1196	tr	1.05	1.14
13	cis-Carveol	Oxygenated monoterpenes	1229	1229	0.14	0.33	0.29
14	Geranial	Oxygenated monoterpenes	1266	1267	0.18	0.42	0.38
15	Bornyl acetate	Oxygenated monoterpenes	1284	1285	3.63	1.59	2.23
16	trans-Linalool oxide acetate	Oxygenated monoterpenes	1287	1288	0.12	0.19	0.24
17	Eugenol	Phenylpropanoids	1356	1358	9.93	11.06	8.88
18	α-Copaene	Sesquiterpene hydrocarbons	1375	1376	tr	0.21	0.16
19	β-Elemene	Sesquiterpene hydrocarbons	1389	1390	4.38	5.37	6.10
20	Methyl eugenol	Phenylpropanoids	1403	1403	0.30	0.50	0.65
21	β-Caryophyllene	Sesquiterpene hydrocarbons	1417	1419	0.29	0.40	0.36
22	α-trans-Bergamotene	Sesquiterpene hydrocarbons	1433	1434	5.03	5.33	8.12
23	α-Guaiene	Sesquiterpene hydrocarbons	1436	1439	1.29	1.16	1.42
24	cis-Muurola-3,5-diene	Sesquiterpene hydrocarbons	1448	1450	0.36	0.40	tr
25	trans-Muurola-3,5-diene	Sesquiterpene hydrocarbons	1451	1452	tr	tr	0.48
26	Humulene (α-Caryophyllene)	Sesquiterpene hydrocarbons	1453	1454	1.02	0.96	1.25
27	trans-Muurola-4(14),5-diene	Sesquiterpene hydrocarbons	1466	1466	0.59	0.64	0.79
28	Germacrene D	Sesquiterpene hydrocarbons	1481	1481	5.94	6.26	6.85
29	Bicyclogermacrene	Sesquiterpene hydrocarbons	1500	1501	0.70	0.82	1.01
30	α-Bulnesene	Sesquiterpene hydrocarbons	1509	1509	1.99	2.09	2.68
31	γ-Cadinene	Sesquiterpene hydrocarbons	1513	1513	3.50	3.48	4.48
32	β-Sesquiphellandrene	Sesquiterpene hydrocarbons	1522	1522	0.25	0.25	0.41
33	trans-Nerolidol	Sesquiterpene hydrocarbons	1561	1563	tr	0.20	0.18
34	5-epi-7-epi-α-Eudesmol	Oxygenated sesquiterpenes	1605	1607	1.97	0.98	1.58
35	1,10-di-epi-Cubenol	Oxygenated sesquiterpenes	1618	1628	1.34	1.44	1.75
36	epi-α-Cadinol	Oxygenated sesquiterpenes	1638	1640	9.92	10.51	13.52
						tr ≥ 0.03	
		Monoterpene hydrocarbons			0.89	0.95	0.70
		Oxygenated monoterpenes			49.01	44.68	35.91
		Phenylpropanoids			10.23	12.61	10.67
		Sesquiterpene hydrocarbons			25.33	27.57	34.28
		Oxygenated sesquiterpenes			13.23	12.92	16.85

RIcalc—calculated Kovats index; RIlit—Kovats index by literature data [27]; tr—traces.

In the cultivar ‘Serafim’, 30 compounds were identified in the essential oil (Table 12). β-Linalool was found in the largest amount with values from 49.52% when applying the microorganism treatment up to 60.80% in the case of organic fertilization. The next important compound was eugenol, with values between 6.81% under the organic fertilization and 10.37% with the microorganism application. Other main compounds were β-elemene, germacrene D, camphor, α-trans-bergamotene, γ-cadinene and α-guaiene.

Table 12. Influence of fertilization on essential oil composition of 'Serafim' (%).

