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IMPACT OF BIOLOGICAL FERTILIZER ON THE ANATOMICAL STRUCTURES OF SHEET FROM LEAF LETTUCE (*LACTUCA SATIVA*)

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Abstract

The different growing conditions and in particular fertilization, affects the physiology, morphology and anatomy of the plants and provides the necessary information to compare their biological plasticity. The aim of the present study was to trace the effect of optimized nutrition in at organic cultivation of greenhouse lettuce using different formulations of organic fertilizers, on the anatomical structure of the leaves in order to increase plant resistance to the abiotic stress. The experimental production of seedlings was conducted at the base of Agricultural University - Plovdiv, Bulgaria. Four bio fertilizers were used in six variants of biological plant cultivation. Morpho-anatomical analyzes of the leaf blate, epidermis and stomata were performed using the methods of comparative anatomy. In all variants of lettuce with organic fertilization applied, a positive effect on the anatomical parameters of the leaves was observed, which was a prerequisite for increasing the biological resistance of the plants.

Key words: *anatomy, biological fertilization, leaf, lettuce.*

INTRODUCTION

Greenhouse vegetable production is associated with the use of significant amounts of fertilizers and pesticides, which increases the risk of soil, groundwater and production of hazardous substances (Chen et al., 2014; Zhang et al., 2017). Today, technologies in agricultural production are closely linked to consumer needs and the demand for products that meet the requirements of food quality and safety (Gheorghiu et al., 2013; Terziev & Arabska, 2015; Day et al., 2008)

Biological analysis of plants is the best method for measuring the effect of the application of various chemical and organic substances in the soil (Basta et al., 2005). A number of studies point to varying degrees of phytochemical availability of organic and inorganic elements in soil (Bell et al., 1991; Brown et al., 1998; Cunningham et al., 1975; Mahler et al., 1987). Factors such as variety, agro-technical practices, climatic conditions, degree of ripeness, harvest time and storage conditions are of particular importance. The change in the individual elements of the technology is an important tool for obtaining healthy and higher biological value plant products with increased amount of bioactive substances. This is a potential for increasing commercial production of leafy vegetables (Dumas et al., 2003; Barba et al., 2016; Ebert, 2014).

Of the leafy vegetables group, lettuce is grown on all continents, but the largest consumers and producers are the United States (91,000 hectares) and Europe (a total EU area of 80 000 ha) (Pink & Keane, 1993). In Europe, it is the most widely used vegetable product for fresh consumption. (Serna et al., 2012). In Bulgaria, salad is a widely grown field and greenhouse culture (Retrieved from http://www.sunoilbg.org/language/en/uploads/files/documents0/document_6638d0d4b9d0a517e62b8bd4aef20ced.pdf)

The objectives of modern lettuce selection are divided into three main areas: (1) resistance to diseases and pests, (2) increased yield and uniformity, and (3) improvement of horticultural characteristics, such as quality and sustainability (Pink & Keane, 1993).

When the nitrate content of lettuce leaves exceeds the daily allowance, consumption of this crop poses a health hazard (Lorenz, 1978; Sanchez et al., 2005). On the other hand, increased nitrate uptake generates more vegetative growth, improves leaf morphology (e.g. length and width), but leaf thickness can significantly reduce (Soundy & Smith, 1992). High nitrogen content in lettuce usually leads to storage difficulties and the ability to quickly rot after harvest. Decay in this crop generates significant financial losses for producers (Hoque et al., 2010; Fageria, 2009).

The quality of the salad can be enhanced by organic farming. The use of low nitrogen organic fertilizers is cost-effective and provides the necessary nutrients for this crop. For the purposes of industrial production, it is important to determine the appropriate organic fertilizers ensuring high yield and quality according to the production line and technology used (Fontes et al., 1997; Heckman et al., 2003).

There are a number of characteristics that make lettuce suitable for biological research. It has a relatively short growing season, forms a large amount of leaf mass with a large leaf area and accumulates the most nitrates in the leaves compared to other leafy vegetables (Pink & Keane, 1993).

Studies by Falla et al. (2000) and Honor et al. (2009) indicate that plant species reveal the quality of the environment in which they develop by altering their leaf anatomy and physiology, and these changes in leaf indices may be used in the reasonable and accurate assessment of habitat quality.

The epidermis, as the outermost layer of the assimilating organs, performing a protective and regulatory function, provides information on the degree of response of the plant species (Aneli, 1975). Changes in the size and number of major epidermal cells are one of the main indicators of the response of a plant organism to favorable or unfavorable changes in the environment, and in particular soil. (Ninova & Dushkova, 1977; Koev et al., 2001; Gostin & Ivanescu, 2007).

As the main structures of the epidermis, carrying out gas exchange and transpiration, the morphology and number of the stomata are also the main object of anatomic-morpholytic analysis for the plant response to abiotic stress (Dimitrova, 2000; Gostin & Ivanescu, 2007).

The purpose of this study is to investigate the effect of optimized nutrition on organic greenhouse salad cultivation with organic fertilizers of different composition on the anatomical structure of the leaves in order to increase the resistance of plants to abiotic stress.

