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THE EFFECT OF SOME BIOFERTILIZERS ON THE BIOCHEMICAL COMPONENTS OF THE TOMATO PLANTS AND FRUITS

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Abstract

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The present study aims at highlighting the major role of ecological agriculture in the current organic farming context, internationally and nationally, and the importance of using natural fertilizers in the vegetable culture.

The research was conducted at the Banu Mărăcine Didactic Station - Romania, in unheated solarium, on some cultivars of tomatoes (Antalya, Cemil and Lorely). There was observed the impact of foliar treatment with three different biofertilizers consisting of humic acids extracted from brown coal, humic acids mixed with polyphenolic extract of *Vitis vinifera* seeds and humic acids mixed with polyphenolic extract of *Vitis vinifera* seeds and Boron, on the content of chlorophyll, total carotene on stages of plant and fruit development and obtained production. It was found that the biochemical changes and obtained production vary depending on the nature of the fertilizer used and of the cultivar, the best results occurring in the variant fertilized with humic acids + extract from the *Vitis vinifera* seeds + Boron (HA + ESVv + B).

Key words: tomato, humic acids, polyphenols, seeds of *Vitis vinifera*, boron

Introduction

The increased yield per area unit in the agricultural production caused an increase of the use of synthetic fertilizers and pesticides in the process of fertilization. The intensive use of them has generated many health problems and environmental pollution thus current researches are heading towards new agricultural practices developed in the so-called organic farming (Chowdhury, 2004).

Worldwide, especially in Europe, a new requirement, quite solid, appeared which turned into a real movement to obtain food products through clean technologies without synthetic chemicals, agricultural products to ensure the consumers health in terms of environmental protection and conservation of natural resources.

The increased interest in the use of natural regulators in the agricultural culture, in the wheat culture (Mona et. al., 2012; Kowsar et al., 2014) and especially in the vegetable culture helped to develop a wide range of fertilizers based on natural components (Pârvan et. al., 2013).

In this paper we present the behavior of tomatoes cultivars (Antalya, Cemil and Lorely) grown in a protected space, with a foliar treatment using three types of biofertilizers of humic acids extracted from brown coal in combination with polyphenolic extract from the seeds of *Vitis vinifera* and boron. This combination shows the individual effects of each component on soil and plants, namely:

- Humic acids are compounds that contribute to the increase of cell membranes permeability in plants (Kaya et al., 2005) and recent studies prove their role on the process of seeds

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germination (Dinu et al., 2014), on raising the seedling growth, on the development and weight gain of the root system in plants (Karakurt et al., 2007; Katkat et al., 2009).

- The action of polyphenols in plants is distinguished by their role in the formation of pigments, in the development and resistance to pathogens and UV protection (Latanzzio et al., 2006).
- The boron influences the flowering, fertilization and seeds development, stimulates the root growth, activates some enzymes (pectase, tyrosinase, invertase, dehydrogenase), and acts indirectly on the photosynthesis accelerating the transport of carbohydrates, especially of the sucrose in leaves. It has a favourable effect on flowering and fruiting of the plants because it stimulates the rapid germination of pollen.

Also, the boron influences the synthesis of the aromatic compounds in plants, the protoplasmic membrane permeability, the translocation of glucose, and the cells division and extent, the cell differentiation, the maturation of the fruits, the accumulation of free auxins and the biosynthesis of nucleic acids (Blevins et al., 1998; Waquar et al., 2012). A deficiency of boron in plants and soil leads to the diminution of the vascularisation process of the plant, the root elongation, slowing the carbohydrates metabolism, reducing the synthesis of nucleic acids etc. (Blevins et al., 1998). In its absence the leaves become vulnerable, their point of growth blacken, and they begin to twist inward. The flowers usually abort and drop. The fruits begin to stain (it starts the tissue damaging).

Materials and Methods

Vegetal material

The research was conducted at the Banu Mărăcine Didactic Station - Romania, in unheated solarium.

Antalya, Cemil and Lorely tomato hybrids were studied. The experiment was arranged in randomized blocks with five variants of 4 repetitions for each variant. The biofertilizer was applied to foliage at a concentration of 10 mL/L humic acid and 15 mL/L for the variants with boron and polyphenol extract. The first foliar treatment was administered at two weeks after planting and the following at a period of 10 days.

