NDVI, chlorophyll and carotenoids content of leaves of Rosa damascena Mill under organic and conventional farming

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Abstract. The aim of our study was to compare NDVI index, total nitrogen, chlorophyll and carotenoids content of leaves of oil-bearing roses cultivated under organic and conventional agricultural systems in order to find impact of farm management on the physiology status of Rosa damascena leaves. The experiment was conducted on six private arable areas with Rosa damascena Mill in Rose valley, Southern Bulgaria. The selected study area size was 5000 m² from each private territory. Three of the oil-bearing rose plantations are certified as organic farming and have been applied an organic agriculture system and the rest of them are characterized as conventional farming. NDVI index of the leaves of Rosa damascena was measured with Plant Pen 310 device in the field. The same leaves were picked up for future laboratory analysis. The total chlorophyll and carotenoids, μg/g dw content of leaves in the 80 % acetone were determined, total nitrogen by Kjeldahl method as well. Linear discriminant analysis (LDA) was performed to classify Rosa damascena leaves on the base of NDVI and photosynthetic pigment content according to relevant farm management. The results from conventional rose bushes leaves contained statistically proven higher total chlorophyll and lower total carotenoids than leaves belonging to organic oil-bearing roses cultivation. It is worthwhile to continue investigations with NDVI leaves measurements as a promising tool for recognition organic or conventional farm production.

1. Introduction

There is a growing interest in using rapid, non-destructive and time-consuming methods for estimation of plant physiological and biophysical parameters, plant health and food quality [1-3]. Normalized Difference Vegetation Index (NDVI) is one of the most widely and frequently used index in the remote sensing research, recently [4,5]. NDVI is the ratio of the amounts of reflectance in the near infrared (NIR) and red (RED) region of the electromagnetic spectra, calculated using the formula \( \text{NDVI} = \frac{(\text{NIR} - \text{RED})}{(\text{NIR} + \text{RED})} \). NDVI thus theoretically takes values ranging from \(-1.0\) to \(+1.0\). Positive NDVI values indicate green, vegetated surfaces, and higher values indicate increases in green vegetation. Reflectance of the red region of the spectrum decreases as solar radiation is absorbed, largely by
chlorophyll, whereas reflectance of the near infrared portion is caused by leaf mesophyll structure [6]. NDVI has also been shown to be related to a number of plant parameters such as leaf area index (LAI), biomass, chlorophyll content and photosynthetic activity. Leaf chlorophyll content is an important physiological parameter which can serve as an indicator of nutritional status, plant stress or senescence. Chlorophyll and nitrogen status in the leaves provides extremely valuable information about plants, which parameters have been used to assess the overall photosynthetic capacity of the canopy and productivity of the plant. [7]

Bulgaria as many other countries has serious traditions in the cultivation of various essential oil plants. Due to the growing interest of essential oil in the world, the arable areas with essential oil plants have increased in the last few years. The industrial cultivation of oil-bearing rose in our country includes Rosa damascena Mill due to the high content and quality of essential oil [8]. The choice of organic cultivation of crops over the conventional one is often associated with higher costs, more risks for agricultural production, but the results are connected with better lifestyle, health and longevity. For instance, many studies have shown that the organic cultivation agricultural system increases the amount of bioactive compounds in fruits [9], mostly due to the effect of plant self-protection against pest and diseases. According to our studies the agricultural system of the oil bearing rose (Rosa damascena Mill.) grown has an effect on antioxidant activity of rose petals. It was found the highest values of total phenols and flavonoids were obtained in the rosewater extract from organically grown plantation in compare to conventional farming [10]. More efficient agricultural practices is a key factor for further increase of the overall rose production, quality and the same time reduction of the production costs. Thus, using fast and non - expensive methods for monitoring vegetation growth of Rosa damascena would be very useful for valuable information obtain of healthy plant status. Basayigit et al., 2013 [11] performed monitoring of oil-bearing rose growth during vegetation period using hyperspectral detection with ASD Field Spec Hand Held spectrometer under the field condition. According to the authors that physical method could be used successfully to identify the growth problems during the vegetative period in early stage and manage them before blooming. Van den Berg and Perkins [12] indicated that the portable chlorophyll meter could been an effective tool for estimating relative chlorophyll and nitrogen content in sugar maple leaves. Other authors [5] found that reflectance from turfgrass was closely related to turfgrass quality or components of quality such as color.

The aim of our study was to compare NDVI index of fresh leaves, total nitrogen, chlorophyll and carotenoids content of leaves of oil bearing roses under organic and conventional agricultural systems in order to find the impact of agricultural system on the physiology status of Rosa damascena leaves.