MATERIALS AND METHODS

The initial phase of the experimental activity was carried out in 2019 by planting a greenhouse experience in the first ten days of month X 2018 and harvested II-III months of 2019 in economic maturity - 200-300 g. A leaf salad-type Batavia variety "Maritima" was used for analysis. The plants were planted in a polyethylene greenhouse at the experimental base of Agricultural University - Plovdiv, Bulgaria.

Seedling production

For the purpose of the experiment, bio-seeds of the above variety were purchased. Seeds for seedling production were sown on October 10. 1000 plants were grown and container technology was applied using 150-ounce stereo boards. An organic seedling mixture was used - 80% Perlite: 20% Lumbricompost developed by the authors (Kostadinov & Filipov, 2013) for bioproduction of seedlings.

Planting

The plants were planted in phase of 4-5 leaf on November 8 in the polyethylene greenhouses of AU-Plovdiv. After the plow was milled and milled, the test surface was profiled on a high flat bed. A 4-row 70 + 30 + 30 + 30/30 cm 30 row was used. The experiment was based on a block method of four replicates of 28 plants per replicate, with a plot size of 3.36 m^2. Internal guards were 8 units/rep. The front and rear guards were 12 each plants. The reported plots included 20 pieces each plants in repetition.

Paid options

The greenhouse experience was laid out with 6 variants with a total area of 450 m^2, of which 375 m^2 with organic fertilization. Irrigation was carried out with a drip system. The following options were used to study the effect of selected biotors on the anatomical arrangement of lettuce leaves under conditions of transition to organic production:

1. Control-MT (mineral fertilization - NPK)
2. Control - not fertilized
3. Italpolina
4. Arkobaleno
5. Lumbricompost
6. Ecoprop NX

The Italpolina and Lumbrikompost granular organic fertilizers have been applied in organic vegetable production, while the Arkobaleno (granular) and Ekoprop NX (liquid) organic fertilizers are less known but promising.

The granulated fertilizers were imported according to a pre-developed scheme as basic fertilization with pre-seed tillage in the following norms: N - 12.5 kg/da, P2O5 - 1.25 kg/da, + K2O - 4.75 kg/da, Italpolina - 25 kg, Arkobaleno - 100 kg/da, and Lumbrikompost - 400 l/da. The organic fertilizer Ekoprop NX dissolved in water was introduced by four times pouring at a dose of 100g / da- from phase 5 leaf at 14-day intervals.

Characteristics of biotores

Italpolina (4N - $4P_2O_5$ - $4K_2O$) - is a dried poultry manure. Due to the high content of organic and active ingredients, in a short time leads to an increase in the microbiological, physical (structure, water retention) and chemical (buffering) properties of the soil. All these benefits help to reduce the loss of nitrogen, phosphorus and trace elements. Italpolina is rich in: organic ingredients, trace elements and beneficial microflora. COMPOSITION: N - 4%, P_20_5 - 4%, K_20 - 4%, M_2O - 0.5%, Water-soluble Fe - 0.8%, Water-soluble B - 0.2%, Organic C - 41%, Organic matter 70, 7%, Humic acids 5%, Fulvo acids 12%, Humidity 12%, pH 7.

Arkobaleno - simultaneously supplies nitrogen, phosphorus, potassium and organic matter (high fraction). Its completely organic form is not subject to washing phenomena. Its organic content is guaranteed as its components are from farms controlled. There is a slow transfer, therefore transmits nutrients to the plant throughout the growing season and allows for a healthy and balanced development. Improves the quality and storage capacity of plants. Allows gradual restoration of soil fertility and humus. Does not contain solid urban waste. Contains: Organic N - 45% - 3.5%; K20 - 3.5%; CaO - 5-8%; MgO - 0.8-1%; Organic C of biological origin - 30%; Organic matter (CX1.724) - 55-60%; Extractable organic matter (% of organic matter) - 30-35%; Humified organic matter (% of organic matter) - 12-14%; Humified organic matter (% of recoverable material) - 38-40%; Humification rate (HR) - 10-13%; Degree of Humification (DM) - 40-42%; Humification Index (Hi) - 1.3-1.4%; Fe - 3100-3200 ppm; B - 40-50 ppm; Cu - 190-200 ppm; Mn - 850-900 ppm; Zn - 550-560 ppm; Humidity - 13-15%; pH (H2O) 6-8.

Lumbrikompost (Organic fertilizer). An eco-friendly bioproduct resulting from the nutrition of organic red California worms. It is homogeneous, odorless. Contains dry matter 44.2%, moisture 55.8%, organic matter 47.24%, organic carbon 27.4%, pH (H2O) 7.85, salts 3.89 mS, total N (abs. Dry) - 1.71%, total P_2O_5 (abs. Dry) - 3.49 %, total K_2O 1.71 (abs. dry), total CaO (abs. dry) 6.25, total MgO (abs. dry) 2.14, Water-soluble (natural moisture) N-NH_4 - 1.75 mg/kg, Water-soluble (natural moisture) N- NO_3 - 804 mg/kg, Water soluble (natural moisture) P_2O_5 - 432.5 mg/kg, Water soluble (natural moisture) K_2O - 3282 mg/kg, Water soluble (natural moisture) CaO - 491 mg/kg, Water soluble (natural moisture) MgO - 353 mg/kg, Water-soluble (natural moisture) Cl - 555 mg/kg, Water-soluble (eu natural moisture) Cl - 555 mg/kg, Water soluble (natural moisture) SO4 - 547 mg/kg, Water soluble (natural moisture) Na - 229 mg/kg, Cd <0.3 mg/kg, Cr 46.8 mg/kg, Cu 124 mg/kg, Ni 24.0, Pb 17.1 mg/kg, Hg <0.05 mg/kg, Zn 295 mg/kg, B 50.8 mg/kg, Fe 8546 mg/kg, Mn 531 mg/kg.

