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COMPARATIVE STUDY OF A NEW VARIETY OF MULBERRY OF THE SPECIES MORUS ALBA L.

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Abstract

The development of sericulture is closely related to the cultivation of mulberry. Mulberry leaf is the main food material for the silkworms *(Bombyx Mori)*. The mulberry leaves contain all the necessary nutrients to ensure normal growth and productivity of the silkworms. The main aim of the present study is to investigate and analyze the presence of some elements in the mulberry leaves of a new variety and to compare their levels to those of a well-known variety (Vratsa 1). The chemical analysis of the two varieties was performed in the analytical laboratory of Agricultural University – Plovdiv. The concentrations of nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), copper (Cu), iron (Fe), manganese (Mn) and zinc (Zn) were studied. It was concluded that the new mulberry variety contains adequate level of food nutrients required for silkworm diet. Moreover, the analysis shows that the content of most of the tested elements in the new variety significantly exceed those of the variety used as a reference. As the new variety can be planted on weaker soils, its range could be expanded and thus to ensure that even less nutrient-rich soils will be used to the best advantage.

Key words: Bombyx mori L., Morus alba L., micro elements, macro elements, heavy metals

INTRODUCTION

The mulberry belongs to the *Urticales* order, the *Moraceae* family and the genus *Morus*, with more than 30 species and 300 varieties. It comes from China and other species originate in other temperate countries and despite this, they can be considered "cosmopolitan" for their capacity of adaptation to different climates and altitudes. There are three main species of mulberry: black (*M. nigra*), red (*M. rubra*) and white (*M. alba*). (Yigit, Akar, Baydas, & Buyukyildiz, 2010).

Mulberries are a good source of vitamins and minerals and contain an especially high amount of anthocyanin (Zhang W, Han F, He J, 2008). Mulberry is a very good source of ascorbic acid, carotene, vitamin B1, folic acid, folinic acid, isoquercetin, quercetin, tannins, flavonoids and saponins. Due to its chemical composition and pharmacological functions it is being utilized as a medicinal plant (Mahesh et al., 2017).

Studies on the nutritive compounds (including crude protein, crude fat, mineral elements, total anthocyanins, total polyphenols, total flavonoids, and total sugars) in the mulberry leaves have been published (Linghong Liang, Xiangyang Wu, Maomao Zhu, Weiguo Zhao, 1 Fang Li, Ye Zou, 2012). The relationship between the mineral contents of white, red and black mulberry fruits and leaves has been also investigated (Yigit et al., 2010).

Mulberry trees have intensive growth and long life. Under favorable conditions and by applying good agricultural practices, mulberry can be exploited for up to 60 years for tree species and 20-30 years for bushes. For the free-growing trees, the average lifespan is 200 years and they can reach 25 m in height.

Analyses of mulberry leaves were carried out in order to determine the seasonal changes in the contents of Ca, K, P, Mg and S (Levickienė, Vaitkevičienė, Jarienė, & Mažeika, 2018). The authors 0 Lquality indicators of soil and determined the best harvest time to maximize the nutrient contents of white mulberry leaves (*Morus alba L.*) from two different cultivars.

Mulberry requires light soils, rich in nutrients and with good water retention. It does not grow well on heavy soils that do not provide normal aeration and favorable thermal regime. There are high requirements to the content of minerals and elements in the soil due to the specific nature of exploitation of this tree species. Mulberry needs micro and macro elements as nitrogen (N), phosphorous (P), potassium (K), calcium (Ca), zinc (Zn), copper (Cu), iron (Fe), molybdenum (Mo), boron (B) and others.

Three approaches are possible in the diagnosis of the nutrient status of fruit trees: (a) visual symptoms of deficiency or excess can be used to identify acute nutritional problems. However, symptoms vary from crop to crop and if more than one element is deficient, visual diagnosis may not work; (b) soil analysis can help to point out possible problem areas (related to pH and salinity), but it is not easy to use this method as a predictor for woody perennials; (c) plants analysis is a powerful diagnostic tool for agronomists, as it allows one to define the species of plants, their adherence type, state of growth, food value, nutrient deficiency and resistance to disease (Yigit et al., 2010).

Mulberry leaves are basic food material for Bombyx mori L. as the leaf mass contains all the nutrients, proteins, carbohydrates, fats, water, minerals and vitamins needed for normal growth and productivity of the silkworms (E. Kipriotis, N. Petkov, D. Grekov, 1999).

The nutritious leaves are the most important growth regulating factors for the silkworms. Being a monophagous insect, silkworms derive almost all the nutrients essential for their survival from the mulberry leaves. Hence, good quality of nutritious mulberry leaves should be fed in abundant quantity for the quality silkworm seed and cocoon production (Singh, Ram, Alam, & Nirmal Kumar, 2016).

