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Sweet Basil (*Ocimum basilicum* L.) Productivity and Raw Material Quality from Organic Cultivation

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Received: 19 April 2019; Accepted: 28 May 2019; Published: 30 May 2019

Abstract: Sweet basil is one of the most important culinary herbs. Currently, its production is carried out mainly in accordance with conventional agriculture. However, its cultivation in organic systems seems to be better adjusted to consumer demands connected with the lack of pesticide residues in foods and their safety. In the present study, two methods of basil cultivation in organic farming system were applied, i.e., in the open air and under foil tunnels. During the experiment, in central European climatic conditions, it was possible to obtain four successive cuts of herb. The herb was subjected to chemical analysis, including determination of the content of essential oil, phenolic compounds, and chlorophylls. Gas chromatography coupled with mass spectrometry (GC–MS) and flame ionization detector (GC-FID) analysis of the essential oil was performed, whereas the fresh herb was subjected to sensory analysis. The cumulative mass of fresh herb was distinctly higher in the cultivation under foil tunnels (44.7 kg·10 m^{-2}) in comparison to the open field (24.7 kg·10 m^{-2}). The content of essential oil, flavonoids, and phenolic acids was also higher in the raw material collected from plants grown under foil tunnels (0.81, 0.36, and 0.78 g·100g^{-1} DW, respectively) than from the open field (0.48, 0.29, and 0.59g·100g^{-1} DW, respectively). In turn, the dominant compound of the essential oil, i.e., linalool, was present in higher amounts in the essential oil obtained from plants cultivated in the open field. The sensory and microbiological quality of herb was comparable for both methods of cultivation. The obtained results indicate that, in central European climatic conditions, it is possible to obtain good-quality yield of basil herb. However, for its better productivity, it seems that cultivation under foil tunnels is preferable.

Keywords: Sweet basil; herb yield; successive harvest; essential oil; linalool; flavonoids; phenolic acids; sensory and microbiological quality

1. Introduction

Sweet basil (*Ocimum basilicum* L.) is one of the most economically important aromatic herbs of the *Lamiaceae* family. The plant originates from southern Asia. Currently, it is cultivated mainly through the Mediterranean regions of Europe, as well as in Asia, and Africa. However, it is also grown in temperate zones. The commercial products obtained from basil are fresh and dry herbs used as seasoning, while its extracts and essential oil are exploited in the food and perfume industries [1].

Medicinal application of the essential oil is also reported [2–4]. The herb contains 0.04%–0.70% essential oil, with linalool, methyl chavicol (syn. estragole), 1,8-cineole, and eugenol as dominant compounds responsible for its specific aroma [1,5–7]. Flavonoids, including quercetin and kaempferol glycosides, as well as phenolic acids with a predominance of caffeic acid, are also present in basil herbs [7]. Among basil cultivars, seven forms were distinguished concerning leaf shape, size, and color, as well as plant height and habit [8]. Numerous types were also described concerning essential oil dominants (chemotypes). Among basil cultivars cultivated in Europe, the most valuable are those rich in linalool and methyl chavicol [9,10]. Linalool is a terpenoid compound that shows antioxidant and antimicrobial activity [11]. In turn, methyl chavicol is a phenylpropene substance (an isomer of anethole) with anti-lipase and anti-inflammatory effects [12,13]. Some toxic activity is related with this compound. Thus, the chemotypes rich in methyl chavicol are not considered appropriate for therapeutic use [13,14].

In Europe, basil is cultivated as a short-lived annual crop. It is very sensitive to low temperatures; thus, in temperate zones, plantations are often established by raising seedlings and then transplanting them into the field, which usually takes place in the second half of May [9]. The plant is also sensitive to water stress. A regular supply of water is necessary to obtain good-quality raw material [15]. However, irrigation, especially via sprinklers, may cause some dangerous diseases and contribute to a decrease in the content of the essential oil. Basil, similarly to other aromatic plants grown for fresh herbs, is mainly cultivated in a system of conventional production, including hydroponics. In such production, application of pesticides against common soil pathogens, such as *Pythium*, *Alternaria*, and *Rhizoctonia*, causing root rot, or other fungi connected with high air humidity, such as *Botrytis*, is necessary [9,16,17]. This may result in the presence of harmful pesticide residues in the resultant fresh herb [18]. Thus, it seems obvious that the cultivation of these plants in an organic production system, is the most desired approach.

As mentioned before, basil is cultivated in many regions of the world. On a larger scale, both for fresh and dry herbs, the plant is grown in the open field, which makes the production cheaper. The advantages of such production include the possibility to cultivate plants over vast areas, easy application of fertilizers or irrigation, and simple, mechanical harvest. On the other hand, in such conditions, it is impossible to control environmental factors, such as light quality, wind, or temperature. In order to produce high-quality crops in organic systems, in a pesticide-free and environmentally friendly manner, a trend to cultivate some species (especially leafy herbs or vegetables) in soil under mesh-protected enclosures recently appeared [19,20]. In this system, to a certain extent, it is possible to control temperature and light or prevent some attacks from insects and fungi. Thus, it may be a promising method to cultivate aromatic herbs originating from the warmer climate in temperate conditions. Until now, comparative studies on both methods of basil cultivation, i.e., in the open field and under mesh-protected enclosures, with the application of organic farming techniques, were carried out mainly in hot regions, such as Mexico, where the enclosures were used mainly for protection from ultraviolet (UV) radiation and pathogens [19].

