

THE EFFECT OF ORGANIC FERTILIZERS ON THE BIOCHEMICAL PARAMETERS OF TOMATO SEEDLINGS

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Abstract. The aim of the paper is to develop a technological flow of ecological production for tomato seedlings, compatible with the environment, by using substances that can be reused in our own household, which would maintain and increase the fertility of the soil on a long term, while ensuring the quality and safety of the consumer. The main objective of the study was to obtain organic seedlings sown in the nutrient substrate using natural fertilizers (aquarium water, poultry manure and manure), thus reducing all forms of pollution that may result from agricultural technology. For all variants of analysed seedlings, a series of biochemical parameters were determined, taking into account the influence produced by the organic fertilizers used on the vegetal material. The biological material was represented by Rila F1 tomatoes. We made 4 experimental variants, of which a control variant, fertilized only once with ammonium nitrate in the amount of 30 g/seedlings, a method commonly used to obtain seedlings. In the other 3 variants we used natural fertilizers to stimulate tomato seedlings, so for variant 2 we used aquarium water as a natural fertilizer, for variant 3 we used a 1:10 dilution of poultry manure, and for variant 4 we used manure. Before planting the seedlings, we made observations and determinations on some biochemical parameters. The content of free water, total water, bound water and dry matter was determined gravimetrically by drying in the oven. For the four experimental variants, the values of total water content ranged from 76.95 to 85.66 without significant differences between them ($p > 0.05$). Even in terms of free and bound water content, we did not notice any significant differences. There are significant differences in the dry matter content (variants V4 and V2 shared with V1 and V3). Regarding the ash content between V2 and V4, there are no significant differences, however between them and the control V1 and even the V3 variant there are significant differences. The chlorophyll content shows significant differences between studied variants and the control. The highest amount of chlorophyll was obtained in case V4 followed by variant V3. The content of xanthophylls and carotenoids does not show significant differences in the analysed samples.

Keywords: tomato seedling, organic fertilizers, biochemical parameters.

Rezumat. Efectul fertilizanților organici pe parametrii biochimici din răsăturile de tomate. Scopul lucrării este dezvoltarea unui flux tehnologic de producere ecologică pentru răsături de tomate compatibile cu mediul, folosind substanțe ce pot fi refolosite în propria gospodărie, care să mențină și să crească fertilitatea solului pe termen lung, asigurând totodată calitatea și siguranța consumatorului. Principalul obiectiv al studiului a fost obținerea de răsături ecologice semănate în substrat nutritiv, utilizând fertilizanți naturali (apă de acvariu, gunoi de păsări și mraniță), astfel reducând toate formele de poluare, care pot rezulta din tehnologia agricolă. Pentru toate variantele de răsături luate în analiză, s-au determinat o serie de parametrii biochimici, urmărindu-se influența produsă de fertilizanții organici utilizați asupra materialului vegetal. Materialul biologic a fost reprezentat de tomate Rila F1. Am realizat 4 variante experimentale, dintre care o variantă martor, fertilizată o singură dată cu azotat de amoniu în cantitate de 30 g/răsad, metodă folosită în mod frecvent în obținerea răsăturilor. În celelalte 3 variante am folosit fertilizanți naturali pentru stimularea răsăturilor de tomate, astfel că pentru varianta 2 am folosit ca îngrășământ natural apa de acvariu, pentru varianta a 3-a am utilizat o diluție de 1:10 a gunoiului de păsări, iar pentru varianta 4 am folosit mraniță. Înainte de plantarea răsăturilor am făcut observații și determinări asupra unor parametri biochimici. Conținutul de apă liberă, apă totală, apă legată și materie uscată a fost determinat gravimetric prin uscarea în cuptor. Pentru cele patru variante experimentale, valorile conținutului total de apă au variat între 76,95 și 85,66 fără diferențe semnificative între ele ($p > 0.05$). Nici în ceea ce privește conținutul în apă liberă și cel în apă legată nu am observat diferențe semnificative. Apar diferențe semnificative în ceea ce privește conținutul de materie uscată (variantele V4 și V2 compartativ cu V1 și V3). În ceea ce privește conținutul în cenușă între V2 și V4 nu sunt diferențe semnificative, în schimb între ele și martorul V1 și chiar și varianta V3 apar diferențe semnificative. Conținutul de clorofilă prezintă diferențe semnificative între variantele studiate și martor. Cantitatea mare de clorofilă a fost obținută în cazul V4 urmată de varianta V3. Conținutul de xantofile și carotenoizi nu arată diferențe semnificative la probele analizate.