Ekoprop NX is a microbiological fertilizer developed specifically for depleted soils. Its composition improves the efficiency of plant nutrition and the efficiency of water absorption. The number of young roots is greatly increasing, which in turn increases the better absorption of nutrients. Fertilizer contains 1% Glumos spp Applied to vegetables at a dose of 100 g / da. After planting, fertilize immediately using a large amount of water. It is applied 3-4 times during the growing season with an interval of 12-15 days. In thinner soils and sensitive crops, the dose is up to 150 g/da. It can also be applied with the last treatment, spreading superficially with subsequent incorporation of 10-15 cm and subsequent treatment after 7-15 days.

Plant material for anatomical studies

The plant material was collected when the plants reached maturity at the end of February - early March 2019. 3 fully developed middle leaves of 10 plants were used, which were fixed in 70% ethyl alcohol. Semi-permanent microscopic preparations were prepared from the middle of the leaf blade. Anatomic-morphological analyzes were performed using the methods of comparative anatomy (Metcalfe & Chalk, 1950) by examining the following indicators:

• Thickness leaf layers µm - TLL;
• Thickness of the upper covering tissue µm - TUE;
• Thickness of the lower covering tissue µm - TLE;
• Number of stomata upper epidermis in 1 mm^2 - NSUE;
• Length of stomata of the upper epidermis µm - LSUE;
• Width of stomata upper epidermis µm - WSUE;
• Number of stomata lower epidermis in 1 mm^2 - NSLE;
• Length of stomata lower epidermis µm - LSLE;
• Width of stomata lower epidermis µm - WSLE;
• Number of epidermal cells of the upper epidermis in 1 mm^2 - NCUE;
• Number of epidermal cells lower epidermis in 1 mm^2. - NCLE.

For each indicator 30 measurements were made.

The results were processed using the Descriptive statistic method, with minimum and maximum values, arithmetic mean, variation coefficient (VC %), mean arithmetic mean error (Sx %) determined. Using the SPSS 20 statistical package, using the Dunkan test, the degree of difference in the

arithmetic mean of the eleven indicators for the six variants examined was determined.
Using a Hierarchical Cluster Analysis, a cluster was created to compare the six Lactuca variants according to the degree of similarity of the measured indicators to compare the link between the groups (link groups) using the Squared Euclidian distance method.
The anatomical analyzes and photographs of each of the variants examined were made using a Magnum T microscope equipped with a Si5000 photographic documentation system at magnifications of x100 to x1000 in the Department of Botany and Methodology of Biology Education at the "Paisii Hilendarski" University of Plovdiv.

RESULTS AND DISCUSSIONS

Leaf lamina

Leaf mesophyll in *Lactuca sativa* is not divided into a palisade and spongy parenhyma (Bolhar-Nordenkampfand and Draxler, 1993), which determined the measurement of the total thickness of the leaf lamina (Figures 1, 2, 3).

