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Antioxidant activity in selected tomato genotypes cultivated in conventional and organic culture systems

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The present study is a compilation of results obtained at the Vegetable Research and Development Station Bacau regarding the influence of the culture system on the quantitative and qualitative yield of tomatoes. The present study provides comparative information regarding yield achievements of tomato genotypes cultivated in two different culture systems (conventional and organic), in order to highlight the suitability of the cultivation system. The best yield results were obtained at a density of 30,000 plants per hectare in both culture systems. All studied genotypes resulted in quantitatively superior yield in the conventional system as opposed to the organic system. Another purpose of this study was to determine the difference in antioxidant activity of tomato genotypes cultivated in the ecological and the conventional culture systems. The results indicate the suitability of the tomato to organic cultivation, highlighting the potential of the tomato species to be utilized as a significant source of natural antioxidants, and also the influence of cultivation systems on the accumulation of antioxidant compounds.

Key words: *Lycopersicon esculentum*, polyphenols, flavones, yield.

INTRODUCTION

Conventional and organic agricultural practices represent dynamic systems that can vary greatly depending upon region, soil quality, prevalence of pests, crop, climate and farming philosophies. Due to these variations, comparisons between different results are very difficult. Conventional agriculture evolved globally in response to the availability of high-yield crop cultivars, chemical fertilizers and pesticides, and progressing irrigation and mechanization (for example, A.E. Mitchell and A.W. Chassy, University of California, Davis, USA, personal communication). Organic farming has evolved due to an increasing market demand for healthy products and due to an interest in enhancing and preserving the quality of the environment. Opinions differ about the nutrient quality of vegetables grown organically. Some claim organic produce is nutritionally superior to conventional produce, while others argue that organic produce is not nutritionally different from conventional, with the exception of the higher price tag.

Kapoulas et al. (2011) observed that, in terms of quality, some studies report a better taste, higher vitamin C content and higher levels of other quality-related compounds for organically grown produce (Mitchell et al., 2007). Several other studies have come to the opposite conclusion, or have found no difference in quality characteristics between organically and conventionally grown fruits and vegetables (Caris-Veyrat et al., 2004).

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The actual global situation currently faces several important issues: (1) The environment is being permanently damaged due to agricultural systems that rely heavily on the use of chemical treatments, and (2) a constantly increasing market demand for good-quality, healthy and products, produced in sufficient quantities has resulted in increased pressure on farmers to improve both tomato yield and quality. The primary objective of tomato growers today is to maximize the harvest of fruit per cultivation area, which has harmed the environment, reduced biodiversity, and has resulted in a larger quantity of lower-quality produce. Due to the importance of environment preservation, as well as the market demand for high-quality healthy products, researchers throughout the world are currently attempting to find solutions that would address both these problems.

The tomato is one of the most valuable fruits currently being grown in the conventional and organic systems, in open fields and also in protected areas. The tomato plays a significant role in human nutrition (Fekadu et al*.,* 2004), because it is a good source of lycopene, vitamins, and antioxidants, which promote good health (Gelmesa et al., 2009). Dumas et al. (2003) noted that many tomato compounds demonstrate antioxidant activity, the most important of which is lycopene, found in abundance in the skin of the fruit. Very large quantities of tomatoes are processed and preserved in a variety of forms. Tomatoes provide nutrients (carbohydrates, proteins, lipids, organic acids), minerals, and vitamins (A, B1, B2, B6, C, PP, E, K), making them one of the most nutritious components of a balanced diet. Today, numerous varieties of tomato, some transgenic, are cultivated in all regions of the world.

Tomato cultivars vary widely in their resistance to disease. The use of new cultivars, employed in intensive production, is not recommended for organic production. A good alternative might be the utilization of traditional varieties, better adapted to particular agro climatic conditions (Díaz del Cañizo et al., 1998). Resistance against the most common diseases (*Verticillium* wilt, *Fusarium* wilt, *Nematodes*, *Tobacco Mosaic Virus*, and *Alternaria*) allows cultivation in the organic system and also plays a major role in obtaining proper yield. Organic farming does not utilize nitrogenous fertilizers, and, as a result, plants respond (to stress) by activating their own defense mechanisms, thereby increasing antioxidant levels (Vallverdú-Queralt et al., 2012). There is consistent growth in consumer demand for organic products, which are different in character from conventional foods but are nevertheless of excellent quality (Rivera and Brugarolas, 2003). This demand is recognized by the fruit and vegetable sectors, which have shown a growing interest in promoting the survival of traditional varieties in order to secure the best methods of producing and marketing quality produce (Gonzalez-Cebrino et al., 2011).