Cuvinte cheie: răsad de tomate, fertilizanți organici, parametrii biochimici.

INTRODUCTION

The excessive use of the synthesis chemicals in intensive production has led to the severe degradation of soil fertility, by severely reducing organic matter and, implicitly, micro flora and micro fauna.

Organic fertilizers are natural while inorganic ones are synthetic. The difference is determined by the carbon, and, more specifically, the carbon hydrogen linkage in organic fertilizers slows the release of the nutrient ions. A slower nutrient release results in more sustained availability of the nutrients. In addition, organic fertilizers may act as an energy source for microorganisms in the soil, which can improve soil structure and plant growth (ALI et al., 2015). The need to use renewable forms of energy and reduce the costs of fertilizing crops has revived the use of the organic fertilizers worldwide (AYOOLA & ADENIYAN, 2006). Large quantities of organic wastes such as poultry manure are available, especially in urban centres and are an effective source of nutrients for vegetables such as tomato (ADEDIRAN et al., 2003). Despite the large quantities of plant nutrients contained in synthetic fertilizers, compared to

organic nutrients, the presence of growth promoting agents in organic fertilizers make them important in enhancing soil fertility and productivity (SANWAL et al., 2007).

Depending on the bonding strength, intracellular water is defined as loosely bound water, cell wall water is categorized as strongly bound water, and intercellular water is known as free water (IMRAN et al., 2017).

Chicken manure had a significant effect on the plant height and root length of Isabella F1 tomato, the leaf area of sun cherry, the fresh and dry weight of the root of Lelord, the fresh and dry weight of the leaves of Sadia F1. on the other hand, the fresh and dry weight of the shoots of the Isabella tomato variety was increased when treated with mixed manure. Agro fish pellet treatment significantly increased the stem diameter of Isabella F1 (KALBANI et al., 2016).

The beneficial effects of foliar bio fertilizer treatments on tomato plants are materialized by increasing cellular chlorophyll synthesis. This increase is detected as a first sign in cultivation, after foliar fertilizer application by converting the leaves colour in a dark green (PFLUGMACHER et al., 2006).

It is known that water enters the composition of all plant cells in large amounts, constituting a continuous internal environment that connects the various organs and tissues of the plant. In the structure of the plant body, water is found in two forms: free water and bound water. The presence of these two forms of water in cytoplasm ensures the dispersion of plasma micelles and the dissolution of some organic and mineral substances beneficial to the growth and development of plants (STANCU & FLEANCU, 2004).

Being weakly retained in the plant body, free water circulates lightly both inside the cell and from one cell to another, ensuring the turgidity of cells. At the same time, the environment in which the biochemical processes take place has a direct influence. Low temperatures down to 10°C cause the freezing of this water, and therefore plants with high free water content are little resistant to low temperatures (STANCU & FLEANCU, 2004; KHAN et al., 2016).

Bound water is made up of non-diffusible molecules and thus, it is plentifully retained by cells. In addition, it does not take part in the biochemical processes or in the dissolution of organic or inorganic substances (CAURIE, 2011; KHAN et al., 2016). Under unfavourable environmental conditions, when the vital activity of the plants is greatly reduced, the content of free water decreases and the content of bound water increases, which leads to higher plant resistance.

The quantitative determination of chlorophyll (Chl a, Chl b), and carotenoids in a whole pigment extract of green plant tissue by UV-VIS spectroscopy is complicated by the choice of the sample, the solvent system, and the used spectrophotometer. The various plant pigments absorb light in overlapping spectral regions, depending on the selected system (LICHTENTHALER & BUSCHMANN, 2001).

This paper was made as a comparative study of tomato seedlings obtained by methods that use natural fertilizers, such as aquarium water, poultry manure and cow manure, concerning the recommendation for using these fertilizers into small, medium and big farms. The purpose of this study is to emphasize the differences caused by these natural fertilizers on the tomato seedlings destined for the protected culture.