Detailed studies on the biochemical composition and pharmacological properties of Mulberry *(Morus spp.)* have been reviewed and revealed that, mulberry leaf with more moisture, protein, sugar, carbohydrates and less minerals and crude fiber content found to be best from the silkworm nutrition point of view (Mahesh et al., 2017).

In different environments, many factors are known to affect the concentration of heavy metals in both soil and plants, including industrialization, traffic density and unknown atmospheric deposits. Although mulberries in some areas are grown without using chemical fertilizers and pesticides, they are thought to have been affected by heavy metals. The concentrations of some heavy metals in mulberries (*Morus Alba L.*) grown at different distances from the roadsides have been studied (Pehluvan, Karlidag, & Turan, 2012).

Soil and climatic conditions in Bulgaria are a prerequisite for the good growth of mulberry and obtaining high yields of leaf mass from it. The rich genetic diversity that exists in Bulgaria is due to the deep long-standing traditions and the interest of the sericulture farmers to increase the yield of cocoons and raw silk. The presence of many farms and especially of individual trees makes it possible to observe and select suitable new varieties of *Morus alba* with high values of the main micro- and macroelements.

The main aim of the present study is to investigate the presence of some elements in the mulberry leaves of a new variety compared to a reference one (for which we have used a variety called Vratsa 1).

MATERIALS AND METHODS

During our field research in the period 2017-2019 we found different varieties with interesting characteristics, which are kept already in our genetic bank or in our sericulture experiment station and are subject of detailed study. One of these varieties was found in the central Rhodope Mountains and was observed to grow on an extremely poor and rocky slope facing south. The variety made a strong visual impression on us and turned out to develop on visibly weak soils. We decided to perform an extensive chemical analysis of the leaf mass to determine the difference, if any, to a reference variety called Vratsa 1.

Vratsa 1 variety, on its turn, is an interspecific hybrid between the *Kinriu* variety and the wild species. It's monoecious and the fruits are black and small. The leaves are large, cordate and entire, with a smooth margin. The variety is high-yielding and suitable for use in the silkworm diet. With the appropriate exploitation and low-stem formation, 1500-1600 kg of mulberry leaf is obtained during the spring, 900-1000 kg during the summer and 800-900 kg per decare during the autumn. The variety was grown at the sericulture experimental station of the Agricultural University of Plovdiv, where the soils are high in humus. No chemical fertilizers or pesticides were used for treating the trees, hence the leaf mass that was obtained can be considered organic.

The new variety that we have been studying was propagated by classical methods by grafting a dormant bud and three of the saplings were planted in different locations within one square kilometer in the area where it was found. The saplings used for sampling were three years old. A representative samples were taken from the lower, middle and upper part of the saplings.

The leaves had a high turgor throughout the growing season, despite the relatively high temperatures, hence it could be used to feed the silkworms on. The leaves were observed to be dark green and with larger leaf size compared to the reference. We noticed that the fruits are also bigger which is basically considered an “unfavorable” characteristic during the silkworm feeding. As our attention was focused only on the quality of mulberry leaves, we have not performed any studies on the size and the chemical composition of the fruits.

The chemical analysis of the two varieties was carried out in the analytical laboratory of Agricultural University – Plovdiv. The content of macroelements (nitrogen, phosphorus, potassium); microelements (calcium and magnesium) and heavy metals (copper, iron, manganese and zinc) were analyzed.

RESULTS AND DISCUSSION

As a result of rising agricultural land prices and the use of fertile soils with high humus content for the cultivation of intensive crops, the less and less nutrient-rich soils will need to be utilized. On the other hand, the use of mulberry as an anti-erosion species requires expanding the range of its cultivation on such poorly stocked soils. All that facts reveal the possibility of mulberry to be grown in these soils / terrains without reducing the content of nutrients in the leaves and, accordingly, to maintain high yields of mulberry leaf.

The nutrients in plants and their roles are influenced basically by the intensity of cation exchange, growing conditions, moisture, pH value and other factors. Therefore soil analyses are not sufficient for a picture of the state of nutrition, whereas the leaves quickly provide information on the macro- and micro-elements (Yigit et al., 2010).

According to the results of the chemical analyses, the new variety (grown on poorly stocked rocky terrains) shows significant differences in the values of the main elements (Table 1).

It was indicated during the visual observations that the leaves throughout the growing season retain their typically green color and high turgor content.

In the sections below the content of the different elements is discussed separately accompanied by a brief description of their role and significance in regards to the quality of the leaves.

