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Original Paper

Impact of biofortification, variety and cutting on chosen qualitative characteristic of basil (*Ocimum basilicum* L.)

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Basil (*Ocimum basilicum* L.) as a member of family *Lamiaceae* becomes favorite part of human healthy diet because of its food taste improvement properties in combination with its powerful antioxidant beneficial effects, confirmed by even more scientific studies. The aim of the paper was oriented to the impact of selenium biofortification on the content of selected qualitative parameters – total polyphenol content (TPC) and antioxidant activity (AOA) – of basil (*Ocimum basilicum* L.) in dependence on grown variety and term of cutting. Field experiment was established in the Botanical garden SAU Nitra in 2014. There were evaluated ´Dark Green´ and ´Red Opal´ varieties in three treatments with four replications. The first treatment was untreated (control). In the second treatment, selenium as sodium selenate was foliar applied at a concentration of 25 mg m^2, double dose 50 mg m^2 was applied in the third treatment. Total polyphenols were determined by the Lachman´s method and expressed as mg of gallic acid equivalent per kg fresh mater. Total antioxidant capacity was measured by the Brand-Williams method using a compound DPPH (2.2-diphenyl-1-pikrylhydrazyl). The selenium biofortification had significant impact on observed characteristics. It caused increasing of AOA values in ´Red Opal´ variety, in the frame of second cutting. Increasing of AOA was noticed after double dose of selenium in case of ´Dark Green´ as well after the first cut. Application of selenium had significant impact on tested level (P <0.001) for ´Dark Green´ variety, where values slowly increased from control treatment, to 1 x Se dose and the highest were after double dose of sodium selenate. From the variety influence point of view, the ´Dark Green´ variety reached the higher values of AOA in comparison with ´Red Opal´. It was also statistically confirmed dependence of variety on TPC content, when ´Red Opal´ reached higher values of TPC in comparison with ´Dark Green´. Term of cutting had significant influence on TPC and AOA according to used statistical analyzes. The values of AOA as well as TPC were higher in case of second cut.

Keywords: basil, *Ocimum basilicum* L., selenium biofortification, polyphenols, antioxidant activity

1. Introduction

Basil (*Ocimum basilicum* L.) as a member of family *Lamiaceae* becomes favorite part of human healthy diet because of its food taste improvement properties in combination with its powerful antioxidant beneficial effects, confirmed by even more scientific studies. A high degree of polymorphism in the genus *Ocimum* determines a large number of subspecies, different varieties and forms producing essential oils with varying chemical composition offering variable level of medicinal potential (Pandey, 2014). Basil possesses central nervous system (CNS) depressant, anticancer, cardiac stimulant, hepatoprotective, hypoglycemic, hypolipidemic, immunomodulator, analgesic, anti-inflammatory, antioxidant, antiulcerogenic, chemomodulatory and larvicidal activities (Mueen, 2015). Basil could be used as an effective source of natural antioxidant and antibacterial additive to protect foods from oxidative damages and foodborne pathogens (Shirazi, 2014). Basil comprises a rich storehouse of phytochemicals including polyphenols, flavonoids and terpenoids which can be exploited as antimicrobials, food preservatives also in some therapeutic purposes (Mishra, 2011). *Ocimum basilicum* L. is the most important species being utilized as a source of essential oil (Said-Al Ahl, 2015). Due to its pleasant aroma and antimicrobial activity, basil essential oil is a major aromatic agent with applications in various industries such as the food, pharmaceutical, cosmetic, and aromatherapy industries (Zheljazkov, 2008). Extracts have more powerful antioxidant activity than the oils (Mastaneh, 2014). In addition to the basic, sensory, role the extract of basil has in the food product, it exerted significant antifungal properties; depending on its concentration (Tanackov, 2011). Purple basil extracts have a higher total phenolic acid content and greater antioxidant activity than do green basil extracts (Brewer, 2011). Total polyphenols and antioxidant activity could be also influenced by biofortification. It is process of breeding nutrients into food crops, provides a comparatively cost-effective, sustainable,

