SZENT ISTVÁN UNIVERSITY

COMPARATIVE ASSESSMENT OF BIOLOGICAL ACTIVE SUBSTANCES OF BASIL (*OCIMUM BASILICUM* L.) TAXA

PhD THESIS

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1. BACKGROUND AND OBJECTIVES

Basil (*Ocimum basilicum* L.) is not only a popular spice and aromatic plant for thousands of years, but in the folk medicine and naturopathy to date from ancient times has been widely used to restrain insects, to treat inflammations and as an appetizer.

These observations are supported by physiological studies carried out since the turn of the millennium, which indicates the broad therapeutic effectivity of the plant: it has analgetic, antiinflammatory, antiviral, antifungal, hepatoprotective, digestive, anticarcinogenic effect, also decreases the level of cholesterol and blood glucose. This diversity of the initial data draws attention that at present time, we are still at the beginning of the clarification of the active substances and its mechanisms.

Many antioxidant compounds (flavonoids, phenolic acids, vitamin C) have been identified in the plant by the current studies (about two decades) of active substances of basil also. It is remarkable that the measured amounts in various publications are heavily deviating, whether it would be essential oil, flavonoids or ascorbic acid. This reason may be due to the high variability of the observed species as well as the different location production of the test materials or the differences of the research methodology. The complex multicriterial evaluation and the experiments conducted by the same research methodology in the same terroir can bring the elimination of heterogeneities of the different taxa.

The purpose of my PhD research – in the continuation of the integral part of the previous studies of the department – was the comparative analysis of the antioxidant compounds of basil taxa which were stored at the gene bank of the Department of Medicinal and Aromatic Plants of Corvinus University of Budapest.

The practical aim of the experiment was to identify the performance indicators of the examined accessions under domestic environment condition. These results may assist the basis for further breeding.

The research comprised the general characterization of taxa, the correlation of its bioactive substances, the comparison of phenological phases and the multicriterial complex evaluation according to the following themes:

1. **General characterization** of the examined taxa based on:
   - essential oil content and composition,
   - antioxidant capacity (FRAP, DPPH),
   - total polyphenol content (TPC),
   - total flavonoid content (TFC),
   - charasteristic flavonoid aglycons (salvigenin- and nevadensin content),
   - vitamin C content.
2. **The correlation study of antioxidant bioactive substances** of the examined taxa based on:
   - essential oil content,
   - antioxidant capacity,
   - total polyphenol content,
   - total flavonoid content,
   - charasteristic flavonoid aglycons (salvigenin- and nevadensin content),
   - vitamin C content.
3. **The comparison of phenological phases** of the examined taxa based on:
   - essential oil content and composition,
   - antioxidant capacity,
   - total polyphenol content,

- total flavonoid content,
- charasteristic flavonoid aglycons (salvigenin- and nevadensin content),
- vitamin C content.

4. Taking into account the performance characteristic at the same time, **multicriterial complex evaluation** of the examined taxa.

2. MATERIALS AND METHODS

2.1. Examined plant materials

The cultivation of the 8 examined basil taxa – 'A-1', 'Arvada', 'Dark Opal', 'Genovese', 'Lengyel', 'Mittelgroβblättriger Grünes' (hereinafter referred to as 'M. Grünes'), 'Piros', 'Rit-Sat' – was done at Soroksár in 2012 and 2013 at the experimental station of the university.

2.2. Preparation of investigated plant materials

In 2012 the examinated taxa's seeds were sown in seed trays and were grown in a greenhouse. The pricking to trays was held at 1-2 foliage leaf stage. The seedlings were planted out to open field at the last decade of May after a week-long training session, 60-60 plants per batches. The spacing was 50 x 30 cm. I supported the adequate water supply of the crops with irrigation till harvest time, and I made mechanical weed control. 30-30 plants per taxon were used to the evaluation. All taxa were uniformly harvested at the time of full bloom.

Fresh plant materials were dried in a natural way in a closed storehouse protected from sunlight by using trays. Before analyses the stems were removed. For measurements only stripped leaves were used in 2012.

In 2013, the plants were grown according to the methods applied in the previous year, however 120-120 seedlings were planted, since harvest occurred

in three different (beginning of flowering, full bloom, overblown) phenophases, with 30-30 plants per taxon.