The aim of our research was to compare the yield and quality of basil herbs cultivated in the open field and under foil tunnels, in an organic farming system, in central European climatic conditions. The evaluation of basil development and productivity was performed in two growing seasons, with four successive herb harvests. The research was carried out mainly on fresh herbs, intended for consumption as a culinary seasoning. Therefore, we primarily assessed the content and composition of the essential oil affecting the aroma of the raw material and its sensory value. Microbiological analysis was also performed, since, in organic farming, where natural fertilizers are used, herb contamination is possible. We also evaluated the content of phenolic compounds (flavonoids and phenolic acids), as well as chlorophyll a and b, which may indicate the physiological condition of the plant, and which, together with the essential oil, determine the biological value of plant raw materials, including the antioxidant activity.

2. Materials and Methods

2.1. Field Experiment and Developmental Observations

The field experiment was established at the Warsaw University of Life Sciences SGGW (WULS-SGGW) experimental station in May 2014 and 2015 (two one-year cycles). In November 2013 and 2014, composted manure (30 t·ha^{-1}) was used on the field to enrich the soil. In spring, the weeds were mechanically destroyed twice, and, one week before planting the seedling, the soil was shredded with a rotary cultivator to a depth of 15 cm. The basil seedlings were produced in the WULS-SGGW greenhouse. The seeds were sown into a substrate composed of organic compost, high peat, and sand (3:1:1). In the phase of single pairs of young leaves, the seedlings were transplanted to a substrate of the same composition, placed in multi-pots. The production of seedlings, including their hardening, took seven weeks. The field experiment was established in the third week of May, using a randomized block design, with three replications. The size of each plot was 10 m^2. The plants were grown with a spacing of 50 × 20 cm. Two variants of cultivation were applied: one group of plants was cultivated in the open field, and the second was cultivated in a tunnel covered with foil. After the seedling was planted, drip lines were laid, allowing regular watering of plants. In the foil tunnel, watering was used in the amount of 150 mm per month, whereas plants grown in the open field, depending on the rainfall, were additionally irrigated to a similar level. Mean temperatures in 2014 and 2015 were recorded (Table 1). Observations concerning plant development and harvest of the herbs were carried out in the first year of cultivation, four times during the vegetation period (every 20 days from the first harvest, as a regrowth), i.e., on 25 June (first cut), 15 July (second cut), 5 August (third cut), and 30 August (fourth cut). The following traits were investigated: plant height (cm), number of shoots per plant, number of internodes per shoot, fresh and dry mass of herbs, and dry mass of leaves, obtained from 10 m^2. The collected herbs were dried at 35 °C. After drying, the leaves were separated from lignifying shoots, weighed, and subjected to chemical analysis.

Table 1. Mean temperatures in the vegetation season of 2014 and 2015 (°C).

Months	2014		2015	
	Open Field	Foil Tunnel	Open Field	Foil Tunnel
May	14	22	13	22
June	16	25	17	26
July	21	29	20	29
August	18	27	22	31
September	15	22	14	21

2.2. Chemical Analysis

Analysis of the total content of essential oil in fresh and air-dried leaves, as well as gas chromatography coupled with mass spectrometry (GC-MS) and flame ionization detector (GC-FID) analysis of the essential oils from fresh herb, was carried out as described earlier by Bączek et al. [21]. The analysis of the total flavonoid (expressed as quercetin equivalent, in g·100 g^{-1}) and phenolic acid content (expressed as caffeic acid equivalent, in g·100 g^{-1}) in air-dried leaves was carried out according to Polish Pharmacopeia 6th edition [22], whereas the content of chlorophyll a and b (in µg·g^{-1}) was assessed using the Lichtenthaler and Wellburn method [23]. All analyses were carried out in triplicate.

2.3. Sensory Analysis

The sensory analysis was carried out according to PN-ISO 11035 and PN-ISO 8589 [24,25] in the sensory laboratory of the Department of Vegetable and Medicinal Plants, WULS-SGGW. A panel of assessors consisting of 15 experts performed the analysis. Quantitative descriptive analysis (QDA) was used. For the analysis, the herbs collected in 2014, from the last term of harvest (fourth cut), were

used. Samples were manually sliced and mixed to obtain a representative sample. Then, 0.5 g of each sample was placed into encoded plastic containers with a capacity of 40 mL, covered from the top to retain the smell. For taste evaluation, potato puree was used as a carrier for basil herbs. In the first step of the evaluation, a "brainstorming" session was organized to select attributes of odor and taste, and to prepare the definitions of each attribute. In the second step of the analysis, randomized, encoded samples were presented to each panelist. Assessment of the intensity of attributes was performed during two independent sessions, using 10-point scales (0 = low intensity, 10 = extreme intensity). The analysis was performed using ANALSENS software (Aspe, Lab, Aswd programs, 6th ed.) developed by CogITos for preparing tests, recording individual evaluations, and statistical processing of the results.

2.4. Microbiological Analysis

2.4.1. Sample Preparation

For the analysis, the herb collected in 2014, from the last term of harvest (fourth cut), was used. Specifically, 25 g of fresh or air-dried basil herb samples were mixed with 225 mL of buffered peptone water (BPW; BTL, Poland) in stomacher bags and homogenized in a stomacher (Seward Stomacher 400 Circulator, UK) for 1 min at 300 units. Then, depending on the microbiological method, the homogenates were decimally diluted in BPW up to 10^{-6}. The preparation of samples for microbiological analysis followed the procedure described in ISO 6887-1:2003 [26].