Organic farming as an approach to sustainable agriculture tries to decrease environmental problems and possible health hazards caused by pesticide residues (Malek-Saeidi et al., 2012). Organic farming does not involve nitrogenous fertilizers or pesticides, which allows plants to strengthen their own natural defenses, thus increasing antioxidant levels. In other words, a stronger, healthier plant is also a more nutritious plant. Very often, consumers associate agricultural chemical treatments with accelerated development of disease (like cancer) or with high levels of pollution of surface and groundwater. These undesirable side-effects draw attention to the present and future dangers of pesticide use in farming, and encourage the development of cleaner, healthier, and more environmentally-friendly alternatives. In recent years, much attention has been devoted to natural antioxidants and their association with health benefits (Arnous et al., 2001). Consequently, an increasing amount of research has been dedicated towards identifying those plants with potential antioxidant activities. Plant phenolics are commonly found in both edible and non-edible plants, and have been reported to have multiple biological effects, including antioxidant activity. The antioxidant activity of phenolics is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donators, and singlet oxygen quenchers. The quantity and quality of phytochemicals detected in tomato fruits is known to depend greatly on genotype and environmental condition (Gahler et al., 2003; Giuntini et al., 2005).

MATERIALS AND METHODS

The experiments were conducted at the Vegetable Research and Development Station Bacau, Romania.

Biological material

The biological material was represented by four different genotypes of *Lycopersycon esculentum* Mill*,* as follows: Moldoveanca (control variant), PL47, PL3, PL6. All genotypes feature a determinate type of growth – suitable for open field cultivation. The tomato fruits were comparable in size and development. Tomatoes were harvested within the period of late July to early August. The plants were harvested at bright red fruit maturity and daily harvests were made periodically. All investigations on antioxidant potential were undertaken at fruits at physiological maturity.

Research protocol

We divided our research into two sections: (1) analyzing the suitability of the cultivation system by comparing the yield obtained in cultivation systems; and (2) detecting antioxidant activity in tomato fruits, as well as the influence of the cultivation system on antioxidant compounds.

Analyzing the suitability of the cultivation system

The experimental station has a certified polygon for organic farming and also land for conventional agriculture. As a result, we conducted our experiments in both the organic and the conventional agriculture fields, using similar environmental conditions. Extreme

Table 1. Graduation of studied factors.

Studied factor	Graduations factor	Code in tables
Culture system	Conventional	a_1
	Ecological	a_2
	Moldoveanca (control)	b_1
Genotype	PL47	b_4
	PL3	b_5
	PL6	b_6
Density (number of plants/ha)	20,000	c_1
	30,000	c_2
	40,000	c_3

Table 2. Concentrations of high performance liquid chromatography (HPLC) reagents used to perform the gradient.

Time (min)	% CH_3COONa 2 mM (pH=3.5)	Percentage (%) CH_3CN
0	98	2
20	86	14
40	80	20
50	70	30
60	75	25
65	98	2
70	98	2

temperatures in the winter can be as low as to - 29°C and in the summer, they can rise to +39°C. Mean air temperature during the growing period was 22 to 23.0°C. Average annual rainfall exceeds 500/550 mm/m^2. Precipitation ranged between 500 and 1100 mm/year. During the vegetation period, the tomato species met the optimum conditions for tomato fruit development in open field. The experiments seeking to identify the tomatoes' genetic resources suitable to organic and conventional farming systems presumed studies about influence of culture system, genotype, and density on yield of the *L. esculentum* Mill. species. Experimental variants were placed in a plot device sub-divided into four partitions. Type of experience A x B x C (2x4x3). The experimental parcels were placed in open field. The graduation for studied factors is presented in Table 1.