MATERIAL AND METHODS

For the performance of the experimental model (Table 1) we used seeds from *Lycopersicon esculentum* MILL, Rila F1 variety, which is an undetermined early hybrid for production in protected areas (greenhouses, solariums) and in the field. The plant presents resistance to tomato mosaic virus, *Verticilium*, fusariosis and nematodes. The seeds were sown on February 24 in a peat-based professional nutrient substrate, Klasman TS3, pH 5,5-6,5, with black peat 20%, 80% blonde peat and microelements, in alveolar trays with 32 cells in order to eliminate plant stress, knowing that a stressed body is more sensitive to pathogens. We studied 4 experimental variants, in 3 repetitions of 10 plants. We applied chemical fertilizers – ammonium nitrate (once 30 g/plant- a method frequently used in seedlings obtaining) /natural products (once every seven days. with 150 ml of aquarium water / plant or poultry manure 150 ml / plant or 100 g cow manure / plant).

Table 1. Experimental variants.

Experimental variants	Used fertilizers
V1	control (ammonium nitrate) 30 g/plant
V2	aquarium water 150 ml/plant
V3	poultry manure (1:10) 150 ml/plant
V4	cow manure 100 g/plant

Maintenance work during the vegetation period was applied uniformly to all studied variants. We applied chemical fertilizers – ammonium nitrate (once 30 g/plant)/natural products (once every seven days with 150 ml of aquarium water/ plant or poultry manure 150 ml/ plant or 100 g cow manure/ plant).

Before planting the seedlings, we made observations and determinations on some biochemical parameters. The content of free water, total water, bound water and dry matter was determined gravimetrically by the drying method in the oven. We took 2 g of plant material collected from the middle floor (m_i), which was put in a porcelain capsule, brought to a constant mass.

Free water is the amount of water that serves for hydrolysis and translocation of the substances accumulated in the leaves, to the other organs of the plant, playing the role of solvent and vehicle (STANCU & FLEANCU, 2004; KHAN et al., 2016). To determine the content in free water, the plant material was dried at 40°C to a constant mass, denoted m_{f1} .

$$\% \text{ free water} = \frac{m_i - m_{f1}}{m_i} \cdot 100$$

Total water represents a supply index for the soils and, if different soils are used, this parameter also shows the difference between them, with regard to the nature and quantity of the substance accumulated per unit of weight. To determine the total water content (humidity), the plant material was dried at 105°C to a constant mass, denoted m_{f2} (GIOSANU et al., 2016; TUDOR-RADU et al., 2016).

$$\% \text{ total water} = \frac{m_i - m_{f2}}{m_i} \cdot 100$$

Bound water at a vegetative moment is an index showing the intensity of the growth process of the plants, with direct effects on the differentiation and accumulation of some nutritive substances that act as vital colloids (CAURIE, 2011; KHAN et al., 2016). The content of bound water in the test sample was calculated as a difference between total water content and free water content according to the relation:

$$\text{Bound water (\%)} = \text{Total water (\%)} - \text{Free water (\%)}$$

Measuring total water, free water and bound water contents for the experimental variants of tomato seedlings in the nutrient substrate by using the above-mentioned natural fertilizers informs us on the plant's resistance to frost and drought. Thus, these biochemical parameters were analysed for all variants, emphasizing the differentiation produced by the fertilizers used on the vegetal material.

It is known that the ash content of plants varies with the species, the age of the plants, the organs under analysis and the environmental conditions in which the plants have grown. Plant leaves contain up to 50% mineral salts of dry matter (STANCU & FLEANCU, 2004). Dry matter content was expressed as a percentage and calculated with the formula:

$$\text{Dry matter (\%)} = 100 - \text{Total water (\%)}$$

Mineral content (ash) was determined by slow calcination at 550-600°C in a furnace, with the calcination of residue resulted after extraction of water (TUDOR-RADU et al., 2016). One gram of plant material (m_i) was calcinated to constant mass to give ash mass (m_{ash}).

$$\% \text{ ash} = \frac{m_{ash}}{m_i} \cdot 100$$

The cells, tissues and organs of plants that perform their nutrition with sunlight, adapted to the photosynthesis process, contain a set of pigments that are involved in this process, known as assimilating pigments. They may be chlorophyllins, carotenoids and phycobials (BULDA et al., 2008). The most well-known chlorophyll pigments are chlorophyll a and chlorophyll b.