The variants were as follows: V1- control (Mt.); V2 - extract from the seeds of *Vitis vinifera* (ESVv.); V3 - humic acid (HA.); V4 - humic acids + extract of *Vitis vinifera* seeds (HA + ESVv.); V5 - humic acids + extract from the seeds of *Vitis vinifera* + Boron (HA + ESVv + B).

The leaves of tomatoes harvested at the beginning of flowering and the fruiting process which reached technological and physiological maturity were used for the study. The leaves and fruits were packed in plastic bags and left overnight in the dark at a temperature of 4ºC.

Determination of chlorophyll a+b and total carotenoid

The weighed samples, having been put separately in 95% in acetone (50 ml for each gram), were homogenized with Braun MR 404 Plus for one minute. The homogenate was filtered and was centrifuged using the Hettich Universal 320/320R centrifuge at 2500 rpm for ten minutes. The supernatant was separated and the absorbances were read at 400-700 nm on Cary 50 spectrophotometer. It was recorded that Chlorophyll a showed the maximum absorbance at 662 nm, chlorophyll b at 646 nm and total caroten at 470 nm and the amount of these pigments was calculated according to the formulas (Nagata, 1992; Dinu et al., 2013; Mladonevic et al., 2014).

$$Ca = 11.75\ A662 - 2.350\ A645$$

$$Cb = 18.61\ A645 - 3.960\ A662$$

$$Cx{+}c = 1000\ A470 - 2.270\ Ca - 81.4\ Cb/227$$

Ca = Chlorophyll a, Cb = Chlorophyll b, Cx+c = Total carotene

For statistical interpretation of the data, the ANOVA method has been used.

Statistical analysis

The data are the values of analytic determinations of samples. Three replicates of each sample were used for statistical analysis. Analysis of the data was performed on the original data by one-way analysis of variance (ANOVA) or regression analysis. Differences at $P < 0.05$ were considered significant.

Results and Discussions

To determine the biochemical changes in the tomato cultivars used in the research, in terms of the composition of applied foliar treatment, we studied the dynamic of the evolution of some biochemical components involved in the plant metabolism (chlorophyll ‘a’, chlorophyll ‘b’ and carotenoids) and their influence on the obtained production (Figures 1, 2 and 3 and Tables 1 and 2).

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

Under the action of the biofertilizers, all the three cultivars show increases in chlorophyll a concentration (the Antalya cultivar has an increase ranging from 1.35% to 18.13%, the Cemil cultivar from 8.6% to 26.71% and the Lorely cultivar from 54.3% to 209.0%) compared to the control sample.

The concentration of chlorophyll “b” in all the three cultivars increased from 1.7% to 13.81% for Antalya cultivar, from 38.03% to 69.57% for the Cemil cultivar and from 56.91% to

61.66% for the Lorely cultivar in comparison to the control plant (Figure 2).

There is a significant increase in the concentration of chlorophyll a and b for Cemil and Lorely cultivars. From the measured values there is noted a significant influence of the fertilizer obtained from HA+ESVv and of that obtained from HA + ESVv + B on the chlorophyll "a" and "b" concentration.

Table 1
The determined biochemical components in tomato fruits, mg/100gFW

Antalya						
Sample	Technological maturity fruit			Fruit physiological maturity		
	Control	Fertilized	Control	Fertilized	Control	Fertilized
V1	51	24.7	6.5	26.2	11.3	8.5
V2	54	23	7.5	28.9	14.2	9.4
V3	50.2	22.9	4.2	26.2	10.8	6.4
V4	47	24.2	6.1	25.5	13.4	8.3
V 5	75.3	34.5	8.1	37.9	15.4	10.1
Cemil						
Sample	Technological maturity fruit			Fruit physiological maturity		
	Control	Fertilized	Control	Fertilized	Control	Fertilized
V1	47	26.3	4.3	0.41	7.2	7
V2	39	20	1.9	0.4	8.4	19.8
V3	37.5	20	1.8	0.42	8.8	16.4
V4	70.4	32.8	7	0.45	9	32.5
V5	68	33.5	7.5	0.42	9.2	31.6
Loreley						
Sample	Technological maturity fruit			Fruit physiological maturity		
	Control	Fertilized	Control	Fertilized	Control	Fertilized
V1	34.2	16	1	0.41	5.2	12.2
V2	36	19.2	1.6	0.4	6.2	14
V3	45.1	22.5	3.8	0.42	6.9	22.5
V4	48.7	27	5.8	0.43	7.2	27.4
V5	46	25.1	3.8	0.42	6.7	34.7