Detection of antioxidant activity

For determination of polyphenols (flavonoids and polyphenol acids) in samples taken during the experiment, we performed analysis by high performance liquid chromatography (HPLC) on methanolic extracts with a DER = 3:100 mg/mL. HPLC analysis consists in a separation on a high performance liquid chromatograph Agilent 1200 type equipped with a reversed phase column Eclipse XDB-C18 (150 mm x 4.6 mm, 5 μm) and UV-VIS diode-array detector. Separation was performed using a mobile phase (concentration gradient) consisting of acetonitrile and 2 mM sodium acetate (adjusted to pH 3.5 with glacial acetic acid). The used gradient concentration is shown in Table 2.

UV detection was performed at multiple wavelengths (220, 250, 260, 280, 320, and 350 nm). To identify peaks, we used the comparison of the retention time of the sample chromatogram with those of standards, and compared the absorption spectra of peaks obtained with those of standards analyzed under the same chromatographic conditions in both cases. Thus, optimizing working conditions for chromatographic separation of standard solutions were injected caffeic acid, chlorogenic acid, o-coumaric acid, ferulic acid, rosmarinic acid, rutoside, hiperozida, luteolin-7-glucoside, apigenin-7-glucoside, quercetol, luteolin and apigenol.

Table 3 displays the values of the retention time to standards. Figures 1 to 5 represent chromatograms obtained for these standards, at a detection wavelength of 320 nm. For standards presented in Table 3, absorption spectrum UV spectra library was recorded and saved, and was then used to further identify these compounds in different samples. For antioxidant analysis of samples of alcoholic extracts, we analyzed by HPLC, using the same conditions. Chromatograms obtained were integrated by comparing the retention time relative to benchmarks, and by comparison spectrum putting into evidence the presence/absence of substances monitored in samples.

For determination of flavones content, we used thin-layer chromatography and we established that the main component was luteolin, expressed in mg luteolin per 100 g fresh substance. The principle of method is based on the presence of specified yellow compounds, developed by extraction of flavonoids in hydrophilic solvents, in the presence of Al^{3+}. The intensity of coloration is photo colorimetrable at spectrometer at wave length λ = 413 nm. The calibration curve was arrived using the standard luteolin, linear measurement range provided by the Beer-Lambert law: 1.66 to 16.64 μg/ml luteolin. On this range, the calibration curve characteristic parameters are: R^2 = 0.9996, correl = 0.9998.

Statistical analysis

Data were analyzed by an analysis of variance ($P < 0.05$) and

Table 3. Retention time values recorded for standards polyphenols used at high performance liquid chromatography (HPLC) analysis.

Nr. Crt.	Standard name	Retention time (min)
1	Chlorogenic acid	11.46 ± 0.57
2	Caffeic acid	14.85 ± 0.74
3	Ferulic acid	22.88 ±1.14
4	Rutoside	27.05 ±1.35
5	Hiperozida	27.35 ±1.37
6	Luteolin-7- glucoside	28.53 ±1.43
7	Rozmarinic acid	29.32 ±1.47
8	O-coumaric acid	29.68 ±1.48
9	Apigenol-7-glucozida	34.53 ±1.73
10	Luteolin	47.93 ±2.40
11	Cvercetol	48.14 ±2.41
12	Apigenol	53.01 ±2.65

Table 4A. Interaction of factor a (culture system), factor b (genotype) and factor c (density) in conventional system.

Symbol	Yield t/ha	Difference (t/ha)	Significance
$a_1b_1c_1$	24.9	- 7.3	000
$a_1b_1c_2$	38.2	+6	***
$a_1b_1c_3$	33.6	+1.4	*
Mean	32.2	-	
$a_1b_4c_1$	38.5	-15,2	000
$a_1b_4c_2$	67.9	+14.2	***
$a_1b_4c_3$	54.6	+0.9	-
Mean	53.7	-	
$a_1b_5c_1$	38.9	-15.9	000
$a_1b_5c_2$	69.3	+14.5	***
$a_1b_5c_3$	56.3	+1.5	*
Mean	54.8	-	
$a_1b_6c_1$	40.6	-15.5	000
$a_1b_6c_2$	69.8	+14.0	***
$a_1b_6c_3$	57.0	+1.2	*
Mean	55.8	-	

DL (p 5%), 0.98; DL (p 1%), 1.92; DL (p 0.1%), 3.55.

means separated by Duncan's multiple range tests using Statistical Analysis System.