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and long-term means of delivering more micronutrients (Saltzman, 2013). Selenization of basil is possible through foliar Se applications, and Se fortification of herbal crops may provide alternative delivery systems in human diets (Kopsell, 2009). Inside plants, inorganic selenium is converted to low molecular weight amino acids and finally into selenoproteins. These proteins are responsible for most of the physiological functions mediated by selenium such as antioxidative action, redox regulation, immune function etc. (Priyadarsini, 2013). The DPPH, ABTS+, FRAP and H_2O_2 assay indicated that *Ocimum basilicum* L. possessed considerable antioxidant activities (Srivastava, 2012). Hydrodistilled extracts from basil and laurel had the highest antioxidant activities of several herbs (basil, laurel, parsley, juniper, aniseed, fennel, cumin, cardamom, and ginger) (Hinneburg, 2006).

The aim of the paper was to search the impact of selenium biofortification on the content of selected qualitative parameters (total polyphenol content (TPC) and antioxidant activity (AOA) of basil (*Ocimum basilicum* L.) in dependence on grown variety and term of cutting.

2. Material and methods

Field experiment was led in a Botanical garden SAU Nitra in 2014. Area is situated in very warm agro-climatic region, very dry sub-region. The average annual temperature is 10 °C. There were evaluated ´Dark Green´ and ´Red Opal´ varieties in three treatments; each treatment was carried out in quadruplicate. The first treatment was untreated (control). In the second treatment, selenium as sodium selenate was foliar applied at a concentration of 25 mg m2 (Se I), double dose 50 mg m2 (Se II) was applied in the third variant. After drying the whole plant, chopped leaves of basil were used for analyzes.

2.1 Total antioxidant capacity

Total antioxidant capacity was measured by the Brand and Williams et al. (1995) method-using a compound DPPH (2.2-diphenyl-1-pikrylhydrazyl). 2.2-diphenyl-1-pikrylhydrazyl (DPPH˙) was pipetted to cuvette (3.9 m^3), and then the value of absorbance which corresponded to the initial concentration of DPPH˙ solution in time Ao was written. Then 0.1 cm^3 of the followed solution was added and the dependence $A = f(t)$ was immediately started to be measured. The solution in the second cuvette was mixed and its absorbance was measured on 515.6 nm in 1, 5 and 10 minutes by the spectrophotometer Shimadzu UV/VIS-1240. The percentage of inhibition reflects how antioxidant compound are able to remove DPPH˙ radical at the given time. Inhibition was calculated in (%) = (*Ao* - *At* / *Ao*) × 100.

2.2 Total polyphenol content estimation (TPC)

Total polyphenols were determined by the method of Lachman et al. (2003) and expressed as mg of gallic acid equivalent per kg fresh mater. Gallic acid is usually used as a standard unit for phenolic content determination because a wide spectrum of phenolic compounds. The total polyphenol content was estimated using Folin-Ciocalteau assay. The Folin-Ciocalteau phenol reagent was added to a volumetric flask containing 100 mL of extract. The content was mixed and 5 mL of a sodium carbonate solution (20%) was added after 3 min. The volume was adjusted to 50 mL by adding of distilled water. After 2 hours, the samples were centrifuged for 10 min and the absorbance was measured at 765 nm of wave length against blank. The concentration of polyphenols was calculated from a standard curve plotted with known concentration of gallic acid.

2.3 Statistical analyzes

The analysis of variance (ANOVA), the multifactor analysis of variance (MANOVA) and the multiple Range test were done using the Statgraphic Centurion XVII (StatPoint Inc. USA).

3. Results and discussion

Antioxidant activity (AOA) was evaluated according to submitted methods within the frame of two cuts and two basil varieties. According to results from Table 1, the highest value (94.16±0.25%) was reached in case of ´Dark Green´ variety in second cut after double dose of

Table 1 Statistical analysis of antioxidant activity (AOA) in two chosen varieties of basil (*Ocimum basilicum* L.)