The drying method of plants was the same as in the previous year. For laboratory tests stems were removed too. To the measurements – unlike in previous years – the stripped leaves and flowers were used together. This does not apply to measurements made at Semmelweis University (DPPH, vitamin C content) where only stripped leaves alone were used in both years.

The nutritional studies were made from mass sample in both years.

2.3. Laboratory measurements

2.3.1. Determination of essential oil content

Essential oil was extracted from a mixture of 20 g dried plant material (30 individuals from each accession) according to the method recommended in the VII. Hungarian Pharmacopoeia (*Pharmacopoeia Hungarica*, 1986). I used 3 repetitions in each variety. Essential oil content was expressed in ml/100g dw.

2.3.2. Determination of essential oil composition

The identification of the sample's essential oil composition was carried out by GC-MS-method based on mass spectra, mass spectra to librarian references (NIST, Wiley and own essential library), the calculation of their linear retention indexes (Van den Dool and Kratz, 1963).

2.3.3. Determination of FRAP antioxidant activity

For the determination of the total antioxidant activity to the dried plant material the modified methodology of Benzie and Strain was used (Benzie and Strain, 1966). The purple colourisation was detected by spectrophotometer at λ=593 nm. Measurements were carried out in 5 replicates in both years. The results was provided in ascorbic acid equivalence (mg AAE/100g dw).

2.3.4. Determination of DPPH antioxidant capacity

For the evaluation of antioxidant capacity the classic DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate)-method was used to (Lugasi and Blázsovics, 2004). The H-donor activity were expressed in I50 values (50% inhibition), which suggesting the amount of sample has caused 50 % colour reduction in the free radical complexes under standard conditions. The loss of dark purple colour was monitored with spectrophotometer. Measurements were carried out in three replicates in 2012 and in five replicates in 2013.

2.3.5. Determination of total polyphenol content

The total polyphenol content were determined by the modificated method of Singleton and Rossi (1965). The reaction indicated blue colour intensity can be determined photometrically at λ=760 nm. The results were determined to GAE mg/g dw. 5 replicates were used in each measurement in both years.

2.3.6. Determination of total flavonoid content

In the absence of announcement of the flavonoid content of basil, we performed the total flavonoid content test expressed in isokvercitrosid to the infertile shoot of *Equisetum arvense* L.. The method is listed in the *Ph. Hg.* VIII. (*Pharmacopoeia Hungarica*, 2003). The flavonoid content was determined spectrophotometrically (λ=425 nm) and expressed as a percentage. The measurements were performed in 3 replicates in 2013.

2.3.7. Determination of salvigenin and nevadensin content

The salvigenin and nevadensin sample preparations and elution program were made according to the method published by Grayer et al. (2004). 200g dried, ground leaf material was used to the experiment. Identification was based on retention times and spectral data in comparison with authentic standards.

Quantification was performed by using the calibration curves. All samples were prepared in triplicate in 2013.

2.3.8. Determination of vitamin C content

Vitamin C content from the dried samples was identified by traditional spectrophotometry with the method of modificated Spanyár-method (Gilingerné and Varga, 2005). The quantity of red colour complex – which is form during the reaction – directly proportional to the vitamin C content. These values were expressed in mg AAE/100g dw. The ascorbic acid measurements were carried out in three replicates in both years.

2.4. Evaluation of the test results

2.4.1. The complex comparison of performance indicators of the examined taxa

The comparison of analyzed taxa was made by the *SRD*-method (*Sum of Ranking Difference*) of which can select the best-performing taxa within a given area and given environmental conditions.

This method can projected different dimension values of different qualities onto a scale, thus making it suitable for comparison. In our case this means that with the transformation of the measured nutritional parameters (%, mg / 100g, etc.) ranking can be set up which taking into account all aspects of the examined taxa (i.e. characteristic).

In the first step *SRD*-method does a reference column from the best data by summarizing the results of each properties in a table. Then compare these rankings values with the rankings set up by the individual varieties. So ranking differences are formed for each attribution (ranking difference of given attribute of given variety). Then it sums up these absolute values (*SRD* value per accession). This value determines how much is the overall difference between

the given variety and the theoretically best variety (zero point). Thus a clear ranking can be set up based on the distance of the zero point.

SRD running on the original data results one *SRD*-value of each varieties. Thereto the uncertainty (deviation of *SRD*-values) of the *SRD* values can be characterize we apply to a LOO (leave one out) cross-validation method. The resulting values (LOO *SRD*-values) have to be re-analyzed by *SRD*-method to take the uncertainty of the *SRD*-values into account.