2.4.2. Mesophilic Aerobic Bacteria, Total Plate Count (TPC)

Homogenates were decimally diluted, and 1 mL of each prepared solution was transferred into the Petri dish and then mixed with 15 mL of plate count agar (PCA; BTL, Poland), in duplicate. The plates were incubated at 30 ± 1 °C for 24–48 h and counted according to ISO 4833-1:2013 [27].

2.4.3. Escherichia Coli

Homogenates were decimal diluted and 1 mL of each prepared solution was transferred into the Petri dish and mixed with 15 mL of Violet Red Bile-MUG Agar (Sigma-Aldrich, Poland), in duplicate. The plates were incubated at 37 °C ± 1 °C for 48 h and counted according to ISO 4832:2006 [28].

2.4.4. Salmonella spp.

Firstly, 25 g of basil was homogenized in 225 mL of BPW and incubated for 1824 h at 37 ± 1 °C. Afterward, 0.1 mL of this suspension was transferred into 10 mL of Rappaport–Vassiliadis medium soya (RVS; BTL, Poland) and incubated at 41.5 ± 0.5 °C for 24 h, and 1 mL of the same suspension was transferred to 10 mL of Muller-Kauffmann tetrathionate-novobiocin broth (MKTTn; Merck, Germany) and incubated at 37 ± 1 °C for 24 h. Afterward, 10 µL of the suspension from RVS and MKTTn was spread onto Petri dishes containing xylose lysine deoxycholate agar (XLD; BTL, Poland) and Hektoen agar (BTL, Poland). The plates were incubated at 37 ± 1 °C for 48 h according to ISO 6579:2002 [29].

2.4.5. Coagulase-Positive Staphylococcus

Firstly, 1 mL of each homogenate was plated onto Baird Parker RPF Agar (BTL, Poland) and incubated under aerobic conditions at 35 ± 1 °C for 24–48 h according to ISO 6888-1:2001/A2:2018-10 [30].

2.4.6. Sulfite-Reducing Clostridium

Firstly, 5 mL of homogenate was transferred into the 50-mL falcon tubes, which were heat-treated in a water bath at 80 °C for 10 min and then immediately cooled in ice. Then, the homogenates decimally diluted and plated onto iron sulfite agar (ISA; Sigma-Aldrich, Poland), in duplicate. After solidifying the medium, 5 mL of ISA was added to create anaerobiosis. The plates were incubated at 30 ± 1 °C for 24–48 h and black spots were counted, according to ISO 15213:2003 [31].

2.4.7. Aerobic Spore-Forming Bacteria and Total Amylolytic Bacteria

Firstly, 5 mL of homogenate was transferred into the 50-mL falcon tubes, which were heat-treated in a water bath at 80 °C for 10 min and then cooled. Then, the homogenates were decimally diluted, and 1 mL of each prepared solution was transferred into the Petri dish and mixed with 15 mL of plate count agar (PCA; BTL, Poland) for total aerobic spore-forming bacteria or with 15 mL of total amylolytic bacteria agar (TABA) supplemented with starch [32], in duplicate. Medium composition was as follows: 5 g·L^{-1} peptone, 3 g·L^{-1} yeast extract, 15 g·L^{-1} agar, and 20 g·L^{-1} soluble starch, pH 6.9. The plates were incubated at 30 ± 1 °C for 48 h. Amylolytic activity of bacteria was observed by the presence of a lysis zone with a halo around the colonies after exposure to Lugol solution [33].

2.4.8. Yeasts and Molds

The homogenates were decimally diluted, and 0.2 mL of each solution was spread on Petri dishes containing 20 mL of dichloran rose-bengal agar base (DRBC; Merck, Germany) for fresh basil herb or dichloran glycerol agar base (DG18; Merck, Germany) for air-dried basil herb, in duplicate. The plates were incubated at 25 ± 1 °C for 72 h for yeast counting and for 120 h for mold counting, according to ISO 21527-1:2008 and ISO 21527-2:2008, respectively [34,35].

2.5. Statistical Analysis

The results were analyzed using Statistica version 13.3 (TIBCO Software) with one-way ANOVA and Tukey's test. They were expressed as means ± standard deviation (SD). Differences between individual means were deemed to be significant at $p < 0.05$.

3. Results and Discussion

3.1. Assessment of Sweet Basil Development

Basil grown in our experiment using organic farming methods developed well, both in the open field and under the foil tunnel. Both cultivation variants, during the period of two months (from the end of June to the end of August), enabled four cuts to be carried out (Table 2). Earlier trials showed that only two–three cuts are possible during one vegetation period [9]. The productivity of plants from our experiment was comparable to or even higher than those cultivated in Greece, where two–four harvests were performed, depending on the cultivation system [5]. In our study, plants grown in the foil tunnel were taller and produced a greater number of shoots per plant than those in the open field (Table 2). Thus, they were characterized by a higher cumulative mass of herb (Table 3).