Table 2
The tomato production of the studied hybrids

	Antalya			Cemil			Lorely		
Var.	Kg/m^2	%	± Relative to control, %	Kg/m^2	%	± Relative to control, %	Kg/m^2	%	± Relative to control, %
V1	22.4	100.0	Mt.	19.3	100.0	Mt.	20.8	100.0	Mt.
V2	30.8	137.5	+ 37.5	23.7	122.7	+ 22.7	21.7	104.3	+ 4.3
V3	32.7	145.9	+ 45.9	27.7	143.5	+ 43.5	24.4	107.6	+ 7.6
V4	30.5	136.1	+ 36.1	28.7	148.7	+ 48.7	25.1	120.6	+ 20.6
V5	34.1	152.2	+ 52.2	29.9	154.9	+ 54.9	26.5	127.8	+ 27.8

The high chlorophyll concentrations are due to the effect of HA+ESV+ B which enhanced the photosynthesis in leaves with a synthesis of sucrose that are rapidly transported to the root and released into their rhizosphere (Macus-Wyner, 1982; Katyal, 1983; Trevisan et al., 2010). The polyphenolic substances contained by ESVv contribute to the formation of assimilating pigments in growth (Latanzzio et al., 2006). The boron activates some enzymes- invertase, pectase, tyrosinase, dehydrogenase, acting indirectly on the photosynthesis accelerating the transport of carbohydrates, especially of the sucrose in leaves. It participates in the oxidation-reduction processes in plants, influencing the formation of chlorophyll process along with other micro-elements (Mn, Cu, Zn), it takes part in the carbohydrates metabolism and participates in the process of their formation, favoring the process of respiration.

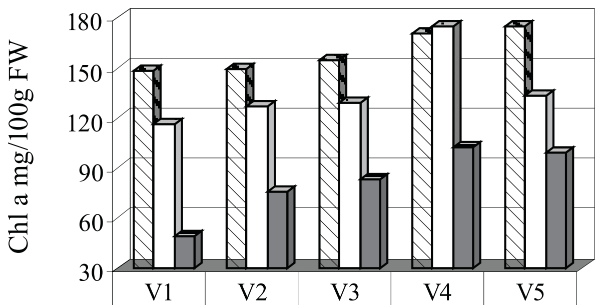
The increasing of the cellular activity and respiration, as an effect of the fertilization, entails an increase in the absorption of water through the vascular system in response to the increased water demand of the leaves. The increased amount of water through the vascular system leads to the nutrients supply of the plant (via the vascular system) (Pflugmacher et al., 2006).

The increase of the total amount of carotene in comparison to the control plant due to the process of fertilization ranges between 3.2% -7.37% for Antalya cultivar, 27% - 57.57% for Cemil cultivar and 32.94 to 50.58% for Lorely cultivar (Figure 3).

A correlation is observed between the values of the assimilating pigments for the Cemil and Antalya cultivars with the values of the total carotenoids. This increase helps to capture the light energy that is transferred to the plant photosynthetic reaction centers (Gross, 1991).

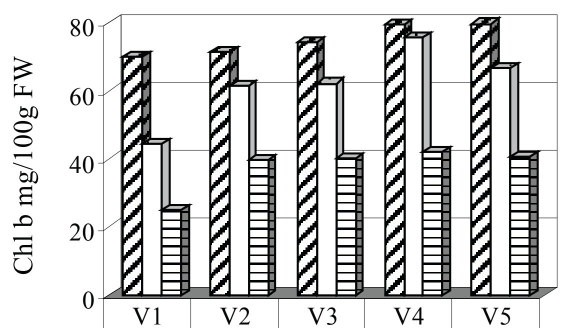
The polyphenols contained in ESVv through their role in the formation of chlorophyll pigments contribute to the formation process of carotenes (Latanzzio et al., 2006).

