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YIELD AND CONTENTS OF SOME BIOACTIVE COMPONENTS OF BASIL (*Ocimum basilicum* L.) DEPENDING ON TIME OF CUTTING

Teofil Gavrić[1]*, Josip Jurković[1], Saud Hamidović[1], Sanel Haseljić[1], Blažo Lalević[2], Azra Čorbo[1], Muamer Bezdrob[1]

[1]University of Sarajevo, Faculty of Agriculture and Food Sciences, Sarajevo, Bosnia and Herzegovina
[2]University of Belgrade, Faculty of agriculture, Belgrade-Zemun, Serbia

Abstract: Basil (*Occimum basilicum* L.) is an annual plant from Lamiaceae family. It is used as a spice, aromatic and medicinal plant. The yield and quality of basil depends on variety of basil, growing technology and environmental factors such as: temperature, rainfall, photoperiod, relative humidity and irradiance. The aim of this study was to determine the yield and contents of some bioactive phytochemical components in basil (*Ocimum basilicum* var. *genovese*), depending on time of cutting. Field experiments were conducted in Butmir, near Sarajevo. In this research yield of fresh and dry mass, contents of essential oil, total phenols, total flavonoids and antioxidant activity were determined. Experimental results suggested that the time of cutting has a significant impact on yield and bioactive components of basil. The yield of fresh mass ranged from 182.13 g (second cut) to 283.16 g per plant (first cut). Contents of bioactive components were also significantly depending on the time of cutting. High total phenols content, total flavonoids and antioxidant activity in basil were recorded in the second cut.

Keywords: basil, yield, essential oil, total phenols, antioxidant activity

INTRODUCTION

Medicinal and spice plants have attracted human attention since ancient times due to a positive impact on human health. One of the most popular herbs in the world, especially in the Mediterranean region, is basil (*Ocimum basilicum* L.). Because of its popularity, basil has another name like "The Herb of Kings″. Basil is native from Asia to India (Bucktowar et al., 2016) but since then it had been naturalized in many regions of the world (Hiltunen and Holm, 1999; Putievsky & Galambosi,1999). Basil is an annual plant from Lamiaceae family. In the genus Ocimum, there are at least 65 species (Makri & Kintzios, 1998), but more than 150 species according to some authors (Zareen et al., 2014; Filip, 2017; Stanojković-Sebić et al., 2017). However, all species possess a great variation in morphological characteristics, contents and chemical composition of essential oil (Nurzyńska-Wierdak, 2011; Rawat et al., 2016).

Basil has many uses in the household where is most often used as a spice plant. It has a strong spicy aroma and a slightly sour taste that enriches the taste of various dishes (Majkowska-Gadomska et al., 2017). Spice of basil is used to flavor marinades, pastes, soups, sauces, meat and cured meat products, tomatoes, cheeses, canned foods and liquoeurs (Frąszczak et al. 2015; Majkowska-Gadomska et al., 2017). Thanks to a wide range of activities of bioactive components such as antimicrobial, insecticidal, antioxidant, essential basil oil is used in food, pharmaceutical, cosmetic and aromatherapy industries. Essential oil produced from this plant have the ability to inhibit several varieties of pathogenic bacteria (Moghaddam et al., 2011: Gaio et al., 2015) and as such can be used in the food industry as a food preservative (Beatović et al., 2013). Basil has also found its application in traditional and homeopathic medicine. It is believed that its consumption has a positive effect on the treatment of various diseases. Basil has been used as a medicinal plant in the treatment of headaches, coughs, toothaches, diarrhea, acne warts, kidney malfunctions, snake bites, insanity, insect stings, cancer, convulsion, deafness, epilepsy and sore throat (Singh, 1999; Venâncio et al., 2011; Bucktowar et al., 2016; Stanojković-Sebić et al., 2017).

Pharmacological properties of each plant, including basil, depend on various bioactive phytochemical components such as total phenols, total flavonoids, alkaloids, tannins because these components produce specific physiological effects in the human body (Zareen et al., 2014). The content of bioactive phytochemical components in basil depends on the variety, growing conditions, altitude, growth phase during harvest, storage, location of growing and weather conditions (Kruma, 2008; Hussain et al., 2008; Nurzyńska-Wierdak, 2011; Scagel and Lee, 2012).