The fast regrowth of basil herb observed in our experiment could be associated with its high light requirements, and in particular the radiation intensity and length of the day. In the mentioned period, from the end of June until August, in the conditions of central Europe, the highest monthly sum of radiation is noted, and the day reaches lengths of up to 18 hours. It was proven that such a long day is one of the factors accelerating the development of basil [9,36]. Another factor important in basil cultivation is temperature. It is well known that the species is susceptible not only to frost but also to cold (from 2 to 0 °C). According to some chamber experiments, the fastest growth of basil was noted at 27 °C with seven harvests performed [9]. It seems that intense cutting of the herb stimulates plants to regrow and enables quick vegetative shoot production, which was visible especially for plants grown under the foil tunnel. These plants, with a temperature about 4–6 °C higher than in the open field, produced a distinctly higher mass of herb (Table 3).

Table 2. Developmental traits.

		First cut		Second Cut		Third Cut		Fourth Cut		Average
Plant height (cm)	F	25.2 c	±3.6	31.3 b	±4.4	35.0 b	±4.3	40.5 a	±4.1	33.0
	T	50.7 a	±3.1	44.2 b	±3.5	43.5 b	±4.6	46.2 b	±3.8	46.2 *
Number of main shoots per plant	F	2.1 d	±0.3	3.0 c	±0.2	4.4 b	±0.3	9.4 a	±0.4	4.7
	T	2.6 d	±0.3	4.7 c	±0.4	5.9 b	±0.4	11.7 a	±0.6	6.2 *
Number of leaf Whorls per shoot	F	5.0 a	±0.2	4.2 b	±0.3	4.4 b	±0.3	4.2 b	±0.5	4.5
	T	6.9 a	±0.4	5.4 b	±0.4	5.6 b	±0.5	4.6 c	±0.6	5.6 *

F: open field; T: foil tunnel; values marked in rows with different letters differ at $p < 0.05$; * $p < 0.05$ (in column).

Table 3. Yield of raw materials.

		First cut		Second Cut		Third Cut		Fourth Cut		Total
Fresh mass of herbs (kg per 10 m^2)	F	2.2 c	±0.9	6.1 b	±2.2	6.3 b	±2.8	10.1 a	±3.3	24.7
	T	11.4 ab	±4.3	9.5 b	±3.5	9.8 b	±3.7	14.0 a	±4.0	44.7 *
Dry mass of herbs (kg per 10 m^2)	F	0.3 c	±0.1	0.7 b	±0.1	0.6 b	±0.1	1.6 a	±0.2	3.2
	T	1.5 a	±0.3	0.9 b	±0.2	1.0 b	±0.2	1.8 a	±0.2	5.2 *
Dry mass of leaves (kg per 10 m^2)	F	0.2 c	±0.1	0.4 b	±0.1	0.4 b	±0.1	0.9 a	±0.2	1.8
	T	0.9 a	±0.2	0.5 b	±0.2	0.6 b	±0.2	1.1 a	±0.2	3.1 *

F: open field; T: foil tunnel; values marked in rows with different letters differ at $p < 0.05$; * $p < 0.05$ (in column).

3.2. Chemical Analysis of Raw Materials

The total content of essential oil in fresh (0.02–0.17 g·100 g^{-1}) and dry (0.21–1.16 g·100 g^{-1}) basil varied depending on the term of harvest. The highest content, irrespective of cultivation method, was observed in the herb from the last cut, and the lowest was observed from the first cut (Tables 4 and 5). These results are in agreement with the previously published results concerning the accumulation of essential oil in fresh basil collected successively from plants cultivated in a greenhouse in Greece [5]. According to Lemberkovics et al. [37], the essential oil content increases during basil ontogenesis. With regard to cultivation method, differences in the content of essential oil between plants grown in the open field and under the foil tunnel were observed especially concerning air-dried leaves, in which the content was significantly higher in the plants collected from the tunnel, especially in the third and fourth cuts (Table 6). The results obtained in this study indicate that the accumulation of essential oil in basil depends on both plant developmental phase and climatic factors, among which temperature seems to be particularly important.

In total, 24 substances were detected in the essential oil obtained from the fresh herb, and these formed 87.51%–97.68% of the identified fractions (Table 4). Among these, the oxygenated monoterpene fraction formed up to 84.85%, with linalool as a dominant compound. This monoterpene alcohol exhibits antihyperalgesic and antinociceptive activity [38]. Along with methyl chavicol, it is considered the most important sweet basil essential oil component, forming up to 90% of the oil. Both compounds are also related to the fine basil aroma [9]. In our study, linalool content increased in tandem with the subsequent four herbal cuts. In the case of plants cultivated in the open field, the content grew from 53.52% (first cut) to 59.50% (fourth cut), and, in thecase of plants cultivated in the foil tunnel, the content grew from 48.50% (first cut) to 54.01% (fourth cut) (Table 4). The increase in linalool content during successive harvests of the fresh herb was described earlier by Tsasi et al. [5]. However, it was observed for one out of four tested varieties of sweet basil. This phenomenon may, therefore, be related to the specific genotype. Other oxygenated monoterpene compounds, identified in our experiment, such as *trans*-methyl cinnamate and 1,8-cineole, were present in basil essential oil in much lower amounts (from 6.46% to 17.17% and from 5.28% to 13.39%, respectively). Methyl chavicol was also observed only in small quantities (from 0.91% to 1.54%). There was no relationship between term of harvest and the content of these compounds in the analyzed essential oils. According to Lemberkovics et al. [37],

during basil ontogenesis, the composition of essential oil does not fluctuate as clearly as its content. In turn, Klimánková et al. [39] claimed that no distinct differences are observed in basil volatile content and their composition between plants under organic and conventional cultivation. Thus, it seems that the composition is related to the genotype, rather than to external factors.