RESULTS

We compared both systems, organic and conventional (Table 4), and we observed a higher level of yield using the conventional culture system (no matter the genotype or density). The yield data obtained in both culture systems confirm the results of other researchers. In regards to optimal density, (Table 4) shows that the best yield results were obtained with a density of 30,000 plants, Figures 1 to 5 represent the chromatograms obtained for standards, at a detection wavelength of 320 nm. Using the same conditions for antioxidant analysis, the samples of alcoholic extracts were analyzed by HPLC, and are presented in Figures 7 to 14.

Our results on the content of chlorogenic acid, caffeic acid, and p-coumaric acid confirm some of the results obtained in other studies: the registered values are quantitatively superior in fruit harvested from the organic system when compared to those cultivated in the conventional

Table 4B. Interaction of factor a (culture system), factor b (genotype) and factor c (density) in ecological system.

Symbol	Yield t/ha	Difference (t/ha)	Significance
$a_2b_1c_1$	20.5	-5.5	000
$a_2b_1c_2$	30.6	+4.6	***
$a_2b_1c_3$	26.8	+0.8	-
Mean	26.0	-	
$a_2b_4c_1$	28.8	-12.1	000
$a_2 b_4c_2$	52.3	+11.4	***
$a_2b_4c_3$	41.6	+0.7	-
Mean	40.9	-	
$a_2b_5c_1$	29.1	-12.8	000
$a_2b_5c_2$	53.4	+11.5	***
$a_2b_5c_3$	43.1	+1.2	*
Mean	41.9	-	
$a_2b_6c_1$	30.4	-12.2	000
$a_2b_6c_2$	53.8	+11.2	***
$a_2b_6c_3$	43.6	+1.0	*
Mean	42.6	-	

DL (p 5%), 0.98; DL (p 1%), 1.92; DL (p 0.1%), 3.55.