Sweet basil is grown sporadically as a medicinal, spicy and aromatic plant in Bosnia and Herzegovina. Basil that is grown in these agro-ecological conditions usually has two cuts (harvest) during vegetation. The first cut of basil is made in July, and the second cut is made in September. But there is not enough scientific research on yield and quality (essential oil content, total phenols, total flavonoids and antioxidant activity) of basil grown in B&H. Regarding the preceding facts the main focus of this research is to determine the yield

Correspondence: Teofil Gavrić, University of Sarajevo, Faculty of Agriculture and Food Sciences, Institute of Field crop production, Zmaja od Bosne 8, 71000 Sarajevo, Bosnia and Herzegovina, Tel. +38733225727, email: t.gavric@ppf.unsa.ba

and contents of some bioactive phytochemical components of basil (*Ocimum basilicum* var. genovese) depending on the time of cutting.

MATERIALS AND METHODS

Plants material. In this study, basil was grown in the experimental field of Faculty of Agriculture and Food Sciences Sarajevo. Seedlings of basil (*Ocimum basilicum* L. var. genovese) were planted at 50 cm × 30 cm plant density on May 10, 2017. The average height of basil seedlings was 10 cm.

Time of cutting (Harvesting). The yield and content of the bioactive phytochemical components is determined depending on the time of the cutting. The first time of cuts was made at the beginning of basil flowering (July 13, 2017). After the first cut, basil regenerated and redeveloped the overground mass and again beginning flowering. Second cut was made on September 27, 2017. After cutting the basil samples were weighed and dried in a dark room at room temperature for 20 days.

Determination of total essential oil. Content of essential oil in basil (Basilici herba) was determined by method recommended in the European Pharmacopoeia (2007) and hydrodistillation was made by Clevenger type apparatus. Briefly, 20 grams of the powdered basil (dry mass) and 400 mL of distilled water was added to flask. After hydrodistillation for 180 minutes volume of essential oils were measured.

Preparation of Extract. The extract was prepared as follows: 0.5 g of dried plant material (*Basilici herba*) was added to 50 mL volumetric flask. Volume of flask was made up to mark with 60% ethanol. After cold extraction the whole mixture was filtered and used for analysis.

Determination of total phenols content (TPC). The total phenols content in the extracts of *Ocimum basilicum* was determined by using the modified Folin–Ciocalteu method (Gavrić et al., 2018a). Briefly, 0.5 mL of extract, blank or standard was added to 5 mL volumetric flask. To the volumetric flask, 0.25 mL Folin-Ciocalteu reagent was added and the mixture was allowed to react. After 3 minutes, 0.75 ml of 20% solution of sodium carbonate was added and after that volume of flask was made up to mark with distilled water. After incubation for 120 minutes at room temperature, absorbance was measured at 765 nm using UV-VIS spectrophotometer (Ultrospec 2100 pro). Total phenols content was expressed as mg gallic acid equivalents (GAE)

Determination of total flavonoids contents (TFC). The total flavonoids contents in the extracts was measured using the modified spectrophotometric method (Velić et al., 2011) as follows: 0.5 ml of ethanol extract, blank or standard was added to 5 ml volumetric flask containing 2 ml of distilled water. To the flask, 0.15 ml of 5% $NaNO_2$ was added and after 6th min, 0.15 ml of 10% $AlCl_3$ was added. At 6th min, 1 ml of 1 M NaOH was added and the total volume was made up to 5 ml with distilled water. The solution was mixed and the absorbance was measured at 510 nm. Determination of total flavonoids compounds was calculated from the calibration curve obtained with (+) - catechin, which was used as standard.

Determination of antioxidant activity. Total antioxidant capacity of the extract of basil was measured using FRAP (ferric reducing antioxidant power) method (Benzie, 1996). Briefly, 240 µL of distilled water, 80 µl of extract, 2.080 µl of FRAP reagent (0.3 M acetate buffer: 20 mM $FeCl_3 \times 6\ H_2O$: 10 mM TPTZ = 10:1:1) ratio was added to erlenmeyer flask. The absorbance at 595 nm was measured after a 5 min incubation at 37 ^oC. Determination of total antioxidants was calculated from a calibration curve obtained with an aqueous solution of $FeSO_4 \times 7\ H_2O$, which was used as a standard.