No	Name	Class	RI_{calc}	RI_{lit}	Chemical	Organic	Microorganisms
1	α-Pinene	Monoterpene hydrocarbons	932	939	0.19	0.21	0.07
2	Sabinene	Monoterpene hydrocarbons	969	974	0.24	0.25	0.13
3	β-Myrcene	Monoterpene hydrocarbons	988	990	0.31	0.33	tr
4	Limonene	Monoterpene hydrocarbons	1024	1028	0.34	0.35	0.21
5	Eucalyptol (1,8-Cineole)	Oxygenated monoterpenes	1031	1030	0.62	0.65	3.95
6	cis-β-Ocimene	Monoterpene hydrocarbons	1041	1037	0.41	0.43	tr
7	Fenchone	Oxygenated monoterpenes	1083	1085	0.34	0.35	0.18
8	Terpinolene	Monoterpene hydrocarbons	1086	1088	0.24	0.25	tr
9	β-Linalool	Monoterpene hydrocarbons	1095	1096	57.49	60.80	49.52
10	Camphor	Oxygenated monoterpenes	1141	1145	1.77	1.87	1.19
11	α-Terpineol	Oxygenated monoterpenes	1188	1188	1.43	1.51	1.22
12	endo-Fenchyl acetate	Oxygenated monoterpenes	220	1221	0.33	0.35	0.37
13	cis-Carveol	Oxygenated monoterpenes	1229	1229	0.26	0.27	0.20
14	Geranial	Oxygenated monoterpenes	1266	1267	0.35	0.37	0.28
15	Bornyl acetate	Oxygenated monoterpenes	1254	1285	0.43	0.45	0.59
16	Eugenol	Phenylpropanoids	1356	1358	8.34	6.81	10.37
17	α-Copaene	Sesquiterpene hydrocarbons	1375	1376	0.20	0.21	0.24
18	β-Elemene	Sesquiterpene hydrocarbons	1389	1390	6.55	6.92	7.78
19	Methyl eugenol	Phenylpropanoids	1403	1403	0.33	0.75	0.02
20	β-Caryophyllene	Sesquiterpene hydrocarbons	1417	1419	1.55	1.33	1.41
21	α-trans-Bergamotene	Sesquiterpene hydrocarbons	1433	1434	0.60	0.64	1.70
22	α-Guaiene	Sesquiterpene hydrocarbons	1436	1439	1.55	1.64	1.90
23	Humulene (α-Caryophyllene)	Sesquiterpene hydrocarbons	1454	1454	0.00	0.00	0.57
24	Germacrene D	Sesquiterpene hydrocarbons	1481	1481	5.24	4.54	6.23
25	β-Selinene	Sesquiterpene hydrocarbons	1489	1490	0.26	0.28	0.45
26	Bicyclogermacrene	Sesquiterpene hydrocarbons	1500	1501	tr	0.08	0.68
27	α-Bulnesene	Sesquiterpene hydrocarbons	1509	1509	2.79	1.95	3.44
28	γ-Cadinene	Sesquiterpene hydrocarbons	1513	1513	1.37	1.45	1.82
29	1,10-di-epi-Cubenol	Oxygenated sesquiterpenes	1618	1628	0.50	0.53	0.72
30	epi-α-Cadinol	Oxygenated sesquiterpenes	1638	1640	3.81	3.02	2.99
						tr ≥ 0.03	
		Monoterpene hydrocarbons			59.21	62.63	49.93
		Oxygenated monoterpenes			5.53	5.84	7.99
		Phenylpropanoids			8.67	7.57	10.40
		Sesquiterpene hydrocarbons			20.13	19.04	26.21
		Oxygenated sesquiterpenes			4.31	3.55	3.72

RIcalc—calculated Kovats index; RIlit—Kovats index by literature data [27]; tr—traces.