2. Material and Methods

2.1. Study area, leaves measurement and sampling
The study was conducted in 2020 on six private oil bearing roses farms in Southern Bulgaria, near Kazanlak valley, also called the Rose valley. According to the climatic zoning of the Republic of Bulgaria, the territory of the Kazanlak valley belongs to the continental climatic region, a moderate continental subregion, a region of the Trans-Balkan lowlands and a region of the eastern Trans-Balkan fields. The Rose valley is situated in the middle of the country between the Stara Planina Mountain on the North and Sredna Gora Mountain on the South. The valley is around 90 km long and around 10 km wide. The climate is transitional continental, relatively mild with an altitude between 400 and 500 m. The winters are warmer and the summers cooler, in this area the average annual temperature is around 11° C, and the annual precipitation is 540 mm.

Three of oil rose private plantations are certified as organic farming with application of the organic agriculture system and the rest of them are characterized as conventional farming. A detailed characterization of the agricultural practices of studied farms is presented in Table 1. Both farms 01 and 02 are characterized as conventional farming, but without soil tillage. There are mulching systems on the soil as a good alternative technique for weed management in oil rose field. Thus Farm 01 and Farm
02 could be classified as farms with a low input farming systems (LIFS), as a kind of conventional farming. According to Parr et al. 1990 [13] the definition of Low Input Farming Systems (LIFS) seek to optimise the management and use of internal production inputs (i.e., on-farm resources) and to minimise the use of production inputs (i.e., off-farm resources), such as purchased fertilizers and pesticides, wherever and whenever feasible and practicable, to lower production costs, to avoid pollution of surface and groundwater, to reduce pesticide residues in food, to reduce a farmer’s overall risk, and to increase both short- and long-term farm probability. In the discussion in the paper Farms 01 and 02 are commented as a conventional with the low-input system. The study area size of every farm was 5000 m². The geographical coordinates of every study area were measured by GPS Garmin.

The study was conducted during the harvesting time of oil bearing rose early in the morning, June, 2020. NDVI index of the leaves of oil-bearing rose bush were measured by Plant Pen 310 in every oil bearing field, randomly. Plant Pen 310 is a portable device compares reflected light at two distinct wavelengths, 635 and 760 nm. The same leaves were picked up for future laboratory analysis, as leaves samples were taken in eight replications per 50 grams from every study area with total 48 samples. While choosing the leaves, attention was paid that there was not dust, pesticide residues and other pollutants on the leaves and problem with biological or mechanical detrimental effect [11].

### Table 1. The name of area, soil type, variety and agricultural practices of the studied farms.

| Farm's number | Area     | Soil type | Variety                                      | Mineral fertilization | Organic fertilization | Irrigation | Soil tillage |
|---------------|----------|-----------|----------------------------------------------|-----------------------|-----------------------|------------|--------------|
| 01            | Damascena 1 | Fluvisols | *R. x damascena f. trigintipetala Dieck*     | yes                   | -                     | yes        | -            |
| 02            | Damascena 2 | Fluvisols | *R. x damascena f. trigintipetala Dieck*     | yes                   | -                     | yes        | -            |
| 03            | Gabarevo  | Fluvisols | *R. x damascena f. trigintipetala Dieck*     | yes                   | -                     | -          | yes          |
| 04            | Skobelevo | Fluvisols | *Rosa damascena x*                           | -                     | yes                   | yes        | -            |
| 05            | Asen     | Fluvisols | *Rosa gallica x*                             | -                     | yes                   | -          | -            |
| 06            | Yasenovo | Fluvisols | *R. x damascena f. trigintipetala Dieck*     | -                     | yes                   | -          | yes          |

*Farms 01 and 02 were conventional with Low– input systems*

#### 2.2. Leaves analysis

The determination of chlorophyll a, chlorophyll b, total chlorophylls and total carotenoids was performed by the method of Lichtenthaler and Wellburn (1983) [14]. The sample was mixed with 80% acetone (1:5 w/v) and extracted in an ultrasonic bath SIEL UST 5.7-150 at 40° C for 15 min. The extraction was performed in triplicate. The combined acetone extracts were measured at three wavelengths 663, 646 and 470 nm against a blank (acetone). The concentrations of chlorophyll a, chlorophyll b, total chlorophyll and total carotenoids were calculated and were presented as (μg/g dw). The leaves samples collected were analysed also for total N, % by Kjeldahl method.

