

THE INFLUENCE OF SUBSTRATE COMPOSITION ON SOME BIOMETRIC AND BIOCHEMICAL PARAMETERS OF *Inimă de bou* TOMATO SEEDLINGS

ULEANU Florina, VÎJAN Loredana Elena, VULPE Mădălina,
SIMINEA Maria Irina, BRATU Ion Gabriel, GIOSANU Daniela

Abstract. Tomatoes are well known vegetables, grown and consumed worldwide due to their nutritional benefits. The purpose of this research was to develop a technological flow for growing tomato seedlings under ecological conditions using nutritious substrates with natural fertilizers (peat, coffee grounds, eggshell, and poultry manure). The biological material was represented by a traditional Romanian tomatoes seedling (*Inimă de Bou*) with indeterminate growth, very productive, recommended for early production. Some biometric and biochemical parameters such as the plant height, the number of leaves per plant, the foliage weight, the foliage volume, the root length, the root weight, the root volume, and the content of free water, total water, bound water, dry matter and have been determined for five variants of *Inimă de Bou* tomatoes seedlings. Analysis of variance (ANOVA) revealed significant differences at some biometric and biochemical parameters among the *Inimă de Bou* tomato seedlings variants determined by the culture substrate composition.

Keywords: substrate composition, biometric parameters, biochemical parameters, tomato seedlings.

Rezumat. Influența compoziției substratului de cultură asupra unor parametrii biometrici și biochimici la răsaturile de tomate *Inimă de bou*. Tomatele sunt legume bine cunoscute, cultivate și consumate în întreaga lume datorită beneficiilor lor nutritive. Scopul acestei cercetări a fost acela de a dezvolta un flux tehnologic pentru cultivarea răsaturilor de tomate în condiții ecologice, folosind substraturi nutritive cu fertilizanți naturali (mraniță, zaț de cafea, coajă de ou și gunoi de pasăre). Materialul biologic a fost reprezentat de un soi tradițional românesc de tomate cu creștere nedeterminată, foarte productiv, recomandat pentru producție timpurie (*Inimă de Bou*). Pentru cele cinci variante de răsaturi de tomate *Inimă de Bou* au fost determinați unii parametrii biometrici și biochimici, precum: înălțimea plantei, numărul de frunze pe plantă, masa frunzelor, volumul frunzelor, lungimea rădăcinii, masa sistemului radicular, volumul sistemului radicular și conținutul de apă liberă, apă totală, apă legată, substanță uscată și pigmenți asimilatori. Analiza varianței (ANOVA) a evidențiat diferențe semnificative la unii parametrii biometrici și biochimici în rândul variantelor de răsaturi de tomate *Inimă de Bou* determinate de compoziția substratului de cultură.

Cuvinte cheie: compoziție substrat, parametrii biometrici, parametrii biochimici, răsaturi tomate.

INTRODUCTION

In time, the increase in the population of the globe, the change of diet and limited opportunities to expand agricultural lands have led to the intensification of agriculture.

Organic farming has emerged as an alternative to intensively practiced conventional agriculture, through the use of large quantities of production boosters, with the aim of continuous growth of the final product for a continuously growing population, mainly urban (SAMUIL, 2007). Organic farming is characterized by the absence of synthetic fertilizers and pesticides, and the frequent use of sources of organic matter to maintain soil fertility (STANHILL, 1990; VAN BRUGGEN, 1995). The principles of organic farming refer to: maintaining soil fertility, protecting the environment, respecting the health of consumers, the global vision on the interactions in nature, the farm - a unit, an organism in balance (DEJEU et al., 1997; TONCEA & STOIANOV, 2002).

The soil is at the centre of the concerns of organic farming, because it is considered a living environment, complex, but still little known, which interacts closely with the plants and animals that populate it. All actions covered by organic farming (soil work, fertilization, choosing products to fight diseases and pests, etc.) aim to increase the microbiological activity of the soil, maintaining and increasing its fertility under essential conditions for the health of plants keeping (AYOOLA & ADENIYAN, 2006).

The main purpose of the study was to find a seedling development solution, transplanted in different nutritional substrates, using natural fertilizers (peat, poultry manure, coffee grounds, and eggshells). Thus, all forms of pollution resulting from agricultural technology and their influence on biometric and biochemical parameters have been reduced. In addition, the influence of different nutrient substrate compositions on some biometric and biochemical parameters were studied.