Table 1. Differences of the elements among the analyzed elements

№	Element	Unit	Difference (Δ)	Higher content of the analyzed element
1	Nitrogen (N)	%	1.81	Sample 1 (New variety)
2	Phosphorous (P2O5)	%	-0.03	Sample 2 (Vratsa 1)
3	Potassium (K2O)	%	-0.38	Sample 2 (Vratsa 1)
4	Calcium (Ca)	mg/kg	6513	Sample 1 (New variety)
5	Magnesium (Mg)	mg/kg	2578	Sample 1 (New variety)
6	Copper (Cu)	mg/kg	6.07	Sample 1 (New variety)
7	Iron (Fe)	mg/kg	41.9	Sample 1 (New variety)
8	Zinc (Zn)	mg/kg	25.95	Sample 1 (New variety)
9	Manganese (Mn)	mg/kg	9.65	Sample 1 (New variety)

Table 2. Content of the macroelements in mulberry leaves

№	Element	Unit	Method	Sample Name	Result	Conditions
1	Nitrogen (N)	%	Inter-laboratory method	Sample 1 (New variety)	3.60	T °C=20±2
				Sample 2 (Vratsa 1)	1.79	
2	Phosphorous (P_2O_5)	%	Inter-laboratory method	Sample 1 (New variety)	0.61	T °C=20±2
				Sample 2 (Vratsa 1)	0.64	
3	Potassium (K_2O)	%	Inter-laboratory method	Sample 1 (New variety)	1.37	T °C=20±2
				Sample 2 (Vratsa 1)	1.75	

The levels of macroelements found in the mulberry leaves (in both new variety and the reference) are shown in Table 2. It can be seen from the results in that there is 1.81 % nitrogen more in the new variety compared to the reference Vratsa 1. Given that nitrogen is essential in the growth and accumulation of vegetative mass as well as in the synthesis of proteins in the plant body, the leaves seem to be suitable for use as a basic food material for silkworms to be fed on.

According to some authors (Ovesenska, 1992), the growth of the tree is slowed down and stopped in case of severe nitrogen deficiency. The leaves roughen quickly, change their color to light green and remain poor in crude protein content. In our visual studies throughout the growing season, despite the high temperatures, we did not notice leaf wilting or discoloration, which indicates that the growth of both leaf mass and annual shoots was observed throughout the growing season.

Another main indicator is the phosphorus content, where a slight decrease (0.03 %) has been observed compared to the reference. Given that the phosphorus content affects fruiting, this small difference would help reduce the acceleration of fruiting and the use of mulberry leaves for a longer period of time during the silkworm rearing. This would not prevent the use of the mulberry leaf together with the unripe fruit, as the fruit will still have a lower water content and this in turn will not lead to an increase in indoor humidity and some diseases could be avoided (e.g. Flacherie).

The role of the phosphorus is to increase the cold resistance, to accelerate fruiting and to stimulate the development of the root system. It has a weak effect on the accumulation of leaf mass, but promotes better absorption of nitrogen. In the absence of phosphorus black spots are formed on the edges of the leaf blade, which can lead to leaf death. Hence, a good balance between nitrogen and phosphorus helps the absorption of other nutrients to be improved.

Potassium affects the formation of starch in the tissues of the tree and leads to faster leaves development and ripening of the fruits in the tree species. It helps to increase the assimilation processes and resistance to fungal diseases. The potassium deficiency can lead to curly leaves accompanied by an appearance of dark brown spots.

The difference between the Vratsa 1 variety and the new variety is 0.38 % in favor of Vratsa 1. However, no signs of nitrogen deficiency were observed on the leaves. All this gives us reason to believe that the new variety can thrive in soils poorly stocked with nitrogen (N), phosphorous (P) and potassium (K).

The concentrations of the microelements in the mulberry leaves are presented in Table 3. In regards to the silkworms rearing, those elements are considered important indicators as Ca and Mg are known to accelerate the growth of silkworms and to reduce the larval duration. Decrease in the intake of these elements can reduce the body weight of silkworms (Chakrabarti, 1997).

As expected, the dominant one in the leaves was Ca. This microelement is well known to influence the overall resistance of mulberry by regulating microbiological processes in the soil and stimulating the formation of root hairs and accelerating the growth and the development of the root system.

Table 3. Content of the microelements in mulberry leaves

№	Element	Unit	Method	Sample Name	Result	Conditions
1	Calcium (Ca)	mg/kg	Inter-laboratory method	Sample 1 (New variety)	16122	T °C=20±2
				Sample 2 (Vratsa 1)	9609	
2	Magnesium (Mg)	mg/kg	Inter-laboratory method	Sample 1 (New variety)	6608	T °C=20±2
				Sample 2 (Vratsa 1)	4030	

The performed analyzes show that the values of the new variety exceed those of the reference variety (Vratsa 1) with 6513 mg/kg. This difference confirms the fact that, regardless of the poor soils in which the new mulberry variety was grown, sustainable fruiting is maintained throughout the growing season. In our opinion, this is due to the increased content of calcium and its direct impact on the development of the root system.