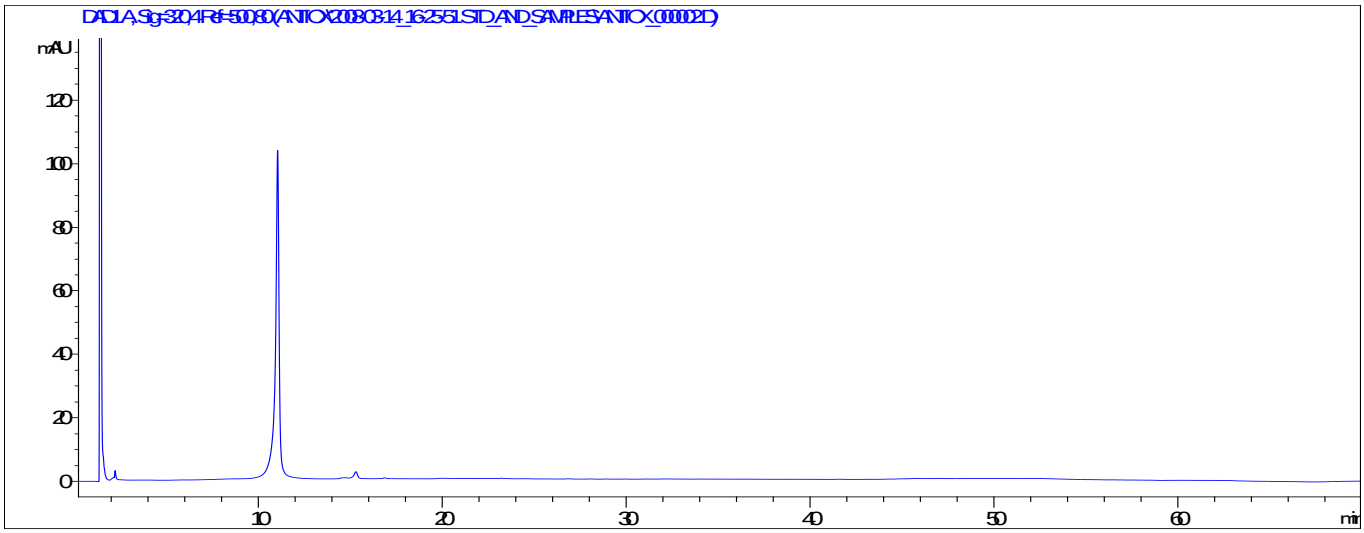

Figure 1. High performance liquid chromatography (HPLC) chromatogram for chlorogenic acid.

system. In case of rosmarinic acid and rutozide, the highest values were accomplished in fruit cultivated in the conventional system. The chlorogenic acid is a phenyl propanoid substance, synthesized in the process of aerobic respiration, and it has been linked by previous researchers to functions of scavenging, free radical, antibiosis, anti inflammation, antivirus, among others. The highest content of chlorogenic acid in our study was detected in organic fruits of the PL 6 genotype (Figure 15). We observed a small variation in content for all genotypes cultivated in the organic system. The same small variation was also registered in genotypes from the conventional system, with one exception: Moldoveanca genotype. No chlorogenic acid content was detected in this genotype. Similar trends were observed in the concentration of caffeic acid and p-coumaric acid in

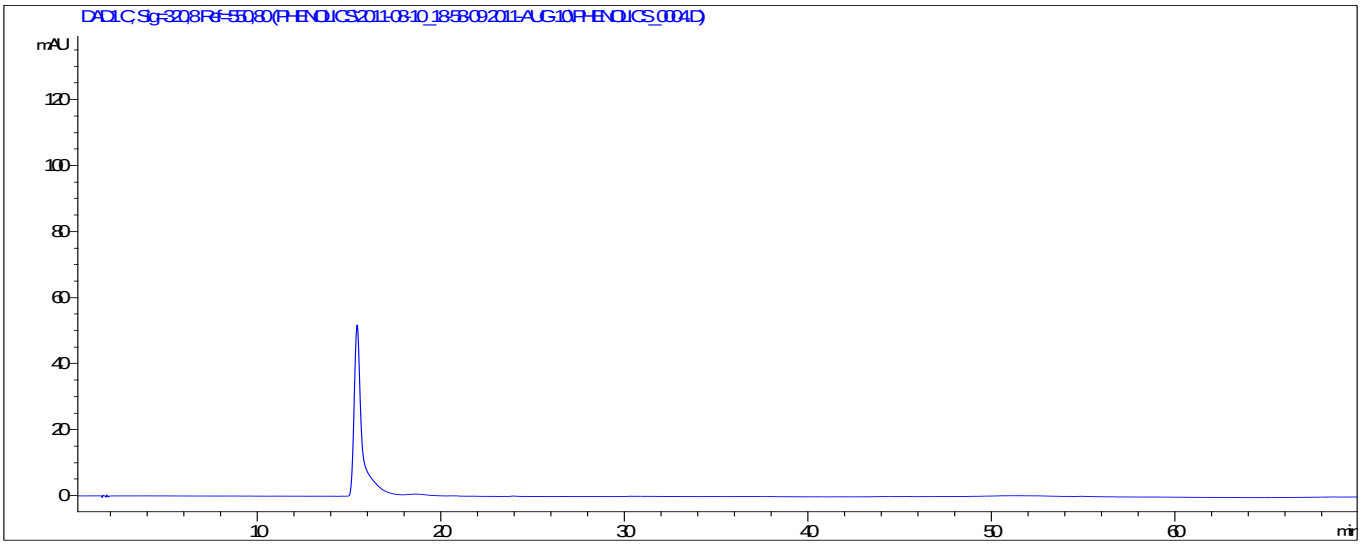

Figure 2. High performance liquid chromatography (HPLC) chromatogram for caffeic acid.