In recent years, much attention was paid to the phenolic profile of aromatic plants. These substances, revealing wide pro-health or even medicinal activity, play an important role for the plants themselves. It was proven that phenolics, especially flavonoids, protect DNA against UV-B radiation [40] and inactivate free radicals responsible for oxidative stress [41]. They can have the characteristics of signaling molecules affecting the expression of genes responsible for plant defense reactions against stress (e.g., salicylic acid). Some polyphenols show allelopathic activity (e.g., chlorogenic, *p*-coumaric, and vanillic acids), inhibit germination of seeds, or limit the development of other species. They may also play an important role in the interaction between the plant and the pathogen [42].

In our experiment, the total flavonoid and phenolic acid contents were determined. For both cultivation methods, the content of flavonoids was the highest in the herb from the last harvest, whereas the amount of phenolic acids was the highest at the first cut. As for flavonoids, their content increased from the first to the last harvest and was higher in plants grown under the foil tunnel (Table 5). According to Mattila et al. [43], the production of flavonols in plant tissues takes place during daytime and is enhanced by light intensity. This, in turn, is combined with their function in plants as quenchers of singlet oxygen. In experiments on rye, it was also observed that, in the early stage of plant development, a protective role against UV-B is played by some phenolic acids. However, during plant development and acclimation to stress, they are successively replaced in this function by flavonoids [44]. This may explain the pattern of accumulation of phenolics as protective substances in plants from our experiment. It was also described that some flavonoids influence the movement of auxin and, consequently, regulate the development of some organs or even the whole plant [45]. Thus, the increase in the content of flavonoids in basil may be related to its quick regrowth.

It is worth mentioning that the content of phenolics in basil depends on other factors, including the level of nutrient compounds in the soil during development [46], presowing treatment of its seeds [47], or light quality during cultivation [48]. Other type of stress, including water shortage, may also influence the accumulation of phenolics and chlorophyll a and b in basil leaves [15,49]. In our experiment, similar to flavonoids, the content of chlorophyll a and b increased significantly from the first to the last term of harvest. However, their amount was distinctly higher in the herbs collected from plants grown under the foil tunnel than in the open field (Table 5). The content of chlorophyll a and b depends on plant species, light regime and its quality, and mineral availability during growth [50]. This is taken into consideration when assessing the overall plant condition and is especially important for production of plants consumed in fresh form. According to Kopsellet et al. [51], the accumulation of chlorophyll a and b in fresh basil depends on both the genotype used and the method of cultivation, wherein cultivation in the greenhouse, with sphagnum peat moss as a medium, resulted in a lower content of pigments in the herb in comparison to open-field cultivation. In our experiments carried out in accordance with an organic farming system, where the same soil was used in both cultivation methods (open field and foil tunnel), a higher content of chlorophyll a and b was detected in plants cultivated under the foil tunnel (Table 5).

Table 4. The total content (g·100 g^{-1}) and gas chromatography composition (percentage peak areas) of essential oils in the fresh herbs.

No.	Compound	RI [a]	Open Field				Foil Tunnel			
			First Cut	Second Cut	Third Cut	Fourth Cut	First Cut	Second Cut	Third Cut	Fourth Cut
1	α-Pinene	1026	0.30	0.41	0.51	0.25	0.26	0.38	0.51	0.52
2	β-Pinene	1114	0.35	0.34	0.46	0.44	0.53	0.52	0.45	0.51
3	Myrcene	1165	1.01	1.08	0.87	0.98	1.02	0.96	1.05	1.12
4	Limonene	1204	0.32	0.43	0.49	0.26	1.92	0.24	0.39	0.41
5	1,8-Cineole	1212	8.20	7.68	7.98	8.06	7.03	7.19	11.71	11.58
6	γ-Terpinen	1245	1.31	0.83	0.82	1.06	0.80	0.96	1.54	2.03
7	*cis*-Ocimen	1252	0.09	0.12	0.11	0.18	0.15	0.12	0.14	0.21
8	*p*-Cymen	1273	0.10	0.14	0.09	0.16	0.03	0.04	0.03	0.01
9	α-Copaene	1488	0.26	0.13	0.12	0.16	0.13	0.08	0.17	0.15
10	Camphor	1509	0.22	0.12	0.15	0.08	0.16	0.10	0.18	0.11
11	Linalool	1541	53.52	55.24	57.18	59.50	48.50	54.01	52.00	52.68
12	Terpinen-4-ol	1590	4.52	2.06	2.44	1.46	1.33	0.82	2.15	1.53
13	β-Caryophyllene	1595	0.40	0.22	0.35	0.27	1.10	0.56	0.37	0.93
14	Methyl chavicol	1673	1.09	0.98	1.54	1.09	1.45	1.22	1.38	1.19
15	α-Terpineol	1681	3.75	2.72	2.51	2.56	2.86	0.80	0.50	0.83
16	Germacren D	1703	3.24	2.18	1.76	2.34	1.12	0.63	0.94	1.00
17	Nerol	1795	0.20	0.98	0.88	1.20	1.73	0.96	0.31	0.45
18	Geraniol	1826	0.19	0.15	0.18	0.11	0.05	0.04	0.04	0.08
19	Methyl Eugenol	2005	0.65	0.45	0.48	0.42	0.65	0.32	0.64	0.43
20	*trans*-Methyl cinnamate	2076	12.39	11.02	15.23	9.22	13.37	18.08	16.50	10.50
21	Eugenol	2144	5.09	3.88	3.83	3.18	3.08	2.47	5.27	3.33
22	Thymol	2163	0.17	0.20	0.19	0.12	0.22	0.05	0.15	0.09
23	α-Bisabolol	2210	0.19	0.14	0.21	0.13	0.66	0.11	0.12	0.12
24	α-Cadinol	2225	0.12	0.17	0.19	0.15	0.05	0.09	0.08	0.13
	Total		97.68	91.67	98.57	93.38	88.20	90.75	96.62	89.94
	Monoterpene hydrocarbons		3.54	4.02	3.56	3.51	4.05	3.99	4.01	4.20
	Oxygenetaed monoterpenes		84.27	80.92	82.80	84.85	83.47	79.68	80.72	83.78
	Sesquiterpenes hydrocarbons		3.77	3.59	3.73	3.56	3.68	3.48	3.13	3.35
	Oxygenated sesquiterpenes		0.35	0.34	0.40	0.25	0.29	0.36	0.38	0.27
	Essential oil content		0.02 b	0.11 a	0.12 a	0.14 a	0.05 b	0.16 a	0.15 a	0.17 a