#### 2.3. Statistical analysis and chemometrics

Chemometrics methods were used for qualitative analysis of data obtained, as PCA and LDA analysis. PCA is a well-known technique for reducing the dimensionality and simplifying the visualization of complex multivariate data sets. PCA transforms the original variables into a smaller number of mutually orthogonal variables called principal components (PCs). PCA is mostly used as a tool in exploratory data analysis and for making predictive models. Linear discriminant analysis (LDA) is a supervised classification method, as it is used to build classification rules for a number of prespecified classes. These rules (model) are later used for allocating new and unknown samples to the most probable class.
Another important application of discriminant analysis is to help in interpreting differences between groups of samples. [15] This method also helps to better understand the distribution of the feature data than PCA analysis. Statistical analysis and chemometrics were done with Unscrambler (Camo, Norway) software packages.

3. Results and discussion

3.1. NDVI, total nitrogen, pigments in Rosa damascena Mill leaves

The NDVI values, total nitrogen, total chlorophyll and carotenoids in examined Rosa damascena Mill leaves are presented in Table 2. The obtained results about NDVI index for all measured leaves in the field showed a difference in the range of values between conventional and organic rose bushes. For instance, the range of NDVI index in leaves of rose bushes from conventional farming (0.61-0.85) was smaller than the leaves from organic farming (0.53-0.85). On the base of those results we suggested that some leaves from organic farming could contain lower chlorophyll and total nitrogen, although of that the mean NDVI values form both agriculture systems were very close. The biochemical leaves analysis confirmed our assuming. According to the values of total chlorophyll and nitrogen content in the leaves was found a significant statistical difference between the organic and conventional agricultural systems. The leaves in conventional rose oil bearing farms contain more total nitrogen and chlorophyll content than these from organic farms. The mean values of total nitrogen and total chlorophyll in the leaves belonging to conventional farming were 2,18 % and 1461.6 μg/g dw and 1,87 %, and 1227.1 μg/g dw to organic farming. Similar results obtained Ponder and Hallmann, 2019 [9] investigated biologically active compounds of raspberry leaves from conventional and organic farming. The authors have found higher total chlorophyll content and individual forms of chlorophylls a and b in raspberry leaves from conventional farming than organic. According to the results we obtained the chlorophyll b content was also higher in rose leaves from conventional farming 568.6 μg/g dw in a comparison to the rose leaves from organic farming 441.2 μg/g dw. The scatter plots of correlation between total N, % and total chlorophyll content, μg/g dw are presented in Figure 1. The relationship between nitrogen and chlorophyll in the leaves is not news, in our study case the values of R was 0,47. The figure illustrated not only lower values for total N and chlorophyll content in the leaves of organic farming and higher values in leaves of conventional farming, but also it was found one common group of values of leaves at both conventional and organic farming. Those samples were collected from Farms 01 and 02 - conventional farming with the low input system. Therefore, both farms are not belong to typical conventional agricultural systems, but are between typical conventional and organic farming. Such kind of conservation agriculture with mulching systems application is a way to increase soil fertility, reduce soil erosion, increase organic matter and improve soil buffer capacity.

The figures 2 and 3 illustrate the normal probability distribution of total N, % and chlorophyll content, μg/g dw in the leaves of organic, low input and conventional farming, which the figures clearly showed the position of the type agricultural system on the base of studied biochemical parameters. Basayigit et al, 2013 [11] monitored the change of chlorophyll content during the vegetation process in the Rosa damascena leaves. They found the weekly change of chlorophyll content during vegetation period, as chlorophyll content reached the highest value in blooming period and maintained this level until the end of harvest. The plant keeping chlorophyll content at the highest level during the blooming period, used all of its energy for blooming metabolism.

In addition to the presence of photosynthetic pigments, as carotenoids also exist in oil bearing rose leaves. Their concentration in leaves depends mainly on the level of chlorophyll. Chlorophyll is associated with the function of carotenoids, which are produced by plants, mainly to protect the photosynthetic system against photooxidation. Carotenoids are synthesized via the general biosynthetic pathway within the chloroplasts of plants. Organic rose leaves in our study contained a significant higher level of total carotenoids 133,3 μg/g dw compared to that of conventional oil bearing rose leaves 120,3 μg/g dw.
Table 2. NDVI values, total nitrogen, total chlorophyll and carotenoids in examined *Rosa damascena* Mill leaves depending on cultivation system.

| Parameters                          | Organic farming (n=24) | Conventional farming (n=24) | p-Value |
|-------------------------------------|------------------------|-----------------------------|---------|
|                                     | range      | mean | range      | mean |         |
| NDVI                                | 0,53-0,85  | 0,77 | 0,61-0,85  | 0,78 | 0,01    |
| Total N, %                          | 1,53-2,26  | 1,87 | 1,94-2,44  | 2,18 | 0,001   |
| Chlorophyll a, μg/g dw              | 527,2-1221,0 | 527,2 | 560,6-1362,5 | 899,5 | N.S     |
| Chlorophyll b, μg/g dw              | 300,4-682,0 | 441,2 | 318,8-1475,3 | 568,6 | 0,01    |
| Total Chlorophyll, μg/g dw          | 827,6-1903,5 | 1227,1 | 879,4-2837,8 | 1461,6 | 0,02    |
| Total Carotenoids, μg/g dw          | 91,4-176,5 | 133,0 | 80,9-145,7 | 120,3 | 0,02    |
| Ratio Carotenoids/Chlorophyll       | 0,07-0,26  | 0,12 | 0,05-0,14  | 0,09 | 0,001   |