As a ranked true location of gene bank stored varieties can only clearly defined by statistical tests therefore Wilcoxon Matched Pairs Test and Sign-Test were used to define the pairwise comparison in rankings of LOO *SRD*norm values. (*SRD*-values typically do not follow a normal distribution).

2.4.2. Statistical analysis

The final results obtained in the experiments worked up to Microsoft Office Excel 2007 software. SPSS 22.0. program package was used to statistical analysis. 95% confidence level ($p \leq 0,05$) was chosen to the data evaluation. Data normality and homogenity of deviation was examined during test condition. Levenne-test was used to examinate the homogenity of deviation while Shapiro-Wilk-test was used to examinate the normality. After the investigation of normality and homogenity of deviation I have used single-factor analysis of variance (ANOVA) when comparing the results. At the pairwise comparisons of treatments Tukey HSD post hoc test was ran in case of homogenity of deviation while Games-Howell test was ran in case of inhomogenity of deviation. On the figures different letters indicates significantly different average.

Pearson correlation coefficient value was used to characterisate the correlation between methods which have antioxidant and free radical scavenging capacity (FRAP, DPPH) with compounds/groups of compounds wich have certain antioxidant activity (vitamin C, total polyphenol and total flavonoid, salvigenin, nevadensin, essential oil).

To evaluate to the multicriterial performance indicators of the examined taxa, statistical evaluations were performed by *SRD* software and Statistica 12.0 program package.

3. RESULTS

3.1. The results of the laboratory measurements

3.1.1. Essential oil content

In 2012 significant difference was detected between taxa at the full flowering stage (taxon: $F(7;16)=14.962$; $p\leq0.001$). The highest essential oil content proved to be ’M. Grünes’ (0.59±0.05 ml/100g) and ’Lengyel’ (0.52±0.05 ml/100g), the lowest quantities accumulated in ’Rit-Sat’ (0.29±0.04 ml/100g), ’Piros’ (0.32±0.04 ml/100g) and ’Genovese’ (0.32±0.04 ml/100g) taxa.

During the experiments of the year 2013 significant difference was shown between the three phenophases (begining of flowering, full bloom, overblown stage) of the examined taxa too (taxon: $F(7;48)=151.024$; $p\leq0.001$) (phenophase: $F(2;48)=657.844$; $p\leq0.001$).

At the begining of flowering the highest yield was matched to ’Dark Opal’ (1.46±0.00 ml/100g), while the lowest to ’Piros’ taxon (0.74±0.06 ml/100g).

At the full bloom period ’Lengyel’ (1.38±0.06ml/100g), ’Dark Opal’ (1.33±0.08 ml/100g) and ’M. Grünes’ (1.31±0.08 ml/100g) had the highest essential oil yield, while ’Genovese’ taxon had the lowest one (0.65±0.00 ml/100g).

At the overblown stage – as like as in the begining of flowering – ’Dark Opal’ (0.92±0.05 ml/100g) reached the maximum and ’Piros’ taxon (0.19±0.08 ml/100g) the minimum quantity of essential oil again.

3.1.2. Essential oil composition

Based on the essential oil composition the 8 studied taxa can be classified into two chemotypes (linaloolic or linalool-methyl-chavicolic). According to the measurements of 2012 ’Arvada’, ’Dark Opal’, ’Genovese’, ’Lengyel’ and ’Rit-Sat’ taxa belongs to the first group and’A-1’, ’M. Grünes’ and ’Piros’ taxa belongs to the second one. The studies conducted in 2013 confirmed this classification.

3.1.3. Total antioxidant activity (FRAP)

In 2012 significant difference was measured between taxa at the full flowering stage (taxon: $F(7;28)=52.161$; $p\leq0.001$). ’Rit-Sat’ (131.90±2.15 mg/AAE/g d. w.) and ’Dark Opal’ (129.38±5.77 mg/AAE/g d. w.) had the biggest antioxidant capacity and ’Arvada’ taxon (56.56±13.46 mg/AAE/g d. w.) had the lowest one.

In 2013 there was a significant difference between taxa and within different phenological phases ((a) begining of flowering, (b) full bloom, (c) overblown stage) (taxon: $F(7;92)=134.230$; $p\leq0.001$) (phenophase: $F(2;92)=4824.545$; $p\leq0.001$).