Values marked in row with different letters differ at $p < 0.05$. [a] Retention index calculated on polar column.

Table 5. Total content of essential oil, phenolic compounds (g·100 g^{-1}), and chlorophyll (μg·g^{-1}) in the air-dried leaves.

		First Cut		Second Cut		Third Cut		Fourth Cut		Average
Essential oil (g·100 g^{-1})	F	0.21 d	±0.03	0.42 c	±0.05	0.57 b	±0.08	0.72 a	±0.07	0.48
	T	0.30 c	±0.06	0.69 b	±0.08	1.10 a	±0.16	1.16 a	±0.14	0.81 *
Flavonoids (g·100 g^{-1})	F	0.15 c	±0.05	0.26 b	±0.03	0.32 a	±0.05	0.39 a	±0.05	0.29
	T	0.24 b	±0.08	0.29 b	±0.05	0.28 b	±0.06	0.64 a	±0.05	0.36 *
Phenolic acids (g·100 g^{-1})	F	0.90 a	±0.05	0.71 b	±0.12	0.46 c	±0.07	0.28 d	±0.06	0.59
	T	0.98 a	±0.08	0.95 a	±0.20	0.65 b	±0.16	0.55 b	±0.09	0.78 *
Chlorophyll a (μg·g^{-1})	F	1.36 d	±0.09	2.74 c	±0.06	3.97 b	±0.07	4.80 a	±0.12	3.22
	T	2.06 d	±0.12	3.76 c	±0.12	4.54 b	±0.07	4.95 a	±0.10	3.83 *
Chlorophyll b (μg·g^{-1})	F	0.38 d	±0.05	0.75 c	±0.05	1.05 b	±0.12	1.53 a	±0.08	0.93
	T	0.65 c	±0.07	1.19 b	±0.08	1.25 b	±0.09	1.63 a	±0.12	1.18 *

F: open field; T: foil tunnel; values marked in rows with different letters differ at $p < 0.05$; * $p < 0.05$ (in column).

3.3. Sensory Analysis of Fresh Herb

The genotype used in our experiment was represented by a linalool chemotype (Table 4), which influenced the sensory profile of the fresh herb. According to D'Antuono et al. (2007), among the most valuable components of sweet basil essential oil, i.e., linalool, eugenol, and 1,8-cineole, only linalool generated positive acceptance of panelists assessing their odor value. However, the balance between the three compounds plays an important role in the appreciation of the odor of basil essential oil. In our experiment, the general intensity of fresh basil herbs collected from both open field and

tunnel cultivation was similar. The rate of the intensity of perception for most analyzed odor notes, i.e., fresh, sweet, floral, minty, acidic, green, herbal, terpenic, and spicy, was also similar. The distinct differences between herbs obtained from the open air and foil tunnel cultivation concerned basilic, anisic, and spicy odor notes, which were higher for the herb collected from plants cultivated under the foil tunnel (Figure 1, Table 6). Of the 14 taste notes, only two differentiated fresh basil, i.e., basilic and spicy. The intensity of their perception was also higher for the raw material collected from plants grown under the foil tunnel (Figure 2, Table 7). This was probably connected with the higher content of the essential oil in this raw material.