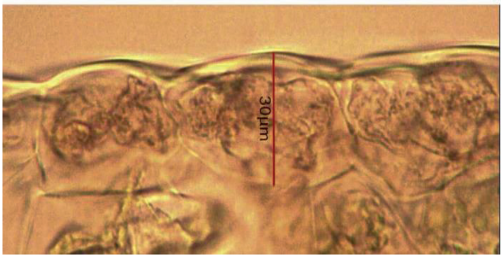

Figure 1. Upper covering tissue

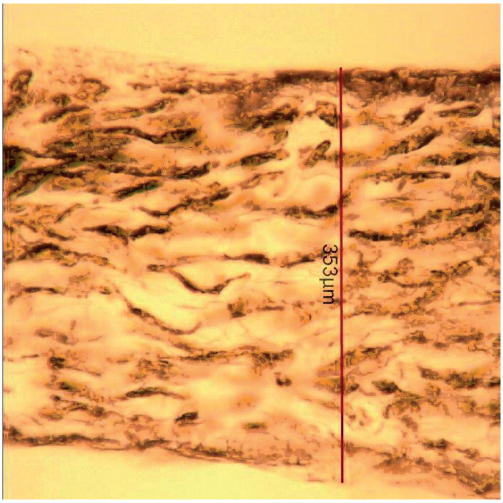

Figure 2. Leaf lamina

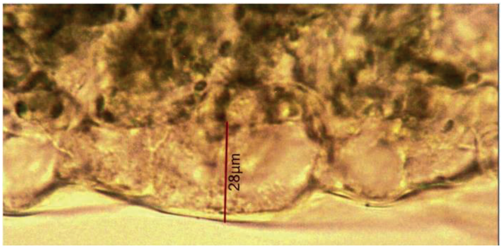

Figure 3. Lower cover tissue

The maximum value for leaf lamina thickness was reported for variant 3 (522.5 μm) and the minimum value for 1 (151.8 μm) (Table 1). In the mean values of this indicator, differences between the two variants are significant and statistically proven (Table 5). For the other variants, a statistically proven difference in the mean thickness of the leaf lamina was reported for variant 5 (234.9 μm), while differences in values for variants 2, 4, and 6 were not significant (Table 5).
For the thickness of the upper covering tissue, a maximum value was reported for variant 2 (38.5 μm), followed by variant 6 (35.2 μm) (Table 1). Minima were reported at 3 (19.8 μm) and 1 (20.9 μm) (Table 1). The highest mean values of this indicator were reported for variants 2, 6 and 4, with the statistical difference between the three variants being insignificant (Table 5). A minimum mean value for the thickness of the upper covering tissue was reported for variant 5, while the difference with the other variants statistically significant (Table 5).
The thickness of the lower covering tissue was also highest in variant 2 (35.2 μm), followed by variant 4 (31.9 μm) (Table 1). Minima were reported for 1 (14.3 μm) and 6 (15.4 μm) (Table 1). Maximum averages for this indicator were reported for variant 4 and 2 (Table 5). The statistical difference between the two variants is insignificant but statistically significant compared to the other four variants (Table 5). A minimum mean with a proven difference was reported for variant 1 (Table 5).
A number of studies have noted a decrease in the size of assimilating organs in plants in adverse environmental conditions. Dineva (2004) reported a nearly double reduction in leaf lamina size for *Fraxinus americana* L. and *Platanus acerifolia* Willd. exposed to road pollution. According to Ilkun (1978), Ninova (1970), Ninova Dushkova (1981), Dineva (2004), Radoukova (2009) unfavorable environmental conditions lead to a significant reduction in the overall thickness of the leaves, and especially that of the cover tissues.
In our study, the reported average of four times the mean leaf lamina at variant 3 (463.2 μm) compared to variant 1 (172.0 μm) is an indicator of the positive effect of Italpolina organic fertilizer.

With respect to the thickness of the upper and lower covering tissues, a clearly visible positive effect is considered in variant 4 where Arcobaleno is used.

The epidermis

Form of major epidermal cells

The degree of folding of the anticlinal cell walls of the major epidermal cells is one of the diagnostic features in examining the response of plants to environmental stressors. Stronger flexion of the anticline walls is an indication of greater sensitivity, i.e. for adverse effects (Ninova & Dushkova, 1978).

The shape and degree of folding of the anticline walls of the major epidermal cells of the upper and lower epidermis in the six variants examined are similar, i.e. are not affected by the nutrition. The major epidermal cells of the two epidermis in the six variants are characterized by a strong folding of the anticline walls. According to the classification of Aneli (1975), they refer to a curvilinear clan with zigzag folded cell walls. (Figures 4, 5), and according to the classification of Sveshtnikov (1970) are defined as curved to strongly curved.

Number of major epidermal cells in 1 mm^2

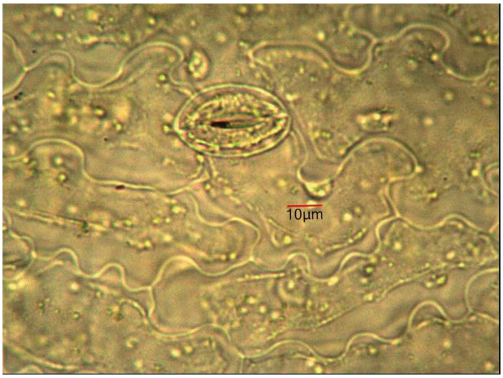

Figure 4. Upper epidermis

Figure 5. Lower epidermis

The smaller size of the major epidermal cells, as well as the increase in their number, are some of the main indicators of the response of the plant organism to adverse environmental changes. (Ninova & Dushkova, 1977; 1978; Koev et al., 2001; Gostin & Ivanescu, 2007; Radoukova et al., 2018; Dospatliev et al., 2018). According to Ninova and Dushkova (1981), low temperatures, excessive lighting, poor nutrition, and environmental pollution are among the factors causing reduce of the cell size.

Maximum values with respect to the number of epidermal cells of the upper epidermis are shown in variant 1 (487.8) and with respect to the lower variant 6 (560.9) (Table 2). The lowest values for the indicator were reported in variant 2 (195.1) for the upper epidermis and variant 1 and 2 for the lower (219.5) (Table 2). The statistically proven highest mean for the number of epidermal cells of the upper epidermis was reported for variant 2 (443.9) and the lowest for 2 (Table 5). In the lower epidermis, the averages of the six variants are statistically proven different. Variant 6 (491) is the maximum, and variant 2 (232) is the minimum (Table 5).

The imported organic fertilizers tend to increase the number of epidermal cells, especially the lower epidermis, with Arkobalenf and Ekoprop NX having the strongest impact.