AOA	Variant	´Red Opal´in %	´Dark Green´ in %
1th cut	control	91.48±0.38^a	93.72±0.26^a
1th cut	Se I	91.23±0.21^a	93.73±0.18^a
1th cut	Se II	91.39±0.34^a	94.33±0.22^b
2nd cut	control	92.78±0.37^b	94.16±0.25^b
2nd cut	Se I	92.73±0.30^b	94.13±0.14^b
2nd cut	Se II	93.66±0.23^c	93.77±0.33^a

*Means (n = 16 values) ± standard deviation
Column values with different lowercase letters in superscript are significantly different at P <0.001 by Tukey HSD in ANOVA

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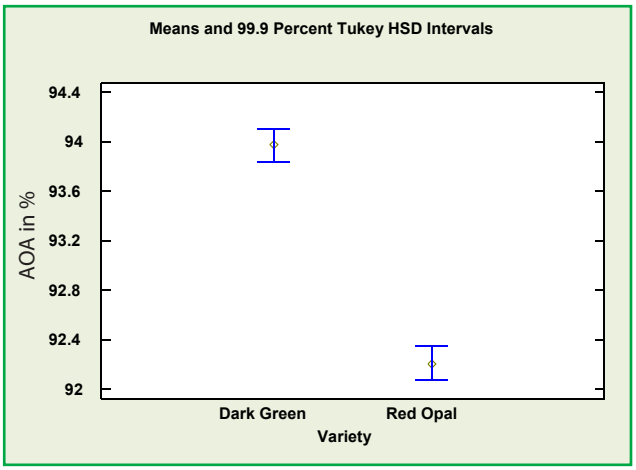

Figure 1 Graphical representation of basil variety influence on antioxidant activity (AOA)

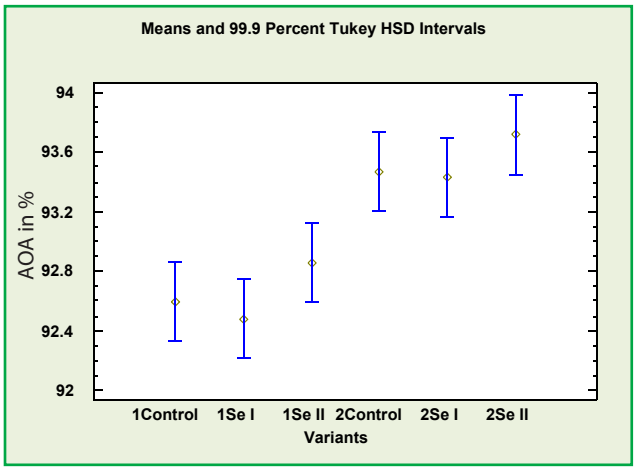

Figure 2 Graphical representation of variant influence on antioxidant activity (AOA)

sodium selenate application, the lowest (91.23±0.21%) for ´Red Opal´ in first cutting with dose of 25 mg m^2 (Se I). Application of selenium had statistically significant impact in ´Red Opal´ variety, in antioxidant activity, in the frame of second cutting, where in control variant there was reached the average value 92.78±0.37% and in case of Se II variant (double selenium application) 93.66±0.23%. Increasing of AOA was noticed after double dose of selenium in case of ´Dark Green´ as well within the frame of first cut. In opposite there was significant difference in AOA values in second cut within the frame of ´Dark Green´ evaluation, but there was AOA decreasing (from 94.16±0.25 for control to 93.77±0.33 for Se II variant).