MATERIAL AND METHODS

For the realization of the experimental model from our research, *Inimă de Bou* tomato seedlings were used. *Inimă de Bou* is a traditional Romanian tomato cultivar with indefinite growth, highly productive, recommended for early production. This cultivar produces big fruits (200–250 g) which have the shape of a heart and their taste is refreshing, sweet with a discreet pineapple aroma.

Seedlings were obtained from seeds (purchased from a speciality store in Pitești) sown in a professional substrate, in 32-cell honeycomb trays, to eliminate plant stress because it is known that a stressed body is more sensitive

to pathogens. The maintenance works during the vegetation period were applied uniformly to all the studied variants. At the appearance of the first true leaves (after three weeks) the seedlings were retransplanted on different nutritive substrates.

Five experimental variants, designated as V1, V2 ... V5 (Table 1) were used. The V1 control variant had as nutritive substrate the professional substrate commonly used for sowing tomato seeds while nutritious substrates with natural fertilizers (peat, coffee grounds, eggshell, and poultry manure) were used in four other variants, in different proportions.

Table 1. Experimental variants.

Experimental variants	Substrate
V1 (control variant)	professional mix substrate, peat, perlite and poultry manure in the proportion of 1:1:1:1
V2	peat and coffee grounds in the proportion of 2:1
V3	peat and coffee grounds in the proportion of 2:1 and eggshell with 1 teaspoon of eggshell
V4	garden soil, peat and coffee grounds in the proportion of 1:1:1
V5	garden soil, peat and coffee grounds in the proportion of 1:1:1 with 1 teaspoon of eggshell

60 days after sowing, when the seedlings had 4-5 well-developed leaves, before being transplanted in the greenhouse, biometric and biochemical determinations were made.

First, the following were determined by direct measurements: the plant height, the number of leaves per plant, the foliage weight, the foliage volume, the root length, the root weight, and the root volume. To perform all biometric analysis, five plants were selected for each variant, removing the plants from the marginal cells of the trays. The plant height and the root length were determined by tape measurement.

The foliage weight, the root weight, the foliage volume and the root volume were determined with an electronic balance with an accuracy of 0.01 g. For example, the root volume was determined by weighing a beaker with water (denoted m_1), where the root system is immersed, ensuring complete coverage by the water but preventing contact with the walls of the vessel (denoted m_2). The weight increase ($m_2 - m_1$), in grams equals the root volume, in mL.

Then, the content of free water, total water, bound water and dry matter was determined gravimetrically by means of oven-drying. We took 2 g of vegetal material (leaves) collected from the middle floor (m_i), which was put in a porcelain capsule, brought to a constant mass. To determine the content in free water, the plant material was dried at 40°C to a constant mass, denoted m_{f1} and the following formula has been used.

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

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).

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

The content of bound water in the test sample was calculated by the difference between the total water content and free water content according to the relation:

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

Finally, the assimilatory pigment content was obtained with a UV-Vis spectrophotometer PerkinElmer Lambda25, using acetone as the solvent for extraction. Thus, we weighed 0.1 g tomato seedlings leaves and we mixed with 10 mL of 100% acetone, filtered and then collected the filtrate in a test tube. We filled the spectrophotometer cuvette with the extract and read the absorbance at the following wavelengths: 663 nm, 645 nm and 470 nm. Using Arnon's relation (LICHTENTHALER & WELLBURN, 1983; WELLBURN, 1994), the contents of chlorophyll a, chlorophyll b and carotenoids (carotenes and xanthophylls) were calculated:

$$\begin{aligned} \text{Chl } a \text{ (mg/L)} &= 11.75 \times A_{662} - 2.35 \times A_{645} \\ \text{Chl } b \text{ (mg/L)} &= 18.61 \times A_{645} - 3.96 \times A_{663} \\ \text{Total Chl (mg/L)} &= \text{Chl } a + \text{Chl } b \\ \text{Carotenoids (mg/L)} &= [1000 \times A_{470} - 2.27 \times \text{Chl } a - 81.4 \times \text{Chl } b] / 227 \end{aligned}$$

All parameters were analysed for all variants, emphasizing the differentiation produced by the used culture substrates. The experimental data processing was carried out by using specific mathematical and statistical methods. For analysing the biometric parameters, five tomato plants were selected and the biochemical analyses were carried out in triplicate. The data were statistically analysed by one-way analysis of variance (ANOVA) using SPSS 16.0 software.