Assimilating pigments have the ability to selectively absorb solar radiation. Determination of the chlorophyll concentration is performed spectrophotometrically, using acetone as the standard (WELLBURN, 1994). Thus, we weighed 0.1 g of leaf and mixed it with 10 ml of 90% acetone, filtered and then collected the filtrate in a test tube. We filled the spectrophotometer cuvette with the extract and read the absorbances at the following wavelengths: 663 nm, 645 nm and 470 nm. Using Arnon's relation (LICHTENTHALER & WELLBURN, 1983), the content of chlorophyll a, chlorophyll b and carotenes and xanthophylls was calculated:

$$\text{Chl a (mg/L)} = 11.75 \times A_{662} - 2.35 \times A_{645}$$

$$\text{Chl b (mg/L)} = 18.61 \times A_{645} - 3.96 \times A_{663}$$

$$\text{Total Chl} = \text{Chl a} + \text{Chl b}$$

$$\text{Carotenes and xanthophylls (mg/L)} = [1000 \times A_{470} - 2.27 \times \text{Chl A} - 81.4 \times \text{Chl B}] / 227$$

We performed the statistical analysis of the obtained results using the analysis of variation in SPSS 16.0 software and compared using Duncan's multiple tests at a level of 5%. We expressed the results of this research as mean \pm standard error (SEM).

RESULTS AND DISCUSSIONS

As for the total water content (Fig. 1) we noticed that the V3 variant (fertilized with poultry manure) has the highest value, over the value obtained by the control variant. V2 and V4 variants recorded lower values of the analysed parameter compared to the control variant. For the four experimental variants, the values of total water content varied between 76.95 and 85.66 without significant differences between them ($p > 0.05$). The capacity of the plant to store the moisture would also increase its water use and convert into the yield per unit of the applied water (BEHESHTI & FARD, 2010). KARASAHIN (2015) reported greater water use efficiency through the use of inorganic fertilizers than the organic manures.

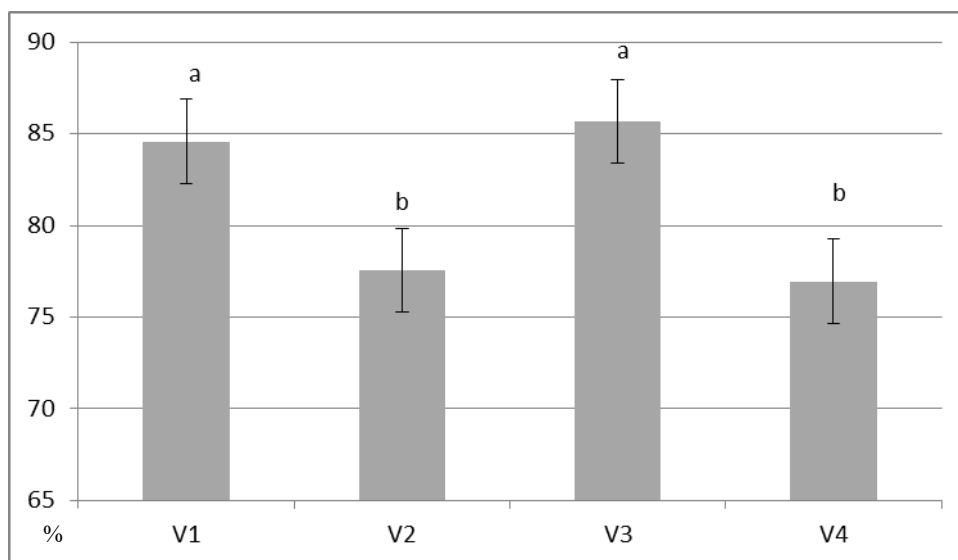


Figure 1. Influence of natural fertilisers on total water content in Rila F1 tomato seedlings naturally fertilized (%).

One emphasized the V3 variant which has the highest value of free water content (Fig. 2) over the control variant. We noticed that V2 and V4 variants recorded lower values of the analysed parameter compared to the control variant but the differences are not significant.