According to Figure 1 (Graphical representation of basil variety influence on antioxidant activity (AOA)) the ´Dark Green´ variety reached the higher values of AOA in comparison with ´Red Opal´. The results are in opposite with research of Brewer (2011), where purple basil extracts had a greater antioxidant activity than do green basil extracts. Following the Figure 2 (Graphical representation of treatments influence on antioxidant activity (AOA)) statistically significant impact of the cut term was confirmed. The values of AOA were higher in case of second cut. It corresponds with findings of Nicoletto et al. (2013) where in terms of cuts (CT), the AOA increased from CT1 to CT2 (+62.4%), then decreased in CT3. Both varieties of *Ocimum basilicum* L. reached higher values of AOA in comparison with *Ocimum sanctum* in task of Mahajan et al. (2014), where AOA – Methanolic extracts were screened for their possible antioxidant activity by DPPH scavenging assay. In DPPH assay, percentage inhibition in *O. sanctum* leaf extract was in range of 18–76% while in seed extract it was 6–29%. As well as in study of Sarfraz et al. (2011), in which total antioxidant activity of *O. sanctum* seed and leaf extract displayed 84.59 and 79.39% inhibition activities respectively. Srivastava et al. (2012) measured AOA in case of four species by 4 different methods and for *Ocimum basilicum* L. it was 93% according to DPPH methods, which corresponds with our results.

Total phenolic content (TPC) ranged in interval from 3.16±0.62 mg g in ´Dark Green´ to 5.90±0.29 mg g in case of ´Red Opal´ according to results from Table 2. Application of selenium had significant impact on tested level (P <0.001) for ´Dark Green´ variety, where values slowly increased from control treatment, to 1 x Se dose and the highest were after double dose of sodium

Table 2 Statistical analysis of total polyphenol content (TPC) in two chosen varieties of basil (*Ocimum basilicum* L.)

TPC	Treatment	´Red Opal´ in mg g DM	´Dark Green´ in mg g DM
1th cut	control	5.65±0.39bc	3.47±0.74a
1th cut	Se I	5.80±0.32bc	3.16±0.62a
1th cut	Se II	5.90±0.29c	3.27±0.42a
2nd cut	control	5.00±0.55a	5.73±0.63b
2nd cut	Se I	5.29±0.45ab	5.54±0.60b
2nd cut	Se II	5.36±0.36abc	5.25±0.78b

*Means (n = 16 values) ± standard deviation
Column values with different lowercase letters in superscript are significantly different at P <0.001 by Tukey HSD in ANOVA (Statgraphic)

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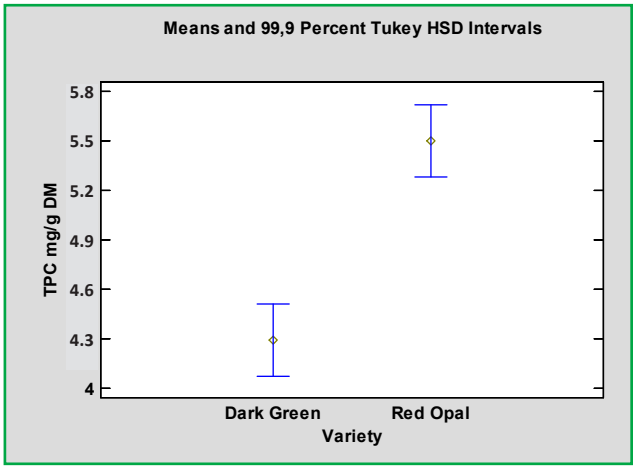

Figure 3 Graphical representation of basil variety influence on total polyphenol content (TPC)