Differences in values among the *Inimă de Bou* tomato seedlings variants were estimated with multiple Duncan tests for a confidence degree of 95 % ($p < 0.05$). The different letters mean the significant differences determined by the culture substrate composition, according to the Duncan test ($p < 0.05$).

RESULTS AND DISCUSSIONS

Tomato (*Solanum lycopersicum* L.) is one of the most cultivated crops in the world, also offering many opportunities for research and marketing. Tomatoes which are consumed fresh must have a special taste and colour, some firmness of the pulp and sometimes a certain size of fruit according to the preference of the consumer (CIOFU et al., 2004). Therefore, the appearance plays an important role in accepting and choosing tomato fruits. However, in order to obtain optimal tomato culture and high-quality tomatoes that satisfy market requirements, the used seedling must meet certain conditions, starting from healthy seeds, which are resistant to pathogens.

The data regarding the analysed biometric parameters 60 days after sowing for all five variants of *Inimă de Bou* tomatoes seedlings are presented in table 2.

Table 2. Biometric parameters of the *Inimă de Bou* tomato seedlings.

Measured parameters	Experimental variants				
	V1 (control variant)	V2	V3	V4	V5
Number of leaves per plant	6 b	4 a	4 a	5 ab	5 ab
Plant height, cm	35 c	14.5 a	15 a	16.2 ab	16.5 ab
Root weight, g	5.13 c	0.36 a	0.57 ab	0.99 b	1.29 b
Root length, cm	19.7 c	9.6 ab	7.5 a	11.5 b	22.3 d
Root volume, cm ³	5 d	1 a	1.5 ab	2 b	2.3 bc
Foliage weight, g	12.58 c	1.01 a	1.21 a	1.48 ab	2.28 b
Foliage volume, cm ³	20 d	1.5 a	2.5 b	2.5 b	5 c

Means within each row followed by the same letters are not significantly different according to the Duncan test ($p < 0.05$). The different letters by each row mean the significant differences, which are determined by the culture substrate composition.

Regarding the number of leaves per plant, 60 days after sowing, it is observed that the differences between the variants are small, the largest number being presented by the V1 control variant (6 leaves) and the lowest number by the V2 and V3 variants (4 leaves). It seems that the frequently used substrate (from the V1 control variant) produces a faster development of the leaves of the *Inimă de Bou* tomato seedlings.

The V1 control variant (professional mix substrate, peat, perlite and poultry manure in the proportion of 1:1:1:1) shows the highest increase in plant height compared to the other variants. Between this variant and all experimental variants there are significant differences. From the four experimental variants, the highest value of plant height is presented by the V5 variant (garden soil, peat and coffee grounds in the proportion of 1:1:1 with 1 teaspoon of eggshell) and the lowest value of plant height is presented by the V2 variant (peat and coffee grounds in the proportion of 2:1), the differences between these experimental variants being insignificant.

Roots are important plant organs because they exert control over whole-plant growth and development by controlling the uptake of mineral nutrients and water from the soil (ZOBEL, 1986; SAINJU et al., 2001; MERRILL et al., 2002). Roots also provide mechanical support to plants and supply hormones that affect many physiological and biochemical processes associated with plant growth and development (RAO & CRAMER, 2003; SCHJOERRING et al., 2019). Root growth is critical to the establishment of the newly seedlings. Seedlings can undergo stress when a planted seedling's root system cannot supply enough water to maintain a proper water balance and ensure survival. Thus, a newly planted seedling's ability to overcome planting stress is affected by its root system size and distribution, root-soil contact, and root hydraulic conductivity (GROSSNICKLE, 2005). Nutrient uptake by plants is related to soil parameters such as nutrient availability, buffering capacity and mobility (NYE, 1966). Inputs of nutrients like phosphorus and nitrogen are essential to increase and sustain the production of vegetables but are often unaffordable for poor farmers (FRIESEN et al., 1997; RAO, 2001) and, therefore, it is important to select the optimal culture substrates for a more efficiently foraging of the plants. Root size may be quantified by weight, length, surface area or volume. XIA et al., 2013 showed that phosphorus and calcium influence the increase in root length and volume in tomatoes.

The V1 control variant shows a more vigorous root, having a higher root weight (5.13 g) compared to the other variants. There are significant differences between this variant and the others. The intermediate values of root weight were found for the V5 variant (1.29 g) and the V4 variant (0.99 g) and the lowest values of root weight were found for the V3 variant (0.57 g) and the V2 variant (0.36 g).