At the beginning of the flowering the highest antioxidant capacity was connected to ’Rit-Sat’ (192.93±13.98 mg/AAE/g d. w.) and ’M. Grünes’ (180.18±13.69 mg/AAE /g d. w.), the lowest one to the ’Piros’ taxon (67.94±8.00 mg/AAE /g d. w.).

At full bloom the highest antioxidant capacity was measured in ’Rit-Sat’ (412.56±23.73 mg/AAE /g d. w.), the lowest FRAP-values in ’Arvada’ (164.18±8.84 mg/AAE /g d. w.) and ’A-1’ taxa (181.32±10.86 mg/AAE /g d. w.), which were in the same group.

During the overblown stage ’Lengyel’ (718.43±19.60 mg/AAE/g d. w.) and ’Rit-Sat’ (692.81±22.22 mg/AAE/g d. w.) showed the highest and ’Dark Opal’ taxon (442.12±11.49 mg/AAE/g d. w.) the lowest values.

3.1.4. DPPH antioxidant capacity

As a results of my experiment there was a significant difference between taxa and within the examined two years (year: $F(1;46)=132.972$; $p\leq0.001$) (taxon: $F(7;46)=4.550$; $p\leq0.001$). In addition the interaction of the taxon and year also showed significant differences (taxon*year: $F(7;46)=5.550$; $p\leq0.001$).

In 2012 the highest antioxidant capacities were shown in case of ’Lengyel’ (1.05±0.07 mg) and ’Genovese’ (1.15±0.04 mg), ’Rit-Sat’ taxon (1.33±0.11 mg) had the lowest one. In 2013 the maximum value of the antioxidant capacity was represented by ’Genovese’ (1.28±0.06 mg) and ’Piros’ taxon (1.39±0.06 mg) showed the minimum value.

3.1.5. Total polyphenol content

In 2012 significant difference was detected between taxa (taxon: $F(7;29)=107.235$; $p\leq0.001$). ’M. Grünes’ taxon (123.77±3.15 mg/GSE/g d. w.) had the highest total polyphenol content, ’Arvada’ taxon (53.61±4.68 mg/GAE/g d. w.) had the lowest in turn. ’Genovese’ (113.01±4.30 mg/GAE/g d. w.), ’Piros’ (120.81±3.30 mg/GAE/g d. w.) and ’Rit-Sat’ taxa (119.83±9.02 mg/GAE/g d. w.) were not significantly different to ’M. Grünes’ taxon.

In 2013 there was a significant difference between taxa and within different phenological phases ((a) begining of flowering, (b) full bloom, (c) overblown stage) (taxon: $F(7;80)=4875.886$; $p\leq0.001$) (phenophase: $F(2;80)=46366.391$; $p\leq0.001$).

At the beginning of flowering ’Dark Opal’ had the maximum total polyphenol content value (119.05±4.83 mg/GAE/g d. w.), meanwhile the lowest ones represented in ’Arvada’ (12.23±1.88 mg/GAE/g d. w.) and ’A-1’ taxa (14.16±1.96 mg/GAE/g d. w.) which were not significantly different from each other.

At full bloom the maximum total polyphenol content was represented in ’Dark Opal’ (121.23±2.85 mg/GAE/g d. w.), the minimum in ’Piros’ taxon (33.25±1.46 mg/GAE/g d. w.).

At the overblown stage the highest total polyphenol content was accumulated in ’M. Grünes’ (477.33±2.25 mg/GAE/g d. w.) and ’Rit-Sat’ (465.80±2.74 mg/GAE/g d. w.) taxa, conversely ’A-1’ taxon had the lowest one (40.38±5.25 mg/GAE/g d. w.).

3.1.6. Total flavonoid content

In 2013, the experiment was completed with the total flavonoid content (%), and I determined the quantitative accumulation of the salvigenin and nevadensin flavonoid aglycones.

In 2013 there was a significant difference between taxa and within different phenological phases ((a) begining of flowering, (b) full bloom, (c) overblown stage) (taxon: $F(7;24)=51.232$; $p\leq0.001$) (phenophase: $F(2;24)=73.695$; $p\leq0.001$).

At the beginning of flowering the highest total flavonoid content belonged to ’Lengyel’ (2.00±0.26 %) and ’M. Grünes’ accessions (1.98±0.24 %), meanwhile ’Dark Opal’ accession had the lowest one (0.05±0.02 %).