Number of stomata in 1 mm^2

Increasing the number of stomata and reducing their size is a major response of plant species to adverse changes in environmental conditions. Similar epidermal syndromes are reported in comparative anatomical studies of the same species under conditions of environmental stressors. (Ninova & Dushkova 1970; 1977; 1978; Dimitrova 2000; Radoukova, 2009)

Lactuca sativa is characterized by amphistomatic leaves and anomocytic type of stomata (Esau, 1977) (Figures 4, 5).

The number of stomata on the upper epidermis is maximal in variant 2 (95.1). In some of the test specimens of variants 1, 3, 4 and 5, visual acuity on the upper epidermis was not reported on the visual field (Table 3). At the mean values of the number of stomata in the upper epidermis, statistically proven maxima are reported at 2 and 6 (Table 5).

In the lower epidermis, maximal number of stomata was reported for variants 1 (243.9) and 2 (195.1). The minimum is reported in variant 5 (24.4) (Table 4). The mean values of this indicator are also highest for variants 1 and 2 (196.7 and 157.7 respectively) and lowest for variant 5 (52.8), with differences statistically significant (Table 5).

Variant 5 reports the maximum length of the stomata on the upper epidermis (10.1 µm), while in terms of width the maximum value of the variant 1 (7.6 µm) is shown (Table 3).

The smaller number of stomata on the lower epidermis reported for variant 5 corresponds to their larger size. The average values for the length and width of the stomata for this variant are highest (Table 5). This determines the use of Lumbrikompost as particularly favorable in terms of the number and size of the stomata. Although less pronounced positive effect is observed in the other 3 organic fertilizers. In both control variants, the reported maximum values in terms of number and minimum in terms of stomata size indicate the presence of environmental stressors, most likely due to a lack of organic matter in the soil.

Cluster analysis shows a clear clustering in standalone groups of variants 3 and 5, as well as 4 and 6. Variant 2 joins the group of 4 and 6, while the strongest degree of difference is shown in variant 1 (Figure 6).

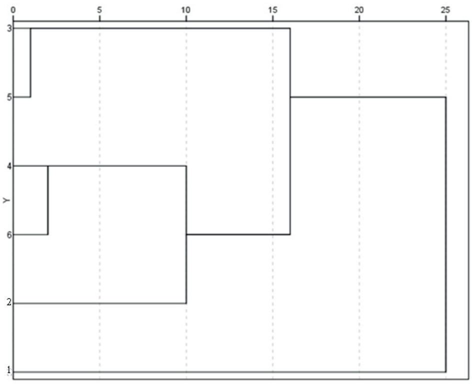

Figure. 6. Hierarchical cluster analysis to compare the relationship between indicators using the square Euclidean distance method

Table 1. Reported values for leaf lamina thickness and cover tissue for the six variants tested (μm)

Thickness of leaf lamina					
Variant	min	x ± Sx	max	VC%	Sx%
1	151.8	172.0±4.9	192.5	8.1	2.8
2	244.2	350±18.4	394	15.8	5.2
3	418	463.2±13.5	522.5	8.2	2.9
4	221.1	382.2±22.7	436.7	17.8	5.9
5	191.4	234.9±11.5	276.1	12.9	4.9
6	303.6	316.9±4.9	343.2	4	1.5
Thick upper cover tissue					
1	20.9	23.6±0.3	25.3	5.8	1.1
2	29.7	31.6±0.44	38.5	7.7	1.3
3	19.8	24.6±0.7	30.8	15.7	2.8
4	27.5	31.4±0.2	33	4.1	0.7
5	22	23.2±0.2	25.3	5.6	1
6	27.5	31.5±0.5	35.2	8	1.4
Thick lower cover tissue					
1	14.3	19.5±0.4	24.2	11.1	2
2	19.8	29.4±0.9	35.2	16.7	3.1
3	18.7	24.2±0.6	29.7	13.9	2.5
4	24.2	29.4±0.4	31.9	6.9	1.2
5	20.9	22.4±0.2	24.2	5.2	0.9
6	15.4	21.8±0.5	24.2	12.1	2.2

Table 2. Reported values for the number of epidermal cells of the upper and lower epidermis in the six variants examined

Number of epidermal cells upper epidermis					
Variant	min	x ± Sx	max	VC %	Sx %
1	356.9	443.9±8.3	487.8	10.3	1.9
2	195.1	211.4±3.4	268.3	8.7	1.6
3	219.5	265.0±5.6	317.1	11.5	2.1
4	317.1	380.5±5.3	414.6	7.6	1.3
5	317.1	334.9±3.1	365.9	5	0.9
6	292.7	338.2±4.6	365.9	7.5	1.3
Number of epidermal cells lower epidermis					
1	219.5	273.9±6.9	341.5	13.9	2.5
2	219.5	232.5±2.3	243.9	5.3	0.9
3	341.5	425.2±7.3	487.8	9.3	1.7
4	365.9	386.2±3.7	414.6	5.2	0.9
5	292.7	323.8±3.2	341.5	5.2	0.9
6	414.6	491.1±7.6	560.9	8.4	1.5

Table 3. Reported values for number and size of stomata (μm) of the upper epidermis in the six variants examined