Statistical methods. All experimental measurements were statistically elaborated with the use of variance analysis for statistical level of significance of 0.05. Statistical analyses were made using SPSS 22 software program.

RESULTS AND DISCUSSION

Monthly precipitation amount and average air temperature for the period of the research is presented in Table 1. Analysis of weather conditions were made on the data for the weather station in Sarajevo (FHMZ, 2017). The data in the table show that during the first three months, temperatures were recorded over 20 ^oC. The temperature lower than 20 ^oC was recorded in the last month of research. There was enough rainfall during the research period, except for the third month (august), when 38.7 mm was recorded. It is considered that the optimal temperature for basil growth is between 20 and 30 degrees (Chang et al., 2005; Kumar et al.,2014). From the above data it can be concluded that the basil after the first cut to second cut (from July 13 to September 27) had relatively unfavorable conditions for growth.

The results of the analysis of the influence of time of cutting on the researched properties of basil are presented in Table 2. On the basis of the data presented in the Table, it can be concluded that the yield of fresh mass was statistically significant depending on the time of cutting. Differences in the yield of fresh mass between the different cuts were 64% in favor of the first cut. A similar effect of cutting time was observed when researched yield of dry mass per plant. Basil is a species from the tropical region (Majkowska-Gadomska et al., 2017) and it can achieve high yields only in such or similar weather conditions. In this study, a short cold period was observed during basil cultivation. Namely, one month before second cut, low temperature was observed (Table 1) and that was the main reason why basil grew slower between the first and the second cuts and why basil had lower yields of fresh and dry mass (Table 2). This observation can be confirmed by research by Chang et al. (2005) which found that basil grown at optimal temperature had a higher dry matter content than plants grown at other temperatures. In this research, it was found that the second cut has lower yields compared to the first cut. However, Hassanain and Abdella (2005) and Hassan et al. (2015) proved that basil can achieve a high yield of fresh mass in second cut during optimum weather conditions.

Essential oil extraction results show a certain difference in oil yield between different cuts although there was no significant difference. The essential oil contents in the first cut was 7.30 and the second was 7.16 mL kg^{-1}. Reduction of essential oil in second cut can be caused by temperature stress before second cut. Namely, according to Wogiatz et al. (2011) and Muráriková et al. (2017) production of essential oil and its composition depends on many environmental factors such as temperature and humidity. Chang et al. (2009) also considers that the temperature during growing has a significant effect on the content of essential oil in basil. They found that basil cultivated at low temperatures has a lower essential oil contents, which is consistent with this research.

Tab. 1.

Average monthly air temperature and amount of precipitation for 2017 year (FHMZ, 2017)

Year	**Month**												**Average monthly air temperature / Amount of precipitation**
	I	**II**	**III**	**IV**	**V**	**VI**	**VII**	**VIII**	**IX**	**X**	**XI**	**XII**	
Average monthly air temperature (^oC)	-4.8	5.2	8.5	9.2	15.3	20.3	21.8	22.6	15.5	10.4	5.4	2.2	11.0
Amount of precipitation(mm)	57.9	69.1	43.6	132.4	73.8	55.0	66.5	38.7	93.2	89.3	74.9	142.9	937.3