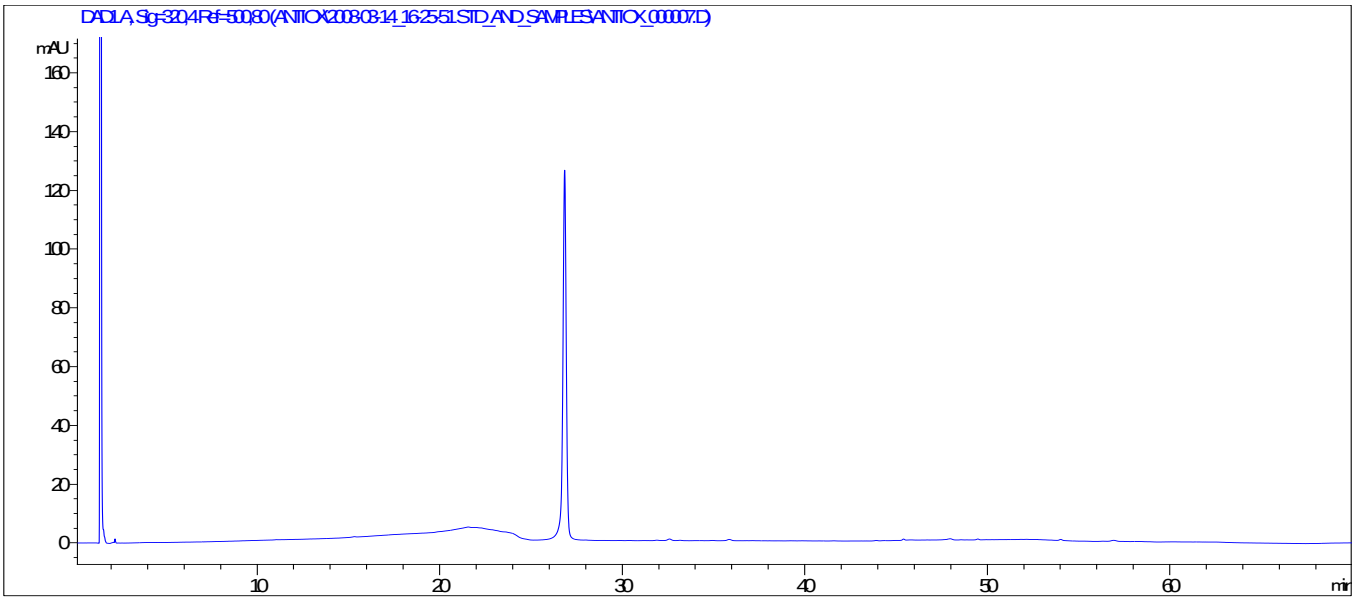

Figure 3. High performance liquid chromatography (HPLC) chromatogram for luteolin-7-O-glucozida.

tomato fruits grown in the two different cultivation systems (Figure 16). All organically-grown genotypes presented higher values of caffeic acid and p-coumaric acid, compared to those cultivated conventionally. In case of caffeic acid, the highest content was detect in fruits on the control variant, genotype Moldoveanca (created and patented in our institute). The influence of genotype is obvious in regards to the accumulation of P-coumaric in genotype PL 6. The difference between the quantities achieved in both culture systems is 6.20 µg/g p-coumaric acid (Figure 17). In regards to rutozide content, we registered a higher level of accumulation for fruits cultivated in the conventional system, which is explained by a stimulation of synthesis by some chemical compounds applied in the conventional system (Figure 18). The trends of assimilation were similar in both systems and the decreased order was: PL 47, PL 6, PL 3 and Moldoveanca. Qualitative and quantitative phytochemical analysis revealed that rosmarinic acid is predominant in all genotypes of tomato extracts (Figure 19). This acid has major antioxidant potential and has been inserted in supplement formula due to its important anti-aging benefits (Archana, 2000; Chattopadhyay et al., 1992). Our results revealed a significant quantity of rosmarinic acid in the fruit of genotypes cultivated in the conventional system. The highest level of rosmarinic acid