At full bloom the highest total flavonoid content was measured in ’A-1’ genotype (2.93±0.32 %). ’Arvada’ (2.04±0.15 %), ’Lengyel’ (2.56±0.23 %), ’Rit-Sat’ (1.88±0.03 %) and ’M. Grünes’ (2.10±0.15 %) genotypes were also represented in this group. ’Dark Opal’ accession (0.31±0.14 %) produced the minimum value.

At the overblown stage the highest total flavonoid content was represented in the group of ’Arvada’ (1.71±0.10 %) and ’A-1’ taxa (1.66±0.11 %), the lowest one was measured in ’Dark Opal’ genotype (0.27±0.06 %).

3.1.7. Salvigenin content

In 2013 there was a significant difference between taxa and within different phenological phases ((a) begining of flowering, (b) full bloom, (c) overblown stage) (taxon: $F(7;44)=2018.910$; $p\leq0.001$) (phenophase: $F(2;44)=5129.605$; $p\leq0.001$).

At the beginning of flowering the highest active agent content was found in ’A-1’ (12.49±0.40 mg/100g), meanwhile the lowest one belonged to ’Dark Opal’ accession.

At full bloom phenophase the highest value was connected to ’M. Grünes’ (21.64±0.14 mg/100g), while the lowest to ’Dark Opal’ taxon (2.50±0.09 mg/100g).

At the overblown stage the highest value was connected to ’A-1’ (7.10±0.27 mg/100g), the lowest ones to ’M. Grünes’ (0.60±0.07 mg/100g) and ’Rit-Sat’ (0.72±0.02 mg/100g) genotypes.

3.1.8. Nevadensin content

In 2013 there was a significant difference between taxa and within different phenological phases ((a) begining of flowering, (b) full bloom, (c) overblown stage) (taxon: $F(7;42)=1906.634$; $p\leq0.001$) (phenophase: $F(2;42)=285.329$; $p\leq0.001$).

At the beginning of flowering the highest nevadensin content was found in ’M. Grünes’ (7.94±0.54 mg/100g), while the lowest one in ’Lengyel’ accession (0.38±0.14 mg/100g).

At full bloom phenophase the highest quantities were connected to ’Piros’ (6.76±0.09 mg/100g) and ’M. Grünes’ taxon (6.72±0.11 mg/100g) which did not differ significantly from ’Piros’ accession. The lowest value was measured in ’Lengyel’ taxon (0.27±0.05 mg/100g).

At the overblown stage the maximum nevadensin content was accumulated in ’M. Grünes’ (6.52±0.08 mg/100g). ’Lengyel’ taxon (0.22±0.02 mg/100g) produced the minimum value.

3.1.9. Vitamin C content

Between the years not (year: $F(1;32)=0.344$; $p=0.562$), but there was a significant difference detected within taxa (taxon: $F(7;32)=4.702$; $p\leq0.01$), the interaction of taxon and year also showed significant difference (taxon*year: $F(7;32)=4.227$; $p\leq0.01$).

In 2012 there was not a significant difference detected within taxa. The highest vitamin C content was measured in ’M. Grünes’ (32.46±5.33 mg/100g), the lowest in ’A-1’ taxon (19.94±1.58 mg/100g).

In 2013 the vitamin C content of ’A-1’ and ’Piros’ accessions was significantly different to each other and to the other genotypes, which were in the same group. The highest vitamin C content was measured in ’Genovese’ (28.21±3.47 mg/100g), while the lowest in ’Lengyel’ taxon (21.35±2.69 mg/100g).

3.2. Evaluation of the test results

3.2.1. Correlation among parameters with antioxidant capacity

Pearson correlation coefficient value was used to characterise the correlation between methods which have antioxidant and free radical scavenging capacity (FRAP, DPPH) with compounds/groups of compounds which have certain antioxidant activity (vitamin C, total polyphenol and total flavonoid, salvigenin, nevadensin, essential oil).

During the experiment in 2012, significant correlation ($r=0.601$) was detected between the FRAP-values of leaves and total polyphenol content at full bloom phenophase.

In 2013 another compounds/group of compounds with antioxidant effect were taken in the experiment. Since this year the plant *herbs* have been consistently studied, DPPH and vitamin C data have not been included in the evaluation, since they only included the leafy part cleanly, and measurements were only made in full bloom.

After a detailed analysis of the phenological phases I determined the correlation between the typical values of antioxidant capacity for each phenophases.