4. Discussion

Currently, the consumer interest towards healthy foods rich in bioactive compounds has increased [38–40]. A strategy to increase these compounds is the application of farming management able to ensure a balance between the quantity and quality of agricultural products [41,42]. The aim of this study was to evaluate the effect of the interaction between cultivar and fertilization type on the morphology, physiology and synthesis of bioactive compounds in basil cultivated in the field. To this end, four basil cultivars ('Aromat de Buzau', 'Macedon', 'Cuisoare' and 'Serafim') were evaluated in combination with three types of fertilization, i.e., chemical fertilization (with a solid chemical fertilizer), organic fertilization (with a chicken manure formulate) and microorganism fertilization (with a microorganisms formulate). As expected, the morphological parameters (plant height, number of stems and leaves and leaf area) were significantly influenced by the cultivar, with 'Macedon' showing the highest plant height (64.67 cm) and number of stems (20.33) and leaves (618.33) and 'Serafim' showing the largest leaf area (4901.67 cm^2 per plant) and the smallest height (39.00 cm). This is due to the great diversity among the existing phenotypically different basil cultivars. For example, Svecova and Neugebauerová [43] investigated 34 cultivars of basil and showed that plant height varied from 143 to 570 mm, while Juskeviciene et al. [44], analyzing ten cultivars of basil, showed that height ranged from 44.0 to 77.6 cm in the greenhouse and from 37.2 to 63.4 cm in the open field. In this study, the fertilization type did not affect the biometric characteristics but significantly influenced the fresh and dry biomass, which attained the highest values with the chemical fertilization. Basil reacts positively to both organic and chemical fertilization. Matlok et al. [45] showed that both plant height and biomass were higher in the Genovese and Violetto cultivars grown on a substrate containing neutral peat (70%), extract of common nettle (10%), horse manure (20%) and organic controlled-release fertilizer Bioilsa N 12.5 compared to plants grown on peat (100%) and under mineral fertilization with ammonium nitrate as a result of the higher nutrient content (N, P, K and Mg) available from horse manure. In a comparison between two basil cultivars, Burducea et al. [46] found that the values of yield and morphological parameters were the highest with chemical fertilization (chemical > AMF > organic > 40 t ha^{-1} biosolids > 20 t ha^{-1} biosolids > control). In the present study, the physiological parameters, the total content of pigments and photosynthesis were significantly affected by the cultivar, thus confirming the results of previous research [13]. The pigment content was stimulated by chemical fertilization and photosynthesis was stimulated by microorganism fertilization. Similarly, photosynthesis and other associated parameters (stomatal conductance and water use efficiency) increased in *Corylus avellana* after inoculation with AMF (*Trichoderma harzianum* and *Glomus intraradices*) [47].

In addition to its use as an aromatic spice within the food and beverage industries, basil is also known as a medicinal plant due to its antimicrobial, antiseptic, antioxidant and anti-inflammatory effects [48]. The chemical compounds which make basil a valuable plant are phenolic compounds, mainly caffeic and rosmarinic acid, rutin and isoquercitrin, and essential oil constituents such as linalool and methyl chavicol [49,50]. Many factors affect the content and composition of phenolic compounds and essential oil profile, such as the cultivar, climate, season, sampling period or plant part used for extraction [51–53]. The fertilization type can also influence the phenolic or essential oil composition [54,55]. For instance, in this study, it was observed that depending on the fertilizer used, the content of each phenolic compound varied. In general, fertilization based on microorganisms enhanced all the phenolic compounds analyzed. For example, the highest value of caffeic acid was recorded in the cultivar 'Cuisoare', and hyperoside and isoquercitrin were accumulated to a more remarkable extent by 'Macedon' and 'Cuisoare' and rutin by 'Cuisoare', while quercetin was accumulated more by 'Aromat de Buzau'. The differences between the cultivars with regard to the phenolic compounds analyzed upon microorganism fertilization suggest the important influence of the genotype on the phenolic profile. The same observation was made by Cruz et al. [56] in a study regarding three basil cultivars and the effect of nitrogen input on different parameters, including phenolic compounds. Additionally, the influence of the cultivar on the synthesis of different phenolic compounds, regardless of the fertilizer applied, was observed for hyperoside, which was detected only in trace amounts in the cultivars 'Aromat de Buzau', 'Cuisoare' and 'Serafim'.