The phenolics are secondary metabolites in plants and they have an essential function in the protection of plants against high or low temperatures (Gavrić et al. 2018a), UV radiation (Kreft et al., 2003; Germ, 2004), droughts (Lim et al. 2012) and high concentrations in plants point to environmental stress factors (Gavrić et al., 2018b). In this research content of total phenols ranged from 29.51 (first cut) to 36.68 mg GAE g^{-1} (second cut). Based on the above data, it can be concluded that basil grown in stressful conditions during the second part of vegetation (between first and second cuts), and it synthesized an additional amount of phenols to protect its from unfavorable environmental conditions. Total flavonoids are a group of phenols compounds, and it was expected that their content in this study depends on the time of cutting as well as it depends on the total phenols content. Thus, the results show that the total flavonoids contents ranged from a minimum of 27.78 mg (first cut) to maximum 33.79 mg CAE g^{-1} (second cut). Taie et al. (2010) found that basil contains from 39.51 to 65.3 mg GAE g of total phenols, while the total flavonoids contents ranges from 7.03 to 12.86 mg QAE g. Javanmardi et al. (2003) also researched the content of total phenols in basil, and the results obtained showed that the content ranged from 22.9 g to 65.5 mg GAE g^{-1} of dry matter. Based on the above data it can be concluded that the content of total phenols and total flavonoids in the researched basil is high and that it does not vary from the values obtained from other authors. This fact is particularly interesting because basil produced in agroecology conditions of Bosnia and Herzegovina can be used as a valuable source of phenolics compounds that have medicinal effect. Namely, it is believed that consummation of food rich of phenols compounds is considered to relieve and heal diseases such as coronary heart disease (Javanmardi et al., 2003), and cancer (Javanmardi et al., 2003; Kumar et al., 2014), high blood pressure (Miranda et al., 2016), neurodegenerative diseases (Tamuly et al., 2013) and diabetes (Tebogo et al., 2016).

Tab. 2.

Yield and contents of bioactive components

Cuts	**Fresh mass, g per plant**	**Dry mass, g per plant**	**Essential oil, mL kg^{-1}**	**Total phenolics, mg GAE g^{-1}**	**Total flavonoids mg CAE g^{-1}**	**Antioxidant capacity, mM Fe^{2+} / g DM**

First cut	283.16[a]	50.55[a]	7.30[ns]	29.51[b]	6.95[b]	0.44[b]
Second cut	182.13[b]	39.10[b]	7.16[ns]	36.68[a]	8.45[a]	0.48[a]
Average	232.65	44.83	7.23	33.10	7.70	0.46

a and b - Statistically significant difference at the 0.05 level
ns - Statistically not significant difference at the 0.05 level

Basil is a rich natural source of antioxidants capable of neutralizing free radicals which may have negative effects on human health. Majkowska-Gadomska et al. (2017) consider that the high antioxidant value of medicinal herbs is due to the presence of organic acids, vitamin C, provitamin A, phenols compounds and anthocyanins. In this research significant difference in antioxidant potential was recorded in dependent to time of cutting (Table 2). Higher values of antioxidant values were recorded in the second cut and they were due to the higher content of phenols compounds, which was confirmed by correlation analysis (Table 3). Date in Table show a strong correlation between total phenols content and antioxidant capacity (0.840) and total flavonoids content and antioxidant capacity (0.925). The results of this study are consistent with the results of many researchers who have noted a highly significant correlation between the antioxidant capacity, the total phenols content and the total flavonoids (Hasna and Afidah, 2009; Tarchoune et al., 2012; Murtić et al., 2014;).

Date in Table 3 also show a strong correlation between green mass and dry mass (0.953), dry mass and essential oil content (0.731), total phenols and total flavonoids content (0.953).

Tab. 3.

The correlation between researched traits

	Fresh mass	Dry mass	Essential oil	Total phenols	Total flavonoids	Antioxidant capacity
Fresh mass	1	0.953**	0.586*	0.451	0.596	0.746
Dry mass	0.953**	1	0.731**	0.322	0.453	0.647
Essential oil	0.586*	0.731**	1	0.214	0.049	0.243
Total phenolic	0.451	0.322	0.214	1	0.953**	0.840**
Total flavonoids	0.596	0.453	0.049	0.953**	1	0.925**
Antioxidant capacity	0.746*	0.647	0.243	0.840**	0.925**	1

* Correlation is significant at the 0.05 level
** Correlation is significant at the 0.01 level

CONCLUSIONS

The yield and contents bioactive components of basil depended on the time of cutting ie weather conditions during growing. During the first cut, thanks to relatively favorable weather conditions it is noted a significantly higher yield of fresh and dry mass compared to the second cut. Differences in the yield of fresh mass between the different cuts were 64% in favor of the first cut. High content of essential oil (7.30 mL kg^{-1}) was recorded in the first cut thanks to relatively favorable weather conditions.

However, the content of bioactive components (total phenols and total flavonoids) and antioxidant activity were statistically significantly higher during the second cut. In this research was also recorded that high contents of total phenols and total flavonoids contribute to high antioxidant activity.

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