Variant	min	x ± Sx	max	VC%	Sx%
Number of stomata upper epidermis					
1	0	30.9±3.3	73.2	8.3	1
2	121.9	156.9±2.8	195.1	9.7	1.8
3	0	26.0±3.7	48.8	7.6	1.1
4	0	30.9±3.5	73.2	6.9	1.2
5	0	21.9±2.4	48.8	6.9	1.1
6	24.4	47.9±3.2	73.2	6.5	1.6
Length of stomata upper epidermis					
1	6.2	7.0±0.09	7.8		
2	6.7	7.9±0.1	8.5	7.2	1.3
3	8.3	8.8±0.05	9.2	2.9	0.5
4	7.4	7.7±0.04	8.1	3.1	0.5
5	8.7	9.3±0.08	10.1	4.6	0.7
6	7.8	8.1±0.04	8.51	2.7	0.4
Width of stomata upper epidermis					
1	4.8	5.3±0.05	7.6	5.2	0.9
2	5.1	5.6±0.04	5.9	4.2	0.7
3	4.6	5.5±0.06	5.8	5.5	0.9
4	5.5	5.7±0.03	5.9	2.8	0.5
5	5.5	5.8±0.05	6.2	4.8	0.8
6	5.3	5.6±0.03	5.9	3.5	0.5

Table 4. Reported values for the number and size of the stomata (μm) of the lower epidermis in the six variants examined

Number of stomata lower epidermis					
Variant	min	x±Sx	max	VC%	Sx%
1	170.7	196.7±3.3	243.9	9.1	1.6
2	97.6	157.7±4.3	195.1	15	2.7
3	48.8	81.3±2.9	97.6	19.8	3.6
4	73.2	86.9±2.2	97.6	14.1	2.5
5	24.4	52.8±3.1	73.2	32.2	5.8
6	97.6	125.2±3.6	146.3	15.9	2.9
Length of stomata lower epidermis					
1	7.4	8.5±0.09	9.2	5.8	1.1
2	7.6	8.3±0.06	8.9	4.2	0.7
3	7.1	7.9±0.06	8.3	3.9	0.6
4	8.1	8.4±0.05	8.7	3.3	0.5
5	8.1	8.5±0.03	8.7	2.2	0.3
6	6.9	7.6±0.05	7.8	3.9	0.6
Width of stomata lower epidermis					
1	5.1	5.4±0.03	5.5	2.9	0.6
2	5.1	5.7±0.06	6.2	5.4	0.8
3	5.5	5.9±0.05	6.4	4.7	0.8
4	4.8	5.4±0.07	5.8	7	1.2
5	5.5	6.2±0.06	6.44	5.6	0.9
6	5.1	5.4±0.06	5.8	5.3	0.9

Table 5. Duncan test for similarity between the arithmetic value of the studied parameters for the six variants p 0.05

Variant	TLL	TUE	TLE	NSUE	LSUE	WSUE	NSLE	LSLE	WSLE	NCUE	NCLE
1	172.0^{d}	$23.6^{b,c}$	19.5^{d}	30.9^{c}	7.0^{f}	5.3^{c}	196.7^{a}	8.5^{a}	5.4^{c}	443.9^{a}	273.9^{e}
2	$350.3^{b,c}$	31.6^{a}	29.4^{a}	156.9^{a}	7.9^{d}	5.6^{b}	157.7^{b}	8.3^{b}	1.9^{d}	211.4^{e}	232.5^{f}
3	463.2^{a}	24.6^{b}	24.2^{b}	26.0^{c}	8.8^{b}	5.5^{b}	81.3^{d}	7.9^{c}	5.9^{b}	265.0^{d}	425.2^{b}
4	382.2^{b}	31.4^{a}	29.4^{a}	30.9^{c}	7.7^{e}	5.7^{a}	86.9^{d}	$8.4^{a,b}$	5.4^{c}	380.5^{b}	386.2^{c}
5	243.9^{d}	23.2^{c}	22.4^{c}	21.9^{c}	9.3^{a}	5.8^{a}	52.8^{e}	8.5^{a}	6.2^{a}	334.9^{c}	324.4^{d}
6	316.9^{c}	31.5^{a}	21.8^{c}	47.9^{b}	8.1^{c}	5.6^{b}	125.2^{c}	7.6^{d}	5.4^{c}	338.2^{c}	491.1^{a}

Average with identical letters have no statistically proven differences

CONCLUSIONS

The lowest overall thickness of the leaf lamina, the upper and lower covering tissues in variant 1 reported a negative reaction to the growing conditions for this variant. The high average values for these indicators reported for variants 3 and 4 determine their response to soil-borne organic fertilizers (Italpolina and Arkobaleno) as favorable.

With respect to the number of epidermal cells, the minimum values obtained for both epidermis in variant 2 are indicative of a positive response to environmental conditions, which contrasts with the values reported for the same variant in terms of both leaf thickness and number. stomata on both epidermis. The maximum number of stomata reported for both epidermis in variant 2 determines the presence of a negative reaction to growing conditions.

The results obtained for variant 5, which shows the smallest number and largest size of stomata for both epidermis, determined the plant response to fertilization with Lumbrikompost as particularly positive. A similar tendency with respect to the stomata is observed in other variants with the application of organic fertilizers, in contrast to the two controls.