At the beginning of the flowering the strongest significant correlation was between the total polyphenol content and essential oil content (r= 0.759). Less significant correlation was detected between the total polyphenol content and total flavonoid content (r=-0.698), between the total flavonoid content and salvigenin content (r=0.590) and between salvigenin content and nevadensin content (r= 0.552), in descending order. The weakest, but still significant correlation was seen between FRAP and total flavonoid content (r=0.518).

Looking at the full bloom phenophase, I found that the strongest correlation was found between the two main flavonoid aglycone of basil (salvigenin, nevadensin) (r=0.683). Similar levels of significant correlations was found during the pairwise analysis of the total polyphenol content and total flavonoid content as well (r=-0.618). I detected a weaker, but still significant relationship between the total polyphenol content and essential oil content (r=0.447), while the least significant correlation (r=0.419) was connected to the pairwise analysis of total flavonoid content and salvigenin content.

At the overblown stage the strongest correlation was observed between the total polyphenol content and salvigenin content (r=-0.802). This was followed by a significant correlation between FRAP and essential oil content (r=-0.774). Weaker but significant relationship appeared between the total polyphenol content and total flavonoid content (r=-0.647) and the total flavonoid content and salvigenin content (r=0.643), which correlated values were similar.

It was characterized by the same similarity: the correlation of nevadensin content and total polyphenol content (r=-0.553) and the nevadensin content and total flavonoid content (r=0.551), which showed weaker significant correlation than the previous ones. The lowest correlation value having a significant relationship was between the FRAP and total flavonoid content (r=-0.423).

3.2.2. Complex multicriterial evaluation of the examined

Characteristic groupings have been identified by the results based on the summarizing of *SRD*-method of the examined taxa. The best performed taxon was 'M. Grünes' respectively. Followed by 'A-1' and 'Dark Opal' taxon forming the same group. The – third – worst performing group was form by 'Arvada', 'Genovese', 'Lengyel', 'Piros' and Rit-Sat'.

The best performed taxon was again 'M. Grünes' taxon through the re-evaluation of *SRD*-method by following the *LOO* cross validation. This was followed by 'A-1', 'Arvada', 'Genovese' and 'Rit-Sat' taxa, which form the same group and then 'Lengyel' and 'Dark Opal' taxa which was significantly different from each other and from the previous ones. The worst performer 'Piros' taxon lies on the XX1 probability level line.

The pairwise comparison of the ranking of taxa was performed by Sign and Wilcoxon pairwise test, which results were the same. Based on Box and Whisker figure and the statistical analysis significantly the best performer was the 'M. Grünes' accession. The worst performers were 'Arvada', 'Genovese', 'Rit Sat' and 'Lengyel' taxa, which did not significantly differ from each other.

3.3. New scientific results

1. I revealed that the composition of essential oil of the studied taxa is a genetically fixed property and it can be classified into two chemotypes. 'Arvada', 'Dark Opal', 'Genovese', 'Lengyel' and 'Rit-Sat' taxa have

linaloolic, while ’A-1’, ’M. Grünes’ and ’Piros’ taxa have linalool-methyl-chavicolic chemotype character.

2. I proved with the evaluation of phenophases that while the essential oil content at the flowering stage, the total polyphenol content is the highest at the overblown stage. Correlate with this, antioxidant capacity characterized by FRAP method culminates in the overblown stage too. ’Lengyel’ (718.43±19.60 mg/AAE/g dry weight) and ’Rit-Sat’ (692.81±22.22 mg/AAE/g dry weight) taxa proved to have the highest antioxidant capacity.

3. I have verified that the total flavonoid content is the highest at the flowering stage. The total flavonoid content in the group of ’A-1’, ’Arvada’, ’Lengyel’, ’Rit-Sat’, ’M. Grünes’ taxa amounted to 1.88±0.03 - 2.93±0.32 %.

4. I revealed that the amount of the two characteristic flavonoid aglycone (salvigenin, nevadensin) of the plant reached its maximum at the flowering stages.

5. I found that within the measured parameters the phenological phase associated change of the amount of essential oil is considered to be genetically fixed. The polyphenol content and the level of accumulation of flavonoids are phenological phase dependent, the scale of the accumulation is taxon dependent.

6. I detected that depending on the purpose of use, the plants should be harvested in different phenophases. The optimal time to extract the highest content of essential oil, salvigenin and nevadensin is at the time

of flowering, while to achieve the maximum antioxidant activity the overblown stage is recommended.