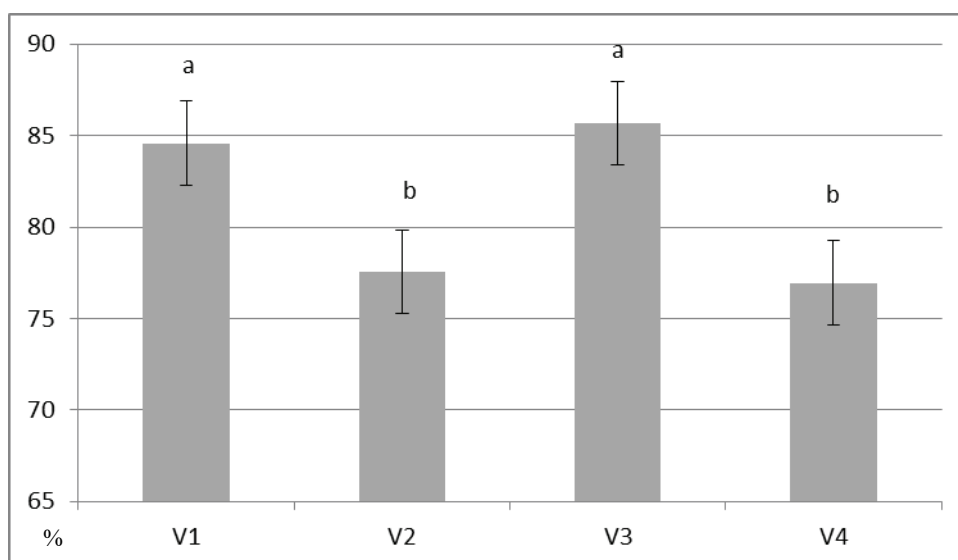


Figure 2. The influence of natural fertilisers on free water content in of Rila F1 tomato seedlings naturally fertilized (%).

The highest value of the bound water parameter (Fig. 3) was recorded in the variant fertilized with aquarium water. Values obtained for all the studied variants exceeded the control variant value in terms of bound water, which confers higher resistance at high temperatures for the above-mentioned experimental variants. We noticed that there are differences between the studied variants, but these are insignificant according to the Duncan test.

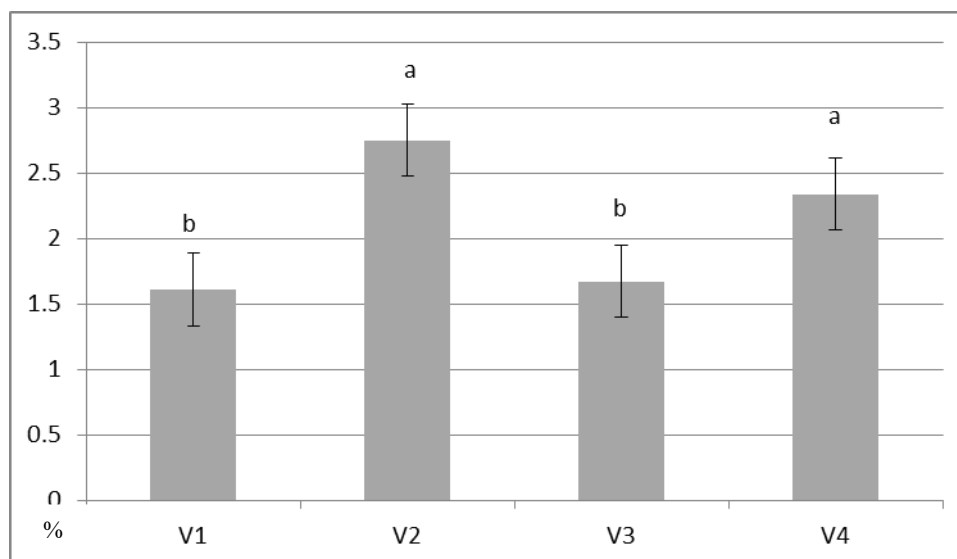


Figure 3. The influence of natural fertilisers on bound water content in Rila F1 tomato seedlings naturally fertilized (%).

For all variants, the content of dry matter, ash and assimilating pigments (Figs. 4, 5, 6) was determined and statistically analysed.