Grouping of variants 3 and 5 and 4 and 6 into separate cluster groups in terms of anatomical parameters is an indicator of a positive response to growing conditions.

The application of organic fertilizers can be defined as favorable to the anatomical structures of the leaves during greenhouse lettuce cultivation.

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REFERENCES

Aneli, N. A. (1975). Atlas of leaf epidermis. *Mecniereba, Tbilisi* (in Russian).

Barba, F. J., Zhu, Z., Koubaa, M., Sant'Ana, A. S., & Orlien, V. (2016). Green alternative methods for the extraction of antioxidant bioactive compounds from winery wastes and by-products: A review. *Trends in Food Science & Technology*, *49*, 96-109.

Basta, N. T., Ryan, J. A., & Chaney, R. L. (2005). Trace element chemistry in residual-treated soil. *Journal of environmental quality*, *34*(1), 49-63. soil.

Bell, P. F., James, B. R., & Chaney, R. L. (1991). Heavy metal extractability in long-term sewage sludge and metal salt-amended soils. *Journal of Environmental Quality*, *20*(2), 481-486.

Bolhar-Nordenkampf, H. R., & Draxler, G. (1993). Functional leaf anatomy. In *Photosynthesis and production in a changing environment* (pp. 91-112). Springer, Dordrecht.

Brown, S. L., Chaney, R. L., Angle, J. S., & Ryan, J. A. (1998). The phytoavailability of cadmium to lettuce in long-term biosolids-amended soils. *Journal of environmental Quality*, *27*(5), 1071-1078.

Chen, Y., Huang, B., Hu, W., Weindorf, D. C., Liu, X., & Niedermann, S. (2014). Assessing the risks of trace elements in environmental materials under selected greenhouse vegetable production systems of China. *Science of the total environment*, *470*, 1140-1150.

Cunningham, J. D., Keeney, D. R., & Ryan, J. A. (1975). Yield and metal composition of corn and rye grown on sewage sludge-amended soil. *Journal of Environmental Quality*, *4*(4), 448-454.

Day, W., Audsley, E., & Frost, A. R. (2008). An engineering approach to modelling, decision support and control for sustainable systems. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *363*(1491), 527-541.

Dimitrova, I. (2000). Foliar diagnostics and analysis of seed viability of *Acer platanoides*L. and *Acer pseudoplatanus* L. in areas with traffic pollution In: N. Pipkov, P. Zhelev, I. Draganova (Eds.). Annual Collection of Scientific Research (*75 Years of Higher Education in Bulgaria. Forest Universitet)*, Sofia, 16-23 (in Bulgarian).

Dineva, S. B. (2004). Comparative studies of the leaf morphology and structure of white ash *Fraxinus americana* L. and London plane tree *Platanus acerifolia* Willd growing in polluted area. *Dendrobiology*, *52*, 3-8.

Dospatliev, L. K., Lacheva, M. N., Ivanova, M. T., & Radoukova, T. I. (2018). Determination of the Total Phosphorus (P) Content and Anatomical Study on Stomata of Plant Species in Urban and Mountainous Environments (Part 2). *Ecologia Balkanica*, *10*(2). (Part 2).

Dumas, Y., Dadomo, M., Di Lucca, G., & Grolier, P. (2003). Effects of environmental factors and agricultural techniques on antioxidantcontent of tomatoes. *Journal of the Science of Food and Agriculture*, *83*(5), 369-382.

Ebert, A. W. (2014). Potential of underutilized traditional vegetables and legume crops to contribute to food and nutritional security, income and more sustainable production systems. *Sustainability*, *6*(1), 319-335.

Esau, K. (1977). *Anatomy of seed plants 2nd Ed,* Wiley New York.

Fageria, N. K. (2016). *The use of nutrients in crop plants*. CRC press.

Falla, J., Laval-Gilly, P., Henryon, M., Morlot, D., & Ferard, J. F. (2000). Biological air quality monitoring: a review. *Environmental Monitoring and Assessment*, *64*(3), 627-644.

Fontes, P. C. R., Pereira, P. R. G., & Conde, R. M. (1997). Critical chlorophyll, total nitrogen, and nitrate-nitrogen in leaves associated to maximum lettuce yield. *Journal of Plant Nutrition*, *20*(9), 1061-1068.;

Gheorghiu, A., Vidrascu, P. A., & Niculescu, M. D. (2013). The development of the Eco-marketing, green performance and corporate responsibility in a competitive economy. *Calitatea*, *14*, 373.

Gostin, I., & Ivanescu, L. (2007). Structural and micromorphological changes in leaves of *Salix alba* under air pollution effect. *Int J Energ Environ*, *1*, 219-26.

Heckman, J. R., Sims, J. T., Beegle, D. B., Coale, F. J., Herbert, S. J., Bruulsema, T. W., & Bamka, W. J. (2003). Nutrient removal by corn grain harvest. *Agronomy Journal*, *95*(3), 587-591.

Honour, S. L., Bell, J. N. B., Ashenden, T. W., Cape, J. N., & Power, S. A. (2009). Responses of herbaceous plants to urban air pollution: effects on growth, phenology and leaf surface characteristics. *Environmental pollution*, *157*(4), 1279-1286.