Regarding the root length, the highest value can be observed in the case of the V5 variant (garden soil, peat and coffee grounds in the proportion of 1:1:1 with 1 teaspoon of eggshell) which exceeded the V1 control variant. It is observed that there are no significant differences between these two variants. Significant differences are observed between these two variants and the other three variants. The lowest value of the root length was registered in the case of the V3 variant (peat and coffee grounds in the proportion of 2:1 and eggshell with 1 teaspoon of eggshell).

Root volume recorded an average value of 5 cm³ for the V1 control variant, the maximum recorded and an average value of 1 cm³ for the V2 variant (peat and coffee grounds in the proportion of 2:1), the minimum recorded. Furthermore, the V5 variant (garden soil, peat and coffee grounds in the proportion of 1:1:1 with 1 teaspoon of eggshell) had an intermediate value (2.3 cm³). There are significant differences between variants.

Based on the values for the biometric parameters of the root it is anticipated that the V1 and V5 variants of *Inimă de Bou* tomato seedlings will have a good evolution, being characterized by a vigorous root system because it is known that the seedlings become strong when they have developed a root system that couples them to the available soil water (GROSSNICKLE, 2005).

Leaves are organs responsible for plant photosynthesis. They provide the conversion of carbon dioxide, water, and UV light into sugar (e.g., glucose). These simple sugars formed via photosynthesis are later processed into various macromolecules (e.g., cellulose) required for the formation of the plant cell wall and other structures. Therefore, the leaves are highly specialized to combine the carbon dioxide, water, and UV light in this process. The sugar is typically transported to the roots and shoots of the plant, to support plant growth and development. Leaves are attached by a continuous vascular system to the rest of the plant so as to ensure the exchange of nutrients, water and end products of photosynthesis (oxygen and carbohydrates in particular) in various parts of the plants (ARBER, 1950; BUCCI et al., 2004).

Regarding the foliage weight and volume, 60 days after sowing, the V1 control variant (professional mix substrate + peat + perlite + poultry manure in the proportion of 1:1:1:1) shows the highest values in the two parameters compared to the other variants. There are significant differences between this variant and all experimental variants. Regarding the four experimental variants, the highest values of the foliage weight and volume are presented by the V5 variant (garden soil, peat and coffee grounds in the proportion of 1:1:1 with 1 teaspoon of eggshell) and the lowest values of these two parameters are presented by the V2 variant (peat and coffee grounds in the proportion of 2:1), the differences between these four variants being small or medium.

For all variants of *Inimă de Bou* tomatoes seedlings, the content of free water, total water, bound water, dry matter, and assimilatory pigments were determined.

It is known that water enters in large amounts in the composition of all plant cells, 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. Free water is the environment in which water is bound together with the macromolecules it hydrates. This is the amount of water that serves for the hydrolysis and the translocation of the substances accumulated in the leaves, to the other organs of the plant, acting as solvent and vehicle (STANCU & FLEANCU, 2004; KHAN et al., 2016). Low temperatures down to 10 °C cause the freezing of the free water, and therefore plants with high free water content are not very resistant to low temperatures (VÎJAN et al., 2019). Figure 1 presents the results regarding the free water content of *Inimă de Bou* tomatoes seedling samples.