It was found that in the case of dry matter, the values obtained for the studied variants are higher than the value obtained for the control variant, with the exception of the V3 variant, fertilized with poultry manure. This result is probably due to the respiratory rate (the intensity ratio of photosynthesis/ breathing intensity) which is a physiological index correlated with the content of dry matter.

Statistically analysing the data from figure 4 we observed that in the case of the results obtained on the variants taken into account there are significant differences regarding the content of dry matter (variants V4 and V2 compared with V1 and V3). The statistical analysis of the data showed that the organic fertilizers do not significantly affect total soluble solids in coriander (AHMAD & UMAIR, 2017).

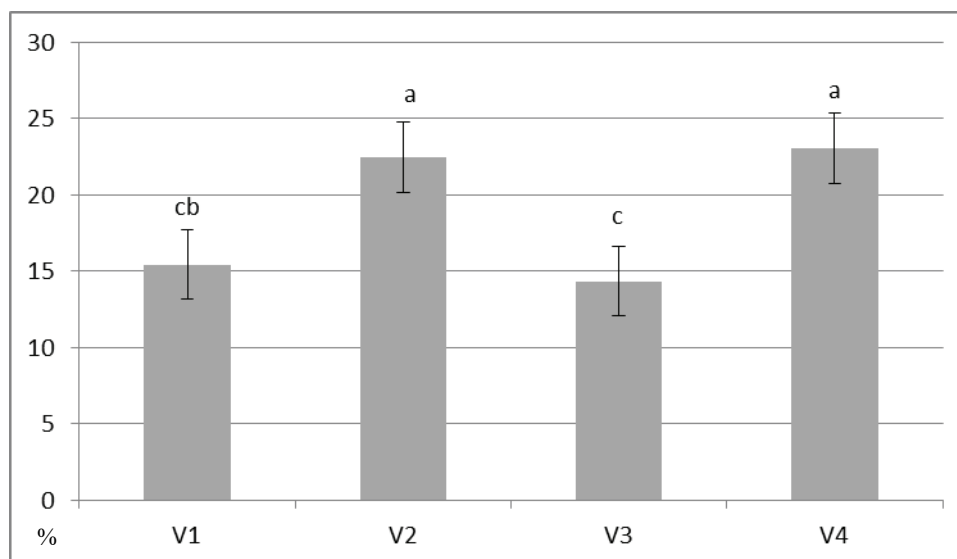


Figure 4. The influence of natural fertilisers on the content in dry matter in Rila F1 tomato seedlings naturally fertilized (%).

In the case of ash content (Fig. 5), the values obtained for all the studied experimental variants are higher than the value obtained for the control variant, this result showing the synthesis of the complex organic substances that make up the plant organism and the proper development of the physiological processes in the seedlings. There are no significant differences between the variants V2 and V4, however there are significant differences regarding the ash content between them and the control V1 and even the variant V3.

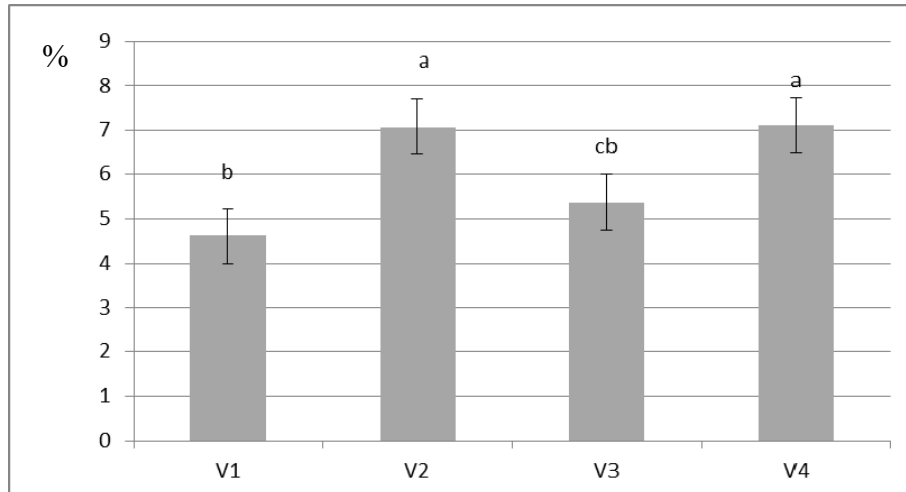


Figure 5. The influence of natural fertilisers on ash content in Rila F1 tomato seedlings naturally fertilized (%).