7. I detected strong, partly phenological phase dependent positive correlation between total flavonoid content and salvigenin content, while negative correlation was detected among total polyphenol content and total flavonoid content.

8. I ranked the studied basil taxa according to its biologically active materials with multicriterial complex evaluation of performance indicators, using *SRD*-method.
   Based on this the best accession was the ’M. Grünes’ taxon.

4. CONCLUSIONS AND PROPOSALS

Examining the different phenophases in 2013 I concluded that the most essential oil accumulation was at full bloom (’A-1’, ’Lengyel’ and ’Piros’ taxa), also it was at the begining of flowering (’Arvada’, ’Dark Opal’, ’Genovese’, ’M. Grünes’ and ’Rit-Sat’ taxa). In practice this means that if the aim of use is to maximize the essential oil content, latter should be harvested at the begining of flowering, the first ones should be harvested at the full bloom phenophase. It is strongly stated that in any case overblown stage had the lowest amount of essential oil, so effect of phenophase prevailed. The decrease, however, was taxon dependent. At the overblown stage essential oil accumulation decreased to two-thirds (’A-1’, ’Arvada’ és ’Dark Opal’ taxa), half (’M. Grünes’ and ’Rit-Sat’ taxa), third (’Lengyel’ taxon), fourth (’Genovese’ taxon), one fifth (’Piros’ taxon) compared to the maximum essential oil content.

The essential oil composition of the studied taxa overall match with the classification of Szabó (2000) (linaloolic and linalool-methyl chavicolic

chemotypes) except that ’Genovese’ and ’Arvada’ taxa proved to be linaloolic chemotype. ’Dark Opal’ taxon linaloolic chemotype is different to the same taxon classification identified by Grayer et al. (1996) which is eugenol-linaloolic (linalool 30.5-38.6 %, 38.9-48.0 % eugenol). One explanation may be the different origin of the gene bank materials. From the results I concluded that the composition of the essential oil of the examined taxa is a genetically fixed property.

During the measurement of the antioxidant capacity can be said generally about the examined taxa that at the begining of flowering FRAP values varied between 67.94±8.00 and 192.93±13.98 mg/AAE/g d. w. limits, while it ranged between 164.18±8.84 and 412.56±23.73 mg/AAE/g d. w. at full bloom. These values were realized between 442.12±11.49 and 718.43±19.60 mg/AAE/g d. w. at the overblown period. Therefore from the begining to overblown period 3-5 fold, even in the case of ’Piros’ taxon almost 10-fold increase was observed, meaning significant phenophase effect prevailed beside minimal taxon effect. This may be explained that the plant possessed higher total polyphenol content at the overblown phenophase, which compounds have proven to have antioxidant effect. Based on the results, we can say that the plant should be harvested at the overblown stage when our goal is the highest total antioxidant capacity.

Comparing the 2012 and 2013 years in full bloom phases of the antioxidant capacity based on DPPH radical scavenging it can be stated that in the case of ’A-1’ and ’Rit-Sat’ taxa there was no significant difference detected. In case of the other taxa I received significantly higher values in the year of 2013 than in 2012, which meant that during 2013 taxa performed worse. So in 2013 more mg of dry material would cause 50 % inhibition to individual taxa. This result can be explained by the fact that in 2012 from the planting of the seedlings to the harvest for investigation (May, June, July), the monthly mean temperatures were warmer than in 2013, so the weaker performance is due to

lower temperature values which are affecting the accumulation of antioxidant compounds. In both years, ’Genovese’ belonged to the group having the highest antioxidant capacity, so at total this taxon produced the best result.

The total polyphenol content of taxa (TPC) in 2013 was different at phenophases; genetic variability obviously was proven. At the beginning of the flowering TPC values varied between 12.23±1.88 and 119.05±4.83 mg/GAE/g d. w. limits, during the full bloom it varied within 33.25±1.46 and 121.23±2.85 mg/GAE/g d. w.. 40.38±5.25 to 477.33±2.25 mg/GAE/g d. w. was measured at the overblown period, so taxa were produced the highest values so far (phenophase effect). The increase is variable per taxon. From the begining to overblown period 3 fold (’A-1’, ’Dark Opal’ and ’Genovese’ taxa) and 9-13 fold (’Arvada’, ’Lengyel’, ’M. Grünes’, ’Piros’ and ’Rit-Sat’) increase was observed. Consequently – if TPC is the base of the use – plant herb should be utilized at the overblown stage.