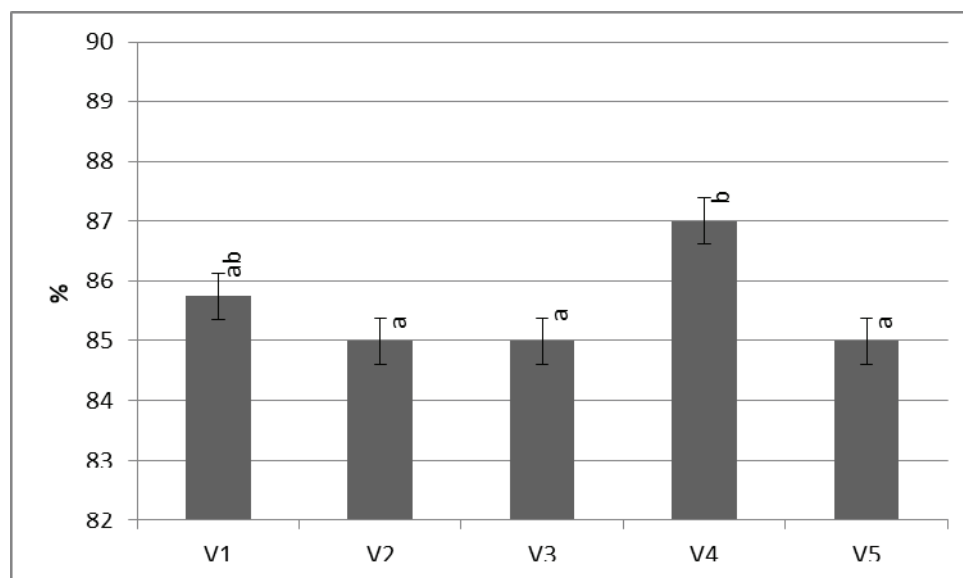


Figure 1. Free water content of the *Inimă de Bou* tomato seedlings depending on the culture substrate Means within each column followed by the same letters are not significantly different according to Duncan test ($p < 0.05$).

It is noted that the free water content for the *Inimă de Bou* tomatoes seedlings variants ranged between 85% (the V2, V3 and V5 variants) and 87% (the V4 variant), as the V1 control variant has an intermediate value (85.75%) at this parameter. Differences between all the variants are relatively small.

Total water represents a supply index for the soils and, because different culture substrates 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 (STANCU & FLEANCU, 2004; VÎJAN et al., 2019). Total water content is determined by measuring the mass of the vegetal material before and after the water was removed by evaporation. The basic principle of this technique is that water has a lower boiling point than the other major components within foods, e.g. lipids, proteins, carbohydrates and minerals. Figure 2 presents the results regarding the total water content of *Inimă de Bou* tomatoes seedling samples.

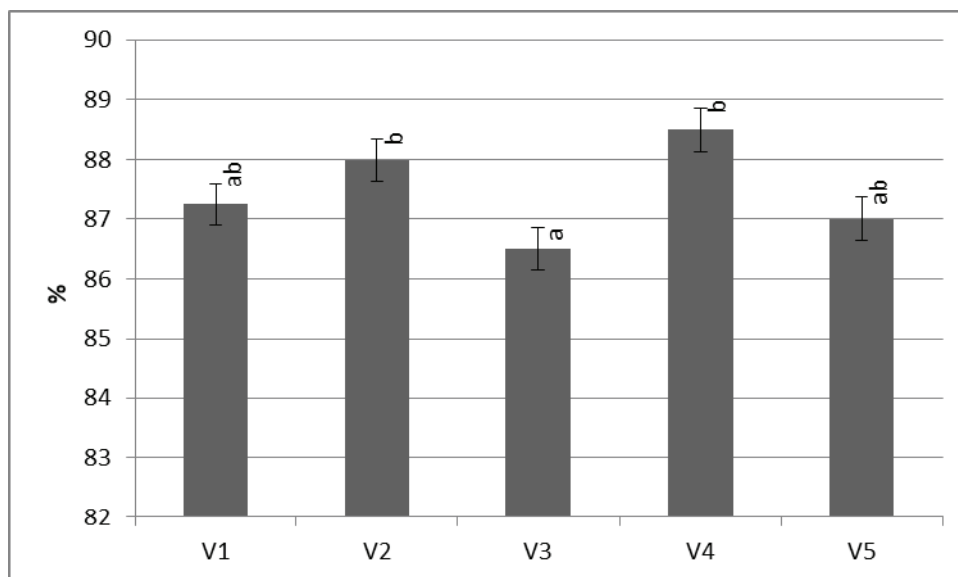


Figure 2. Total water content of the *Inimă de Bou* tomato seedlings depending on the culture substrate. Means within each column followed by the same letters are not significantly different according to Duncan test ($p < 0.05$).

It is noted that the *Inimă de Bou* tomatoes variants have a water content ranging from 86.5% (the V3 variant) to 88.5% (the V4 variant), and the V1 control variant has an intermediate value (87.25%) in this interval. Differences between all the variants are relatively small.

Bound water is made up of non-diffusible molecules and thus, it is plentifully retained by cells. In addition, this water does not take part in 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. Therefore, bound water at a vegetative moment is an index which shows 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). Figure 3 presents the results regarding the bound water content of *Inimă de Bou* tomato seedling samples.