It is known that Chl a and Chl b absorb with narrow bands (maxima) in the blue (near 428 and 453 nm) and red (near 661 and 642 nm) spectral ranges. With the increasing polarity of the solvent, the red absorption maximum of Chl a shifts from 660 to 665 nm and the blue absorption maximum of Chl a shifts from 428 to 432 nm. The same also applies to Chl b, which shifts from 642 to 652 nm and 452 to 469 nm (LICHTENTHALER, 1987). For our experimental variants, the absorption spectrum of extracts of green leaves of the tomato seedlings containing a mixture of Chls a and b and total carotenoids (Fig. 6) is dominated by the absorption of Chl a at A430 (blue) and A662 (red). One observes that Chl b and the carotenoids absorb broadly in the blue region (400 to 500 nm). Therefore, the quantity of pigments assimilating in the leaves of tomato seedlings was higher for the experimental variants compared to the control variant. Our results support the preponderance of the anabolic side of the plants metabolism with direct implications on the growth and development much better of the seedlings fertilized with natural products. It was found that the chemicals applied to fertilization restrict the growth of seedlings affecting photosynthesis. The organic fertilizer was found non- significant regarding chlorophyll content by AHMAD & UMAIR in 2017.

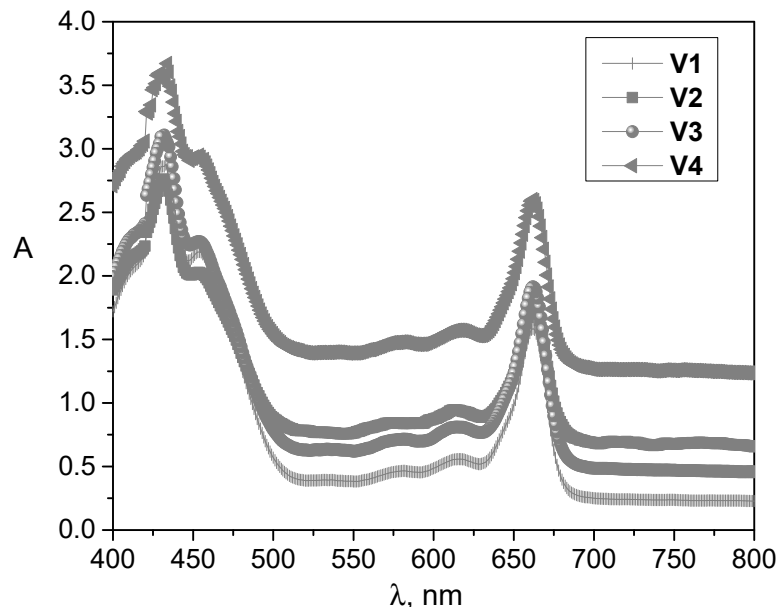


Figure 6. The influence of natural fertilisers on the content of assimilating pigments in Rila F1 tomato seedlings naturally fertilized (λ, nm).

The chlorophyll content (Fig. 7) shows significant differences between V1 and V2, V3, V4. The high amount of chlorophyll was obtained in case V4 followed by variant V3. The smallest amount of chlorophyll was recorded in V1.