The influence of the foliar treatment with HA+ESVv and of that obtained from HA+ESVv+B is also observed in the formation of total carotenoids. The boron by accelerating the assimilation process of carbohydrates in the plant, act on the enzyme system and maintain the function of the plasma membrane contributing to the increase of plants metabolism during the formation of the carotenoids (Xu et al., 2007).



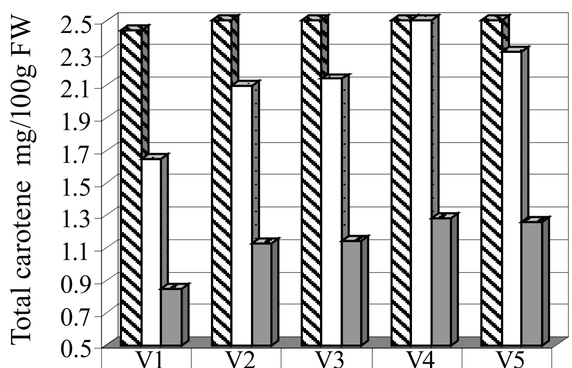
	V1	V2	V3	V4	V5
Antalya	147.8	149.8	154.5	170.2	174.6
Cemil	116.4	126.5	129.3	174.5	133.6
Loreley	48.8	75.3	82.6	102.4	98.7

Fig. 1. The determination of chlorophyll "a" in the leaves of tomato cultivars



	V1	V2	V3	V4	V5
Antalya	70.2	71.4	74.2	79.6	79.7
Cemil	44.7	61.7	62.2	75.8	66.7
Loreley	25.3	39.7	40.1	42.3	40.9

Fig. 2. The determination of chlorophyll "b" in the leaves of tomato cultivars



	V1	V2	V3	V4	V5
Antalya	2.44	2.52	2.54	2.6	2.62
Cemil	1.65	2.1	2.14	2.6	2.31
Lorele	0.85	1.13	1.14	1.28	1.26

Fig. 3. The determination of the total carotene content in the leaves of tomato cultivars

The polyphenols contained in ESVv through their role in the formation of chlorophyll pigments contribute to the formation of carotenoids (Latanzzio et al., 2006).

The humic acids along with other components with the effect on the cell membrane permeability growth, on the metabolism and development of major active regions in the growth and development of plants (roots, leaves) (Kaya et al., 2005) lead to the increased carotene content in plants.

The effect of the growth process of carbohydrates assimilation by plants contributes to the enhancing of the synthesis in chloroplasts and chromoplasts of carotenoids, after the process of biodegradation of carbohydrates.

In order to achieve the best possible correlation between the treatments and the response of the plants to the treatment there were carried out determinations on the fruits of tomatoes which reached the technological and physiological maturity (Tables 1-2).

The treatments applied to the three cultivars of tomatoes (Antalya, Cemil and Lorely) stimulate differently the formation of carotenoids in fruits which reached the technological and physiological maturity. Antalya cultivar fertilized with ESVv and HA+ESVv+B presents total carotenoids concentration values in the fruits which reached a technological maturity (7.5 mg/100 g FW and 8,1 mg/100 g FW) and physiological maturity (9.4 mg/100 g Fw and 10.1mg/ 100 gFW), values which are higher than those of the control plant (6.5 mg/100 g FW and 8.5 mg/ 100 gFW). It is noted the negative effect of fertilization with HA and HA+ ESVv in the formation of the carotenoids in fruits, having an opposite effect in the formation of chlorophyll pigments.

The Cemil cultivar has an increase of the total carotenes content in the tomato fruits which reached the technological maturation fertilized with HA+ ESVv (7.0 mg/100 Fw) and HA+ESVv+B (7.5 mg/100 g FW) in comparison to the control plant (4.3 mg/100 g FW). The tomato fruits which reached the physiological maturity have a high content of total carotenoids for all the used fertilizers ranging between 19.8 mg/100 g FW - 32.5 mg/100 gFW in comparison to the control plant (7 mg/100 gFW).

The Lorely cultivar has a positive response to all the used fertilizers having a total carotenoids concentration in the tomato fruits which reached the technological maturation ranging between 1.6 mg/100 gFW and 5.8 mg/100 gFW in comparison to the control plant (1 mg/100 gFW) and in the tomato fruits which reached the physiological maturation values of 14 mg/100 gFW-34.7 mg/100 g FW in comparison to the control plant (12.2 mg/100 gFW).