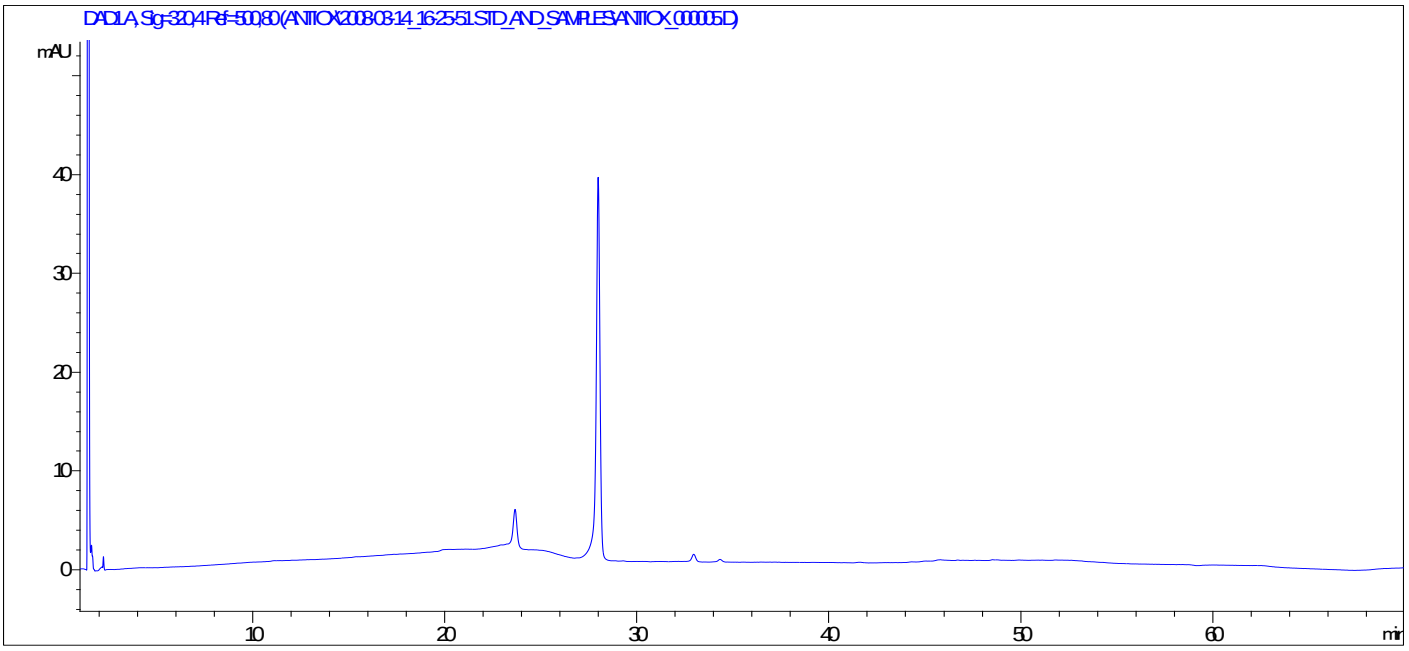

Figure 4. High performance liquid chromatography (HPLC) chromatogram for rutozide.