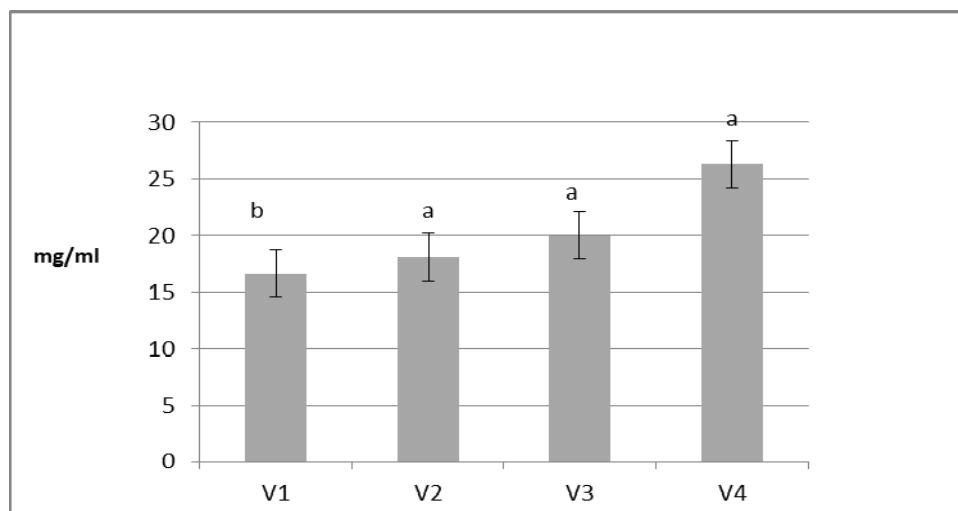


Figure 7. The influence of natural fertilisers on chlorophyll content in Rila F1 tomato seedlings naturally fertilized (mg/ml).

From the data of the Fig. 8 we can conclude that, as far as the content of xanthophylls and carotenoids is concerned, significant differences are not seen in the analysed samples.

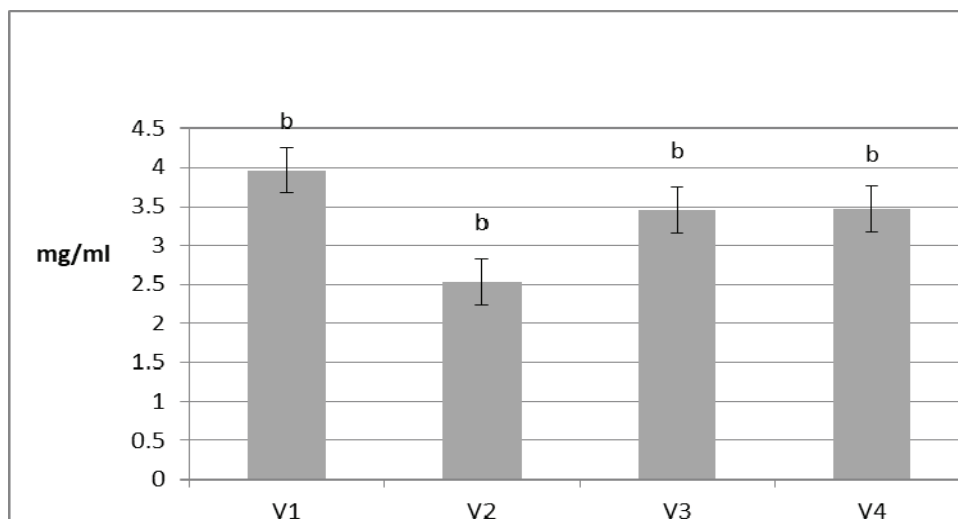


Figure 8. The influence of natural fertilisers on xanthophylls and carotene content in Rila F1 tomato seedlings naturally fertilized (mg/ml).

CONCLUSIONS

For the four experimental variants, the values of total water content ranged from 76.95 to 85.66 without significant differences between them ($p > 0.05$). Even in terms of free and bound water content, we did not notice any significant differences. There are significant differences in the dry matter content (variants V4 and V2 shared with V1 and V3). Regarding the ash content, there are no significant differences between V2 and V4, however there are significant differences between them and the control V1 and even the V3 variant. The values obtained for all the studied experimental variants are higher than the value obtained for the control variant, as this result shows the synthesis of the complex organic substances that make up the plant organism and the proper development of the physiological processes in the seedlings.

The chlorophyll content shows significant differences between studied variants and the control variant. The highest amount of chlorophyll was obtained for V4 followed by variant V3. The content of xanthophylls and carotenoids does not show significant differences in the analysed samples. Due to the fact that the nutrients from the natural fertilizers are assimilated more slowly by the plants they do not cause substantial increases of the studied parameters in the young plants, but only small changes were detected in values compared with the chemical fertilization.

The use of bio fertilizers in the production of Rila F1 tomato seedlings has played an important role in their composition through the intake of mineral substances, so that the use of chemical substances in the fertilization of tomato seedlings can be successfully replaced in the production of Rila F1 tomato seedlings with positive results on environmental protection.

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