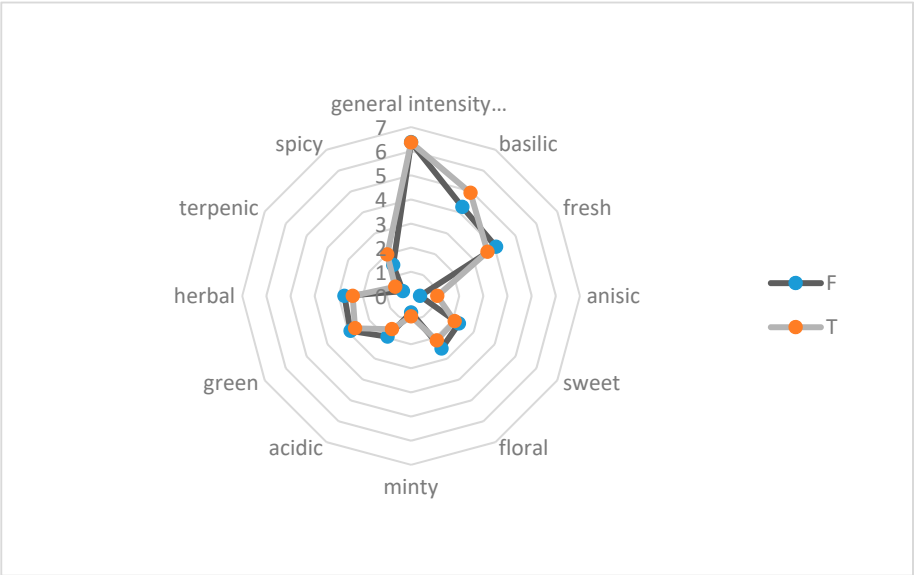

Figure 1. Diagram of sensory profile of fresh herb odor.

Table 6. Odor notes.

	General Intensity of the Odor	Basilic	Fresh	Anisic	Sweet	Floral	Minty	Acidic	Green	Herbal	Terpenic	Spicy
F	6.37	4.26	4.07	0.37	2.29	2.52	0.69	1.95	2.90	2.76	0.39	1.48
T	6.36	4.93 *	3.66	1.09 *	2.09	2.13	0.85	1.59	2.68	2.42	0.76	1.98 *

F: open field; T: foil tunnel; * $p < 0.05$ (in columns).

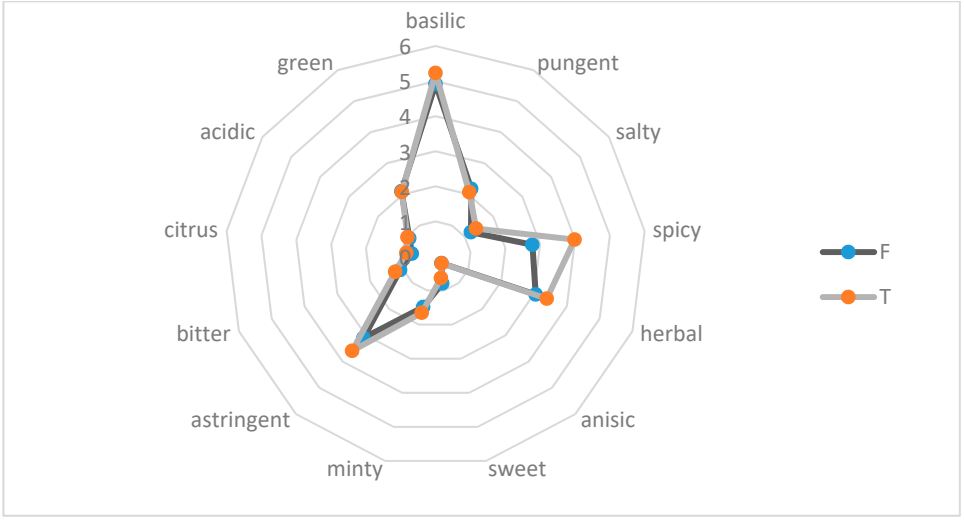

Figure 2. Diagram of sensory profile of fresh herb taste.

Table 7. Taste notes.

	Basilic	Pungent	Salty	Spicy	Herbal	Anisic	Sweet	Minty	Astringen	Bitter	Citrus	Acidic	Green
F	4.92	2.18	1.22	2.78	3.05	0.25	0.79	1.48	3.10	1.08	0.68	0.91	2.10
T	5.23 *	2.07	1.40	3.99 *	3.39	0.26	0.63	1.65	3.59	1.23	0.84	0.97	2.08

F: open field; T: foil tunnel; * $p < 0.05$ (in columns).

3.4. Microbiological Analysis of Fresh and Air-Dried Herb

The microbiological quality of aromatic herbs is determined by the hygienic status and environmental conditions of the region they originate from and where they are pre-treated. The method of cultivation may also influence their microbiological quality, especially when natural fertilizers based on animal excrements are used. Therefore, contaminations with undesirable microflora may occur at each stage of their production process, i.e., during cultivation, harvest, and processing, as well as during storage, distribution, or use by consumers [52]. Table 8 presents the results of microbiological contamination of fresh basil herb and Table 9 presents the results of the air-dried herb. *Salmonella* and coagulase-positive *Staphylococcus* were not found in any of the samples. *E. coli* was present in samples of fresh basil cultivated both in the open field and foil tunnel. However, their number did not exceed 90 cells in 1 g of herb. *E. coli* was not found in the dried basil. The samples of herb collected from plants grown in the open field were characterized by a higher number of mesophilic aerobic bacteria (TPC) of 6.11 $\log_{10}$ colony-forming unit (CFU)·g^{-1}, compared to those from the foil tunnel (5.12 $\log_{10}$ CFU·g^{-1}). The content of mold and yeast in 1 g of fresh basil exceeded 4 $\log_{10}$ CFU. However, a smaller number of fungi was determined in dried samples, and it did not exceed 3.5 $\log_{10}$ CFU·g^{-1}. The contamination of sulfite-reducing *Clostridium* was very low and did not exceed 10^2 CFU·g^{-1}. The content of aerobic spore-forming bacteria was 4.37 to 4.87 $\log_{10}$ CFU·g^{-1} for fresh samples and significantly lower (3.27–4.04 $\log_{10}$ CFU·g^{-1}) for dried ones. In the case of basil cultivated in the open field, a high content of spores (over 56%) consisted of amylolytic bacteria. However, in dried samples, the content decreased to over 11%. The samples of fresh and air-dried basil originating from the foil tunnel contained similar numbers of these bacteria.