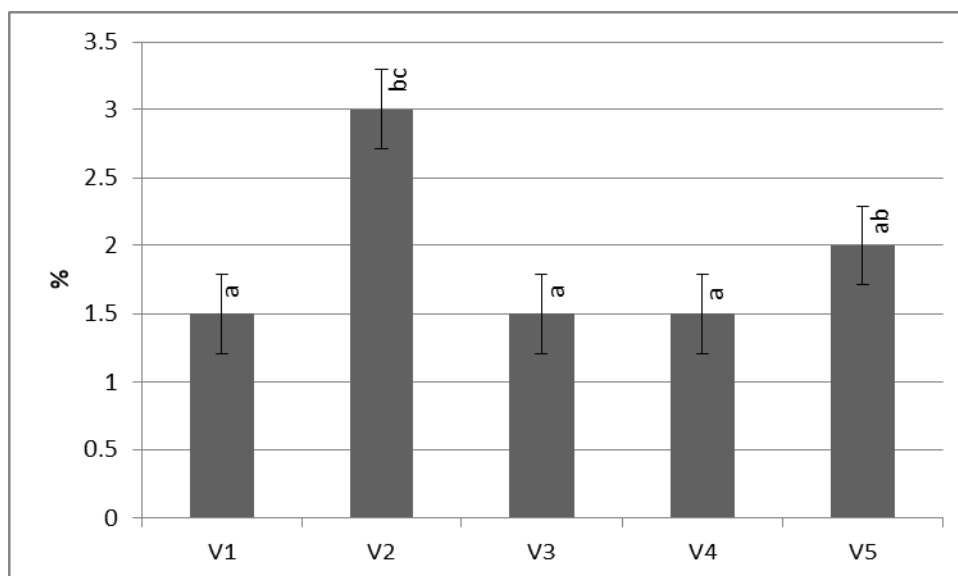


Figure 3. Bound water content of the *Inimă de Bou* tomato seedlings depending on the culture substrate. Means within each column followed by the same letters are not significantly different according to the Duncan test ($p < 0.05$).

Regarding the bound water content, 60 days after sowing, the studied variants of *Inimă de Bou* tomato seedlings presented three values: 3 % at the V2 variant (peat and coffee grounds in the proportion of 2:1), 2 % at the V5 variant (garden soil, peat and coffee grounds in the proportion of 1:1:1 with 1 teaspoon of eggshell) and 1.5 % for the three other variants, including the V1 control variant. Differences between all the variants are relatively small. Based on the values for bound water content it is anticipated that the V2 and V5 variants of *Inimă de Bou* tomato seedlings will have a good resistance to high temperatures during summer (STANCU & FLEANCU, 2004; VÎJAN et al., 2019).

In the case of the dry substance (Fig. 4), the values obtained for the experimental variants are higher than the value obtained for the V1 control variant, except for V2 and V4 variants. Insignificant differences are found between variants. This last aspect is probably due to the respiratory rate (the ratio of photosynthetic intensity/ intensity of breath), this physiological index being correlated with the value of the dry substance.

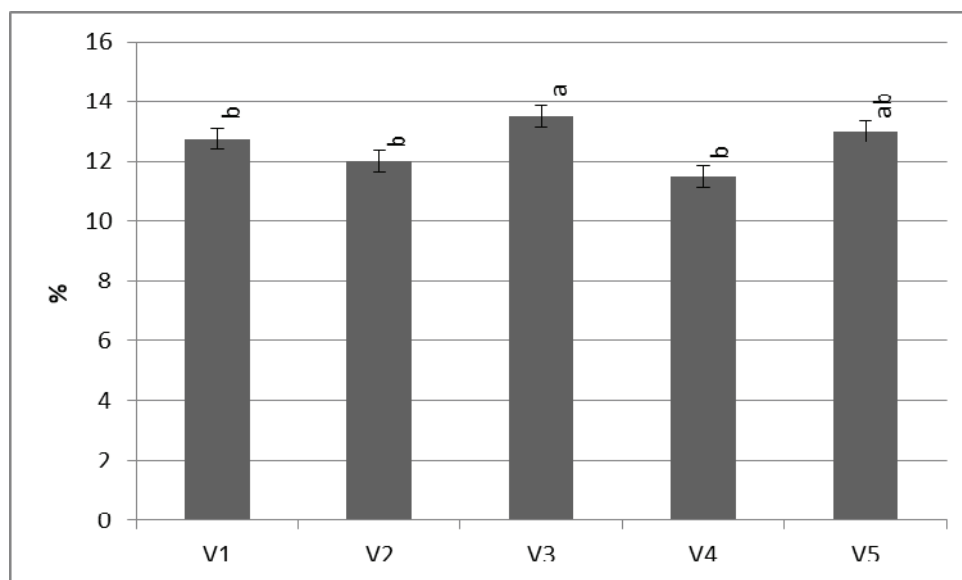


Figure 4. Dry matter content of the *Inimă de Bou* tomato seedlings depending on the culture substrate Means within each column followed by the same letters are not significantly different according to the Duncan test ($p < 0.05$).