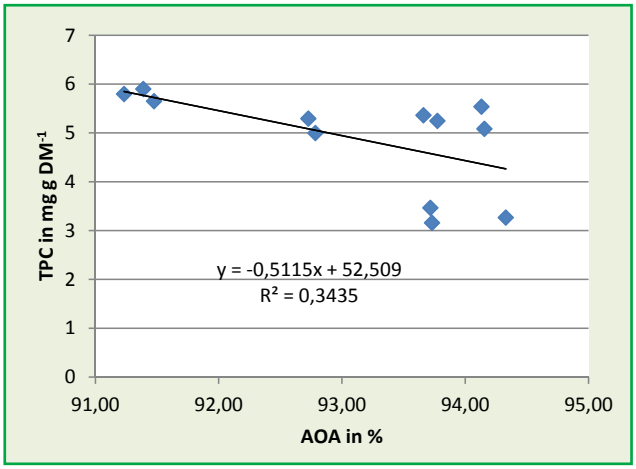

Figure 5 Correlation analyses of TPC and AOA in two basil varieties

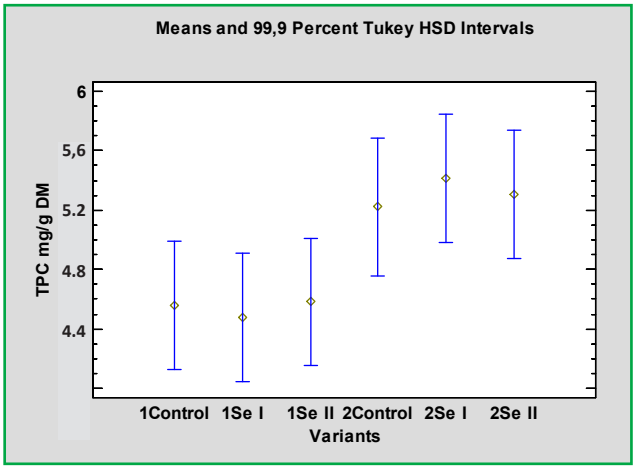

Figure 4 Graphical representation of treatments influence on total polyphenol content (TPC)

selenate (5.65±0.39 < 5.80±0.32 < 5.90±0.29 in case of first cut, 5.00±0.55 < 5.29±0.45 < 5.36±0.36 in case of second cut) respectively.

Our results from Figure 3 (Graphical representation of basil variety influence on total polyphenol content (TPC)) confirmed dependence of variety on TPC content, ´Red Opal´ reached higher values of TPC in comparison with ´Dark Green´. It corresponds with results of Brewer et al. (2011), cut where purple basil extracts have a higher total phenolic acid content than do green basil extracts. Results from our research are similar to observations reported by Sarfraz et al. (2011), in which TPC estimated in *Ocimum* sp. leaf, seed extracts were 3.97±0.14 to 5.67±0.77 and 5.67±0.77 mg GAE/g DW respectively, which exhibited that seed extract, had higher phenolic content than the leaf extract. TPC measured by Mahajan et al. (2014), with Folin-Ciocalteu method, displayed that phenolic content in the leaf extract of *O. sanctum* (4.49–9.31 μg GAE mg^{-1} extract) was higher than those present in its seed extract (4.10–9.05 μg GAE mg^{-1} extract).). According to Srivastava et al. (2012) *O. basilicum* L. had the highest of total phenolic content (305.11mg GAE g^{-1}), highest content of anthocyanins (15.427 mg Kg) and total flavonoids (9.692 QE mg g), when comparing to *Piper betle* L., *Grewia asiatica* L., *Lantana camara* L. Following Figure 4 (Graphical representation of variant influence on total polyphenols content (TPC)) the values of TPC, as in case of AOA, were higher in second cut. Application of selenium had significant impact on tested level ($P < 0.001$) for ´Dark Green´ variety, where values slowly increased from control variant, to 1 x Se dose and the highest were after double dose of sodium selenate (5.65±0.39 < 5.80±0.32 < 5.90±0.29 in case of first harvest, 5.00±0.55 < 5.29±0.45 < 5.36±0.36 in case of second cut) respectively.

Extracts of many members of the *Labiatae* (*Lamiaceae*) family (oregano, marjoram, savory, sage, rosemary, thyme, and basil), which are antioxidative, have a high total phenol content (Chen et al., 2007. It supposed the correlation between AOA and TPC, confirmed by some scientific studies. But results are contradictory. In basil, a significant correlation exists between the total phenolic content and antioxidant activity (Juliani et al., 2002). According to correlation analyze pictured on Figure 5, it was not find any dependence between AOA and TPC data from our trial. Dorman et al. (2003) observed that, while these antioxidant characteristics are not entirely related to the total phenolic contents, they do appear to be strongly dependent on rosmarinic acid, the major phenolic component present.

4. Conclusions

Term of cutting and variety had significant influence on TPC and AOA. The selenium biofortification had as well significant impact to increasing of observed characteristic in some cases, which is why there is strong recommendation of multi-annual results estimation according to submitted methodology.

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