European Union (EU) legislation has no definite microbiological standards for herbs [53]. According to the Codex Code of Hygienic practice [54], which states that herbs should not contain any toxin-producing elements, all samples of the analyzed basil samples met the requirements. Specifically, *Salmonella* spp. should be completely absent in 25 g of the sample [55], and the maximum levels of *E. coli* should not exceed 10^2 CFU·g^{-1} [56]. The level of mold contamination of the basil also met the requirements of the EU commission [56]. According to Stankovic et al. [57], the presence of basil herbs next to black pepper (ground and whole corns) and dill available on sale in healthy food stores and supermarkets, is most often microbiologically unacceptable. Most spices are significantly contaminated with spore-forming bacteria of the genus *Bacillus* [53,58]. We showed that the method of cultivation did not influence the content of spore-forming aerobic and anaerobic (sulfite-reducing) bacteria in basil. The factor significantly affecting the majority of microbiological parameters was drying. The air-dried raw materials were free from *E. coli*, which means that the drying method used and proper storage improved their microbial quality. This is important, especially in the context that some of the foodborne pathogenic strains of *E. coli* (EIEC, ETEC, APEC, STEC, and others) indicate a potential health risk associated with contaminated products [59]. Low water activity (a_w) of the air-dried herb is a factor preventing the development of pathogenic or spoilage microflora, both bacteria and mold. In turn, mold growth carries the risk of mycotoxin contamination, which makes the post-harvest treatment of herbs very important.

Table 8. Quantitative microbial contamination of fresh herb; TPC—total plate count; n.d.—not detected; CFU—colony-forming unit.

	Mesophilic Aerobic Bacteria (TPC)	***Escherichiacoli***	***Salmonella* spp.**	**Coagulase-Positive *Staphylococcus***	**Sulfite-Reducing *Clostridium***	**Aerobic Spore-Forming Bacteria/*Bacillus***		**Molds and Yeasts**
						Total Plate Count	**Total Amylolytic Bacteria**	
	CFU·g^{-1}							
Open field	1.29×10^6	9.0×10^1	n.d. in 25 g	n.d. in 0.1 g	6.50×10^1	2.36×10^4	1.23×10^4	2.81×10^4
Foil tunnel	1.33×10^5	9.0×10^1	n.d. in 25 g	n.d. in 0.1 g	3.50×10^1	7.52×10^4	2.90×10^3	1.50×10^4

Table 9. Quantitative microbial contamination of air-dried herb.

	Mesophilic Aerobic Bacteria (TPC)	***Escherichiacoli***	***Salmonella* spp.**	**Coagulase-Positive *Staphylococcus***	**Sulfite-Reducing *Clostridium***	**Aerobic Spore-Forming Bacteria/*Bacillus***		**Molds and Yeasts**
						Total Plate Count	**Total Amylolytic Bacteria**	
	CFU·g^{-1}							
Open field	5.45×10^4	n.d. in 0.1 g	n.d. in 25 g	n.d. in 0.1 g	1.0×10^1	1.85×10^3	2.20×10^2	2.05×10^3
Foil tunnel	2.34×10^5	n.d. in 0.1 g	n.d. in 25 g	n.d. in 0.1 g	1.0×10^1	1.10×10^4	7.14×10^2	3.30×10^3

4. Conclusions

The results presented above indicate that, in central European conditions, it is possible to obtain a high yield of basil herb using an organic farming system. However, the intensity of herb regrowth and its cumulative weight are related to the manner of cultivation. In a system where typical greenhouse production, including hydroponics, is forbidden, cultivation under a foil tunnel in the soil is a promising approach for such a demanding species. In these conditions, a high basil yield was obtained, and this was connected with a higher temperature during its vegetation period compared to open-field cultivation. In these conditions, the herb was characterized by a high quality based on the content of essential oil, phenolic compounds, and chlorophyll a and b. The quality was also related to the sensory value of the herb and its microbiological purity. On the other hand, it is worth noting that cultivation in the open field was also connected to a relatively high yield with the possibility of achieving four cuts. This may be related to high temperature and the monthly sum of radiation during the vegetation period from June to the end of August in our climatic conditions, which changed in recent years. Thus, in accordance with the latest information on the future of agriculture in the context of climatic change [60], the central European region may be an interesting place to cultivate high-temperature-demanding species, including some aromatic plants grown in high amounts in specific areas of southern Europe.

Despite the promising results obtained in this work, they should be verified in production experiments in organic farms. It would also be necessary to verify the suitability of different varieties and forms of basil in this production system.

Author Contributions: K.B. and Z.W. contributed to the experimental design. K.B. carried out developmental observations, collected raw materials, and performed chemical analysis. O.K. carried out GC–FID analysis of the essential oils. K.B. and O.K. performed sensory analysis. M.G. and I.G. carried out microbiological analysis and described the procedure in the manuscript. K.B. and Z.W. drafted the manuscript.

Funding: The study was supported by the Polish Ministry of Agriculture and Rural Development, within the project titled "Practical aspects of organic cultivation of vegetables and herbs under covers or in a greenhouse" (No. HORre-029-27-21/14(29)).

Conflicts of Interest: The authors declare no conflicts of interest.

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