The total carotenes content in the tomatoes fruits which reached the physiological maturation present in all the three cultivars higher values as compared to the control sample and to the samples which reached the technological maturity.

The carotenoids are accumulated during the fruit ripening due to the disappearance of chlorophyll and chloroplast transformation in chromoplasts and have a direct effect on the fruit colour which range from green to yellow, from yellow to orange, from orange to pink and from pink to red (Raffo, 2002). There is an intensification of the red colour at the cultivars treated with fertilizers containing ESVv that through their content in polyphenols (gallic acid, flavan 3-ol monomers: catechin, epicatechin, galocatechin, epicatechin 3-Ogalat, dimers, trimers and polymers of procyanidins) have an antioxidant activity 20 times greater than that of vitamin C and 50 times stronger than that of vitamin E, providing protection to the carotenoids against the oxidation reactions (Biță-Dumitru et al., 2012).

The strong vegetative plant growth stimulation through the combination of the constituent elements of fertilizers is responsible for the production obtained from the three tomato cultivars, Table 2.

It is observed in the experiments that the production is different depending on the nature of the studied fertilizer. The different production is the result of the stimulating effect of the fertilizer which manifests both on the vegetative growth and plant development, but also on the fructification.

The combination of HA+ESVv+B had the best effect on the growth of tomato production with a percentage ranging between 27% and 54.9% depending on the cultivar. The presence of boron with the other constituents contributed to a better and balanced source of nutrients matching the nutritional requirements of tomatoes. The direct or indirect involvement of Boron in many physiological and biochemical processes during plant growth, enzyme system and nutritional system (Cakmak, 1997; Goldbach, 2001; Brown et al., 2002; Wimmer et al., 2009) contributed to the development of plants with increased metabolism and achievement of higher production. The presence of a large number of biological compounds (sugars, alcohols, organic acids) in the cytoplasm (Raeven, 1980; Hu, 1994) can form complexes with boron (Hu, 1994) with a role in the plant nutrition and production.

The effects of boron on the tomato plants are cumulated during vegetation with the direct effects produced by HA and ESVv. The direct effects produced by the humic acids are different biochemical actions exerted on the cell wall, membrane or cytoplasm and are mainly of hormonal nature (Varanini, 2001; Chen et al., 2004). The effects of ESVv distinguish by their role in the formation of pigments, in the development, in the resistance to pathogens and UV protection (Latanzzio et al., 2006).

A significant increase in production was recorded also in the combination HA+ESVv with a production increase compared to the control sample ranging between 20.6% and 48.7% for the three cultivars. If in the HA fertilization, the Lorely cultivar shows an increase compared to the control plant with 7.6% lower than the Antalya cultivar (45.9%) and Cemil cultivar (43.5%). The increased production of tomatoes fertilized with HA is consistent with the reports made by Dogan and Demir (2004) who reported that the application of HA resulted in a higher yield compared to the control plant, and after the fertilization with ESVv the Lorely cultivar shows an increase compared to the control plant with 4.3% lower than the cultivars of Antalya (37.5%) and Cemil (22.7%).

Conclusions

The treatment of the plants during vegetation with natural foliar biofertilizers, extract from the seeds of *Vitis vinifera* (ESVv); humic acids (HA); humic acids + extract from the seeds of *Vitis vinifera* (HA + ESVv) and humic acids + extract from the seeds of *Vitis vinifera* + Boron (HA + ESVv + B) stimulates the processes of growth and influences essentially the accumulation of assimilating pigments acting directly on the production of tomatoes. The best results were recorded from the use of cumulative fertilization of HA + ESVv + B resulting production increases of 27.8% for Lorely, 52.2% for Antalya and 54.9% for Cemil.

Using boron in combination with humic acids and polyphenolic extract from the seeds of Vitis vinifera has the most suitable effects for growing the tomatoes. The data show the effectiveness of organic foliar fertilizers that contribute to the optimal plant growth and development and to the correction of nutritional deficiencies, supporting the formation and development of the root and foliar system, of the reproductive organs, of the flowers, fruits and seeds, of the crop production increases and of the yields of superior quality.

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