Phenophase effect prevailed at total flavonoid content; the maximum yield is linked to the full flowering stage. At the overblown period the rate of decline is taxon dependent, demonstrating that the examined taxa differ genetically. From the correlation of decreased total flavonoid content and increased (at the same time) total polyphenol content it can be concluded that at the overblown period the amount of phenolic acids rose sharply in the plant, because total polyphenol content measurement result includes both of flavonoids and phenolic acids. In that case, if the total flavonoid content is the base of the consumption, taxa should be harvested in full bloom stage.

The trend in change of salvigenin content in phenophases parallels with the total flavonoid content thus phenophase effect prevail. The highest values of salvigenin was detected at the begining of flowering (’A-1’, ’Arvada’, ’Dark Opal’, ’Lengyel’ and ’Rit-Sat’) or at full bloom (’Genovese’, ’M. Grünes’ and ’Piros’). The lowest salvigenin content was measured at the overblown stage in case of all tested taxa, measured values decreased to two-thirds (’Arvada’), half

('A-1'), third ('Dark Opal', 'Genovese', 'Piros'), one fifth ('Lengyel'), one seventh ('Rit-Sat'), thirty-sixth ('M. Grünes') compared to the maximum values. Examining the results obtained firstly stated that the increase-decrease of salvigenin is taxon dependent, on the other hand for the purpose of extracting the optimal amount of active agent the begining of flowering and full bloom should be recommended, depending on taxon.

Examining the changes in the nevadensin content, phenophase effect also occurred, but to a lesser extent than in case of salvigenin. The highest values of salvigenin was detected at the begining of flowering ('A-1', 'Lengyel' and 'M. Grünes') or at full bloom ('Dark Opal', 'Genovese', 'Piros' and 'Rit-Sat'). Nearly all the taxa produced the lowest nevadensin content at the overblown stage. This trend parallels to the total flavonoid content also, though the rate of decrease is not as significant as in case of salvigenin content. Measured quantitative values of nevadensin decreased to four-fifths ('M. Grünes'), two-thirds ('Dark Opal', 'Genovese'), three fifths ('A-1', 'Lengyel', 'Rit-Sat'), and fourth ('Piros'), compared to the maximum values. It can be concluded, first, that the increase-decrease of nevadensin is taxon dependent too, on the other hand, if the goal is to extract the maximum amount of nevadensin, then the begining of flowering or the full bloom should be recommended.

Comparing the vitamin C content of the tested basil accessions in 2012 and 2013, not significant, nor tendentious differences can be established between the two years. Taking the measured quantities into account (19.94±1.58 mg/100g and 32.46±5.33 mg/100g intervals) the ascorbic acid content of basil though can be recommended as significant if comparing with the fruits, but this is purely numerical comparison, whereas adequate intake of vitamin C from basil to the human body is not so effective such as *Citroideae*.

Based on the correlation between methods which have antioxidant and free radical scavenging capacity with compounds/groups of compounds wich have certain antioxidant activity in case of all three examined phenophases strong positive correlation has been established between the total flavonoid content and the salvigenin content. There is a negative correlation between the total polyphenol content and total flavonoid content.

Harvest proposals related to the (listed) phenophases (of course) only based on the results of research carried out in this work. Further experiments with extended periods of time is necessary to draw the final conclusions, because only the combined analysis of data from several years create a real image such as taxon- and year effects together with what kind of weight affects the results.

My work also draws attention to the fact that taxa should be selected with multicriterial *SRD*-method, because we can characterize most of their their biological potential in this way. In this context I supported, on the basis of biologically active substances 'M. Grünes' performed the best taxa, so this taxon may be offered for growing in this examinated conditions.

We could get more results with the combined instrumental and sensory evaluation of the studied taxa in connection with its sensory characteristics and consumer preferences.

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B. Bernhardt, Gy. Fazekas, M. Ladányi, K. Inotai, É. Zámbori-Németh, J. Bernáth, K. Szabó (2014): Morphological-, chemical and RAPD-PCR evaluation of eight different *Ocimum basilicum* L. gene bank accessions. Journal of Applied Research on Medicinal and Aromatic Plants, 1(1), E23-E29. DOI:10.1016/j.jarmap.2014.03.002

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