Hoque, M. M., Ajwa, H., Othman, M., Smith, R., & Cahn, M. (2010). Yield and postharvest quality of lettuce in response to nitrogen, phosphorus, and potassium fertilizers. *HortScience*, *45*(10), 1539-1544.

http://www.sunoilbg.org/language/bg/uploads/files/documents__0/document_6638d0d4b9d0a517e62b8bd4aef20ced.pdf

Ilkun, G. M & Bukolova, T.P. (1974). Changing the structure of pine needles under the influence of fluorine and chlorine. *Ukrainian Botanical Journal, 31(5),* 624-629 (in Russian).

Ilkun, G. M. (1978). Pollutants of atmosphere and plants. *Kiev: Naukova Dumka* (in Russian).

Koev K., I. Dimitrova, & A.Koleva. (2001). The air pollutant effect of Maritsa - East EDC varied the *Robinia pseudoacacia* and *Betula pendula* lists. In: D..Temniskova (Ed.) (*Publications from the Sixth National Conference on Botany*), Sofia. SU. s. 481-487 (in Bulgarian).

Kostadinov, K. P., & Filipov, S. V. (2013). Effect of composition of mixture on reproductive manifestations in greenhouse tomato. *New Knowledge Journal of Science/Novo Znanie*, *2*(4).

Lorenz, O. A. (1978). Potential nitrate levels in edible plant parts. In *Soil–Plant–Nitrogen Relationships* (pp. 201-219). Academic Press.

Mahler, R. J., Ryan, J. A., & Reed, T. (1987). Cadmium sulfate application to sludge-amended soils I. Effect on yield and cadmium availability to plants. *Science of the total environment*, *67*(2-3), 117-131.

Metcalfe, CR., Chalk, L. 1950. *Anatomy of the Dicotyledons. I-II*. Oxford. 1126 p.

Ninova D. (1970). On the anatomical indices of smoke resistance in some tree plants. *Forestry science, 7*(4), 9-18 (in Bulgarian).

Ninova D., & Dushkova, P. (1981). Trends in the anatomical and physiological variability of tree plants in industrial areas. *Scientific works of Plovdiv University* "*Paisii Hilendarski*", *19*(4) 73-83 (in Bulgarian).

Ninova, D., & Dushkova, P. (1977). Structural and functional investigation of plants in industrial rayons. I. Epidermal-stomatographic analysis of oak plants in rayon on a non-ferrous metal plant "D. Blagoev". *Forestry science*. *6*, 32-39 (in Bulgarian).

Ninova, D., & Dushkova, P. (1978). Structural and functional investigation of plants in industrial rayons. II. Epidermal-stomatographic analysis of the darvesian plant in rayon of the world metal complex "D. Blagoev". *Forestry science, 1*, 3-16 (in Bulgarian).

Pink, D. A. C., & Keane, E. M. (1993). Lettuce: *Lactuca sativa* L. In *Genetic improvement of vegetable crops* (pp. 543-571). Pergamon.

Radoukova, T. (2009). Anatomical mutability of the leaf epidermis in two species of Fraxinus L. in a region with autotransport pollution. *Biotechnology & Biotechnological Equipment*, *23*(sup1), 405-409.

Radoukova, T. I., Lacheva, M. N., Ivanova, M. T., & Dospatliev, L. K. (2018). Determination of the Total Nitrogen (N) Content and Anatomical Study on the Leaves Epidermal Cells of Plant Species in Urban and Mountainous Environments (Part 1). *Ecologia Balkanica*, *10*(2).

Rai, P. K. (2013). Environmental magnetic studies of particulates with special reference to biomagnetic monitoring using roadside plant leaves. *Atmospheric Environment*, *72*, 113-129.

Sanchez, C. A., Krieger, R. I., Khandaker, N., Moore, R. C., Holts, K. C., & Neidel, L. L. (2005). Accumulation and perchlorate exposure potential of lettuce produced in the lower Colorado River region. *Journal of agricultural and food chemistry*, *53*(13), 5479-5486.

Serna, M., Hernández, F., Coll, F., & Amorós, A. (2012). Brassinosteroid analogues effect on yield and quality parameters of field-grown lettuce (*Lactuca sativa* L.). *Scientia Horticulturae*, *143*, 29-37.

Soundy, P., & Smith, I. E. (1992). Response of lettuce (*Lactuca sativa* L.) to nitrogen and phosphorus fertilization. *Journal of the Southern African Society for Horticultural Sciences*, *2*(2), 82-85.

Sveshnikova N. I. (1970). About terminologii of leaves epidermy modern and fossil conifers. *Botanical journal 55*(4), 510-523 (in Russian).

Terziev, V., & Arabska, E. (2015, June). Enhancing competitiveness and sustainability of agri-food sector through market-oriented technology development in Agricultural Knowledge and Innovation System in Bulgaria. In *Proceedings of the III International Scientific Congress Agricultural Machinery, Varna, Bulgaria*.

Zhang, R. H., Li, Z. G., Liu, X. D., Wang, B. C., Zhou, G. L., Huang, X. X., ... & Brooks, M. (2017). Immobilization and bioavailability of heavy metals in greenhouse soils amended with rice straw-derived biochar. *Ecological Engineering*, *98*, 183-188.

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