Magnesium (Mg) is a crucial structural component of the chlorophyll molecule and is necessary for the functioning of plant enzymes in the production of carbohydrates, sugars and fats (Weyers, J.;Paterson, 2002).

In the samples that were analyzed, its content varied between 4030 mg/kg (for the reference variety) and 6608 mg/kg (for the new variety), which means 2578 mg / kg more Mg in favor of the new variety.

Table 4. Content of heavy metals in mulberry leaves

№	Element	Unit	Method	Sample Name	Result	Conditions
1	Copper (Cu)	mg/kg	Inter-laboratory method	Sample 1 (New variety)	10.1	T °C=20±2
				Sample 2 (Vratsa 1)	4.03	
2	Iron (Fe)	mg/kg	Inter-laboratory method	Sample 1 (New variety)	74.01	T °C=20±2
				Sample 2 (Vratsa 1)	32.11	
3	Zinc (Zn)	mg/kg	Inter-laboratory method	Sample 1 (New variety)	41.11	T °C=20±2
				Sample 2 (Vratsa 1)	15.16	
4	Manganese (Mn)	mg/kg	Inter-laboratory method	Sample 1 (New variety)	35.4	T °C=20±2
				Sample 2 (Vratsa 1)	25.75	

The levels of heavy metals in the mulberry leaves are given in Table 4. These elements have an important role in the mulberry growing, as in case of specific mineral deficiency in the soil, such as iron or zinc, the leaves produce insufficient chlorophyll and turn yellow (chlorosis).

According to (Vassilev, A., 2010), the soil contamination with copper (Cu) is associated not only with the extraction and processing of copper ores, but also with the use of copper-containing pesticides in agriculture. It was found that the use of copper-containing fungicides in plant protection increases the copper content. Our studies show that the copper content in the new mulberry variety exceeds by 2.5 times the values of the reference (Vratsa 1) with a difference in the values of 6.07 mg/kg.

In regards to the iron content, it was noticed to be higher in the new variety with 41.9 mg/kg (compared to Vratsa 1 variety). This was confirmed also by our visual observations since throughout the growing season we did not notice yellowing of the periphery of the leaves, which is a typical sign of iron deficiency or the appearance of chlorosis on the upper leaves of the shoot.

Zinc (Zn) is an element that has an important role in the metabolism: when there is an increased amount of zinc, it can accumulate in cells causing toxicity and metabolic disorders (Albergoni, V, E. Piccinni, 1980).

The content of zinc in the new variety is relatively high with values of 41.11 mg/kg while in

Vratsa 1 variety it is only 15.16 mg/kg.

(Benţea et al., 2012) conducted a study on the effects of zinc (Zn) used as a dietary supplement in the diet of silkworm *(Bombyx mori L.)*. According to the same authors, the content of zinc (Zn) in the diet does not show a negative effect, but has the opposite effect. There is a significant increase in the weight of the larvae, has a positive effect on the silk gland mass, increases the fresh cocoon weight, the cocoon shell weight and the filament length. This gives us a reason to think that the increased zinc content in the new variety would not be a disadvantage if the mulberry leaves are used in the silkworm diet.

Manganese (Mn) is an essential element for the synthesis of chlorophyll. It is immobile and its principal function is to activate some of the enzyme systems in plant physiology and to some extent regulation of Fe metabolism. In addition, it has a close relation with N metabolism (protein), assimilation of carbohydrates and formation of vitamin C (MahadevaA, 2016).

The results that we have obtained indicate that the manganese (Mn) content in the leaf mass from the two varieties ranged from 25.75 mg/kg to 35.4 mg/kg. The significantly higher amount of Mn was found in the leaves of the new variety.

We have assumed that some of these values might appear as a result of some soil differences. The new variety may be planted on weaker soils, thus its range could be expanded. Based on the promising results that we have obtained, we would propose also different methods of propagation to be explored. Moreover, the quality of the fruits of the new variety could be studied in order to use them as a food product for humans.

CONCLUSIONS

The results obtained by the chemical analyses supported the hypothesis that the new mulberry variety contains adequate level of food nutrients required for normal silkworm body functioning. In terms of numbers, it can be concluded that the new variety shows significantly higher values of macro- and microelements (including heavy metals) in the leaves compared to the reference. The results of the chemical analysis show that the difference in the levels of the elements ranges from 1.81 % (for the nitrogen content) to 6513 mg/kg (for the levels of calcium). Decreasing of the values was observed only for potassium, which is with 0.38% more in Vratsa 1 variety compared to the new variety that has been studied.

In summary, there is a balance of the chemical elements in the new variety compared to the reference one (Vratsa 1 variety). Hence, the new variety might be proposed for registration within the national and European lists.

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