Figure 1. Scatter plots of correlation between total N, % and chlorophyll, μg/g dw content in the leaves of conventional and organic oil bearing rose farming.
Figure 2. Normal probability distribution of total N,% content in leaves of organic, low input and conventional farming.

Figure 3. Normal probability distribution of total chlorophyll content, μg/g dw in leaves of organic, low input and conventional farming.

3.2. PCA and LDA analysis
On the basis of the values of NDVI index, total N, total chlorophyll and carotenoids content for oil bearing leaves Principal component analyses were performed to investigate the leaves samples distribution in a multidimensional space (Figure 4). In the score plot, the grouping of objects can be recognized. Factor 1 explained 96% of the data variance, and formed one group clearly with conventional farming, but the rest of them – organic and low input system seem mix in the multidimensional space.
Figure 4. Score plot of leaves samples on the first two principal components according to the values of NDVI index, total N, total chlorophyll and carotenoids content.

LDA is based on the assumption that the samples lie closer in measurement space, therefore they are likely to belong to the same category. Multidimensional space is constructed containing the scores corresponding to each class. Each class model treats new samples separately, and an assessment of class membership is made on the basis of the distance of any given sample to the center of the class. Each sample could be assigned to a single class, more than one class, or none of the defined classes. The Discrimination plot is a visualization of the LDA results for the training samples. Every sample is displayed, color-coded by class, and the axes are for two of the classes in the model. The leaves samples are categorized according to the using agricultural system: conventional; conventional with low input system; and organic system. It was performed LDA analysis on the base according to the values of NDVI index, total N and total chlorophyll content in *Rosa damascena* leaves. The results are graphically presented in LDA discrimination plot (Figure 5). Accuracy of discrimination of the leaves according to the using agricultural system was very high 91.67%. In LDA discrimination plot the leaves belonging to conventional and organic fields formed clearly two separate groups and one among them with leaves belonging to the conventional low input system. The confusion matrix (Table 3) is a matrix used for visualization for classification results. It carries information about the predicted and actual classifications of samples, with each row showing the instances in a predicted class, and each column representing the instances in an actual class. According to results obtained all 8 leaves at conventional system were predicted properly as conventional and from all 24 leaves at organic farming, 20 were predicted as organic and the rest 4 were non correctly recognized as leaves in low input system. The all 16 leaves samples at low input system were nicely attributed to the low input class.

LDA discrimination plot on the base of the values of total N in the leaves is presented on Figure 6, where the accuracy of discrimination of the leaves according to the using agricultural system was also high 81.25%. The accuracy of discrimination is relatively high using values of total chlorophyll – 64.58 (Figure 7) and the lowest accuracy of discrimination was found using NDVI index – 41.67% (Figure 8).
Figure 5. LDA discrimination plot of *Rosa damascena* leaves according to the values of NDVI index, total N and total chlorophyll content.

Table 3. Confusion matrix - discrimination of *Rosa damascena* leaves according to the values of NDVI index, total N and total chlorophyll content.

| Agricultural system/class | n, actual | low input | conventional | organic |
|---------------------------|-----------|-----------|--------------|---------|
| Low input                 | 16        | 16        | 0            | 4       |
| Conventional              | 8         | 0         | 8            | 0       |
| Organic                   | 24        | 0         | 0            | 20      |

Figure 6. LDA discrimination plot of *Rosa damascena* leaves according to the values of Total N, %.
Figure 7. LDA discrimination plot of *Rosa damascena* leaves according to the values of total chlorophyll content, μg/g dw.

Figure 8. LDA discrimination plot of *Rosa damascena* leaves according to NDVI index.

4. Conclusion

It was found an impact of agriculture system on the physiological status of *Rosa damascena* leaves. The rose oil bearing leaves from conventional farms contained more total nitrogen and chlorophyll content in comparison to organic farms. Organic rose leaves were characterized by a significantly higher content of total carotenoids. The data of total N, total chlorophyll content and NDVI index could be used to develop different LDA models with relatively high accuracy of determination for *Rosa damascena* leaves discrimination depending on the type of applied agricultural system.
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