As in the case of phenolic compounds, the fertilization type influenced the qualitative and quantitative composition of the essential oil. For instance, some components were produced only when specific fertilizer types were applied: cis/trans-muurola-3,5-diene in 'Aromat de Buzau' with chemical fertilization; cis-thujone and geranyl acetate in 'Macedon' with organic fertilization; trans-muurola-3,5-diene in 'Cuisoare' with microorganism fertilization; β-myrcene, cis-β-ocimene and terpinolene in 'Serafim' with chemical and organic fertilization (Tables 9–12). As for the essential oil composition, there can be variations depending on the fertilization type but also on the cultivar; β-linalool was produced to the highest extent under organic fertilization in the cultivars 'Aromat de Buzau' and 'Serafim' and with microorganism fertilization in 'Macedon' or chemical fertilization in 'Cuisoare'. Moreover, by analyzing the essential oil composition, it was observed that the main components differed depending on the cultivar; β-linalool and methyl chavicol in 'Aromat de Buzau', neral and geranial in 'Macedon' and β-linalool in 'Cuisoare' and 'Serafim'. The highest values of most of the main components, regardless of the cultivar, were obtained under organic and microorganism fertilization, except β-linalool in 'Cuisoare' which showed the highest content with chemical fertilization. Knowledge of the qualitative and quantitative composition, depending on the fertilization and cultivar, is a very important aspect to obtain essential oil rich in specific important components for different medical purposes. For example, methyl chavicol, which has antioxidant and anti-lipase activities [57], was best produced by the cultivar 'Aromat de Buzau' under microorganism fertilization; β-linalool, with antimicrobial (e.g., *Candida albicans*, *Staphylococcus aureus* and *Escherichia coli*), antioxidant, anti-inflammatory and anticancer activities [58], had the highest content in 'Cuisoare' under chemical fertilization; and neral was only synthesized by 'Macedon', with the most remarkable production under organic fertilization.

The PCA and, more specifically, the eigenvector values related to this study revealed that both cultivar and fertilization are based on PC1, which produced the greatest effect on the parameters examined. PCA is a powerful statistical technique that can highlight, for example, the influence of different fertilization types on plants, as was shown in the case of basil fertilized with biosolids [19] or Chinese chives (*Allium tuberm*) under the action of slow-release fertilizer [59]. The fertilization type—for example, chemical or organic (manure-based)—directly influences the microorganism communities in the substrate and the enzymatic activity in the soil with the role of plant growth stimulation or protection [60]. On the other hand, 'Serafim' showed completely different results compared to the other cultivars, which suggests that it is a cultivar with exclusive properties. 'Serafim' is a purple-leafed cultivar, which makes the pigment content higher than that in green cultivars due to the additional presence of anthocyanin compounds. For example, Šamec et al. [61], through the PCA of the physical, chemical and phytochemical parameters of four cultivars of strawberry, were able to highlight specific cultivar properties by grouping in the left side of the PCA plot the color parameters L* and C* and in the right side the polyphenolic compounds, which indicated that the cultivars with a higher polyphenolic content are darker and more colorful.

5. Conclusions

In this study, the four basil cultivars examined showed different biometrics and growth parameters in terms of plant height, number of stems and leaves, leaf area and dry biomass, whereas the fertilization type only affected the fresh and dry biomass, with the highest amounts obtained with chemical fertilization. Either the cultivar or the fertilization type significantly influenced the physiological parameters, such as the total content of assimilatory pigments and photosynthesis, the phenolic compounds investigated (caffeic acid, hyperoside, isoquercitrin, rutin and quercitrin) and the essential oil composition. Fertilization with microorganisms led to the production of beneficial phenolic compounds and essential oil components in larger amounts compared to organic and chemical fertilization. The latter enhanced the biomass yield, whereas organic fertilization in Serafim elicited a large leaf surface, which is desirable for food or decoration purposes.

In the present research, the genotype proved to be a factor showing a major influence, regardless of the fertilization type, which is essential to achieve specific targets such as a larger amount of a certain component of the essential oil (microorganism fertilization), a higher yield (chemical fertilization) or a larger leaf surface (organic fertilization).

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy12123219/s1, Table S1: The correlation matrix between the analyzed variables of basil.

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