Regarding the values for the content of the assimilating pigments from the leaves of *Inimă de Bou* tomatoes seedling samples (table 3) the highest values can be observed in the case of the V1 and V5 variants. Between these two variants and other three experimental variants there are significant differences.

Table 3. Content of the assimilating pigments from the *Inimă de Bou* tomato seedlings leaves depending on the culture substrate.

Measured parameters	Experimental variants				
	V1 (control variant)	V2	V3	V4	V5
Chl a (mg/L)	16.47 d	5.79 b	4.72 ab	3.53 a	11.09 c
Chl b (mg/L)	7.99 b	8.16 b	8.04 b	4.26 a	13.78 c
Total Chl (mg/L)	24.46 c	13.95 b	12.76 b	7.79 a	24.87 c
Carotenoids (mg/L)	3.44 c	1.05 a	0.91 a	1.21 ab	1.24 ab

Means within each row followed by the same letters are not significantly different according to the Duncan test ($p < 0.05$). The different letters by each row mean the significant differences, which are determined by the culture substrate composition.

These results support the preponderance of the anabolic side of the plants metabolism with direct implications on the growth of tomato seedlings replicated on substrates with different composition. Therefore, there is a direct correlation between this physiological index and the content of the dry substance, which highlights the high proportion of anabolism leading to faster growth of bio-fertilized seedlings.

CONCLUSIONS

The analysis of the biometric characteristics of *Inimă de Bou* tomato seedlings variants indicates that the V1 control variant (professional mix substrate, peat, perlite and poultry manure in the proportion of 1:1:1:1) generates the best development of plants, being followed by the V5 experimental variant (garden soil, peat and coffee grounds in the proportion of 1:1:1 with 1 teaspoon of eggshell). In the other three variants of *Inimă de Bou* tomato seedlings, the composition of the culture substrate did not improve the plant development. Because these three experimental variants (V2 ... V4) have in their composition peat (present in all variants) and coffee grounds (present in variants V2 ... V5) we

can conclude that some nutrients in the coffee grounds had a negative effect on the tomato seedlings development. However, a positive aspect, unfortunately insignificant, for the usage of the coffee grounds in the culture substrate composition is determined by the increase of the plants resistance to high temperatures, as a result of the increase of the bound water content in these tomato seedlings.

The analysis of the biometric and biochemical parameters for the V4 and V5 experimental variants of *Inimă de Bou* tomato seedlings, which are differentiated by the presence of a teaspoon of eggshell in the V5 variant, indicates the positive contribution of the eggshell in the culture substrate.

Comparing the results obtained for the V3 and V5 experimental variants, which are differentiated by the presence of garden soil in the V5 variant, it is visible that garden soil had a positive contribution at the seedling development.

Due to the complex composition of the V1 control variant (professional mixing substrate, peat, perlite and poultry manure in a ratio of 1:1:1:1) it is not possible to specify which composite of the culture substrate is the most beneficial for the development of tomato seedlings, but it can be emphasized that the whole substrate is optimal for this crop. However, taking into account that some quite good results were also obtained in the V5 experimental variant (garden soil, peat and coffee grounds in the proportion of 1:1:1 with 1 teaspoon of eggshell), we intend to analyse the effects produced on tomato seedlings by the culture substrates composed of peat, garden soil or professional mix of substrates, perlite or poultry manure or perlite and poultry manure, with or without a teaspoon of eggshell, in order to determine whether there is a culture substrate of better quality than the one which is most frequently used, established as the optimal based on our results.

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Uleanu Florina, Vîjan Loredana Elena, Vulpe Mădălina, Siminea Maria Irina, Bratu Ion Gabriel, Giosanu Daniela
 University of Pitești, Str. Târgu din Vale, No. 1, 110040, Pitești, Romania.
 E-mails: uleanuflorina@yahoo.ro; vloredana2005@yahoo.com, loredana.vijan@upit.ro

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