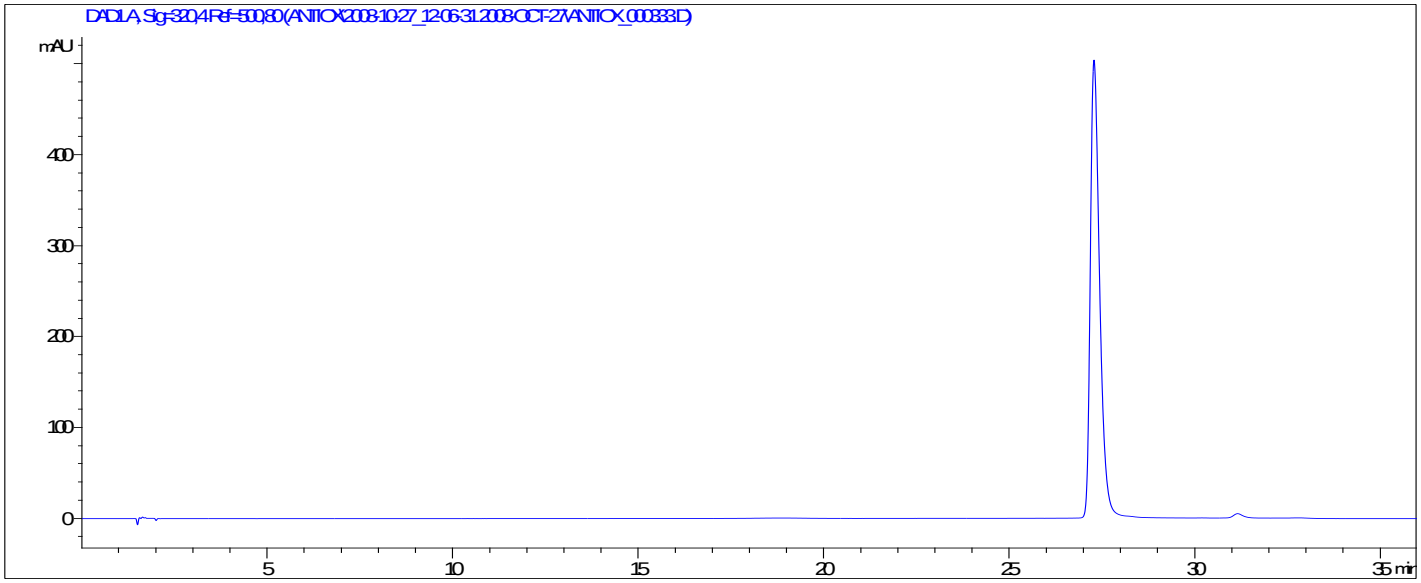

Figure 5. High performance liquid chromatography (HPLC) chromatogram for hiperozida.

was detected in fruit of genotype PL 47. In both systems, the level of accumulation is similar in all genotypes and decreases as follows: PL 47, Moldoveanca, PL 6, and PL 3. Figure 20 presents the variation of luteoline content in our four studied genotypes, cultivated conventionally and organically. In the organic system, all genotypes registered higher values of luteoline. The influence of the cultivation system is obvious for genotype PL 3. In the organic system, this genotype accumulated 2.2 times more luteoline than in the conventional system. In the other genotypes, the luteolin content in the organic system was 1.45 to 1.70 times higher than in the conventional system.

DISCUSSION

Organic horticulture represents a sustainable alternative agricultural model, with the potential to provide both

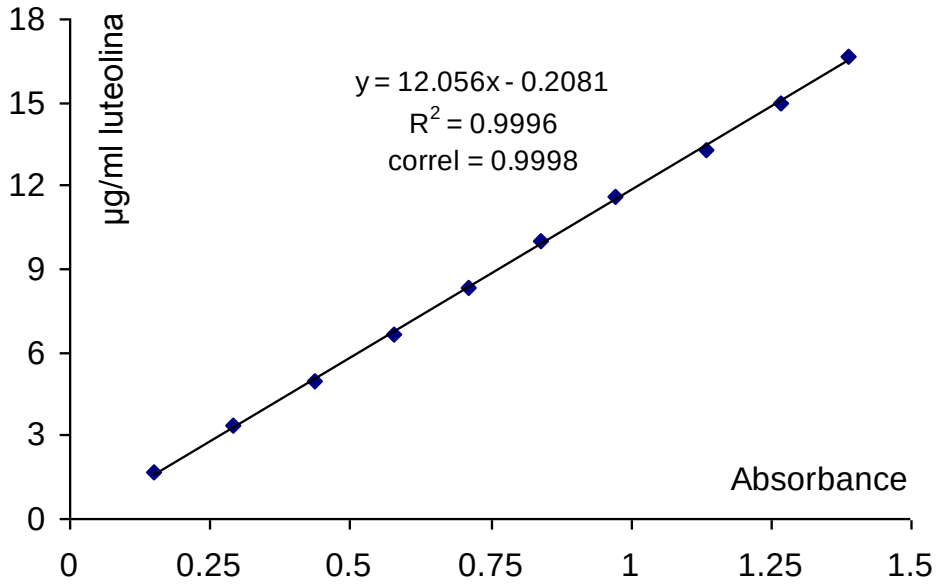

Figure 6. Calibration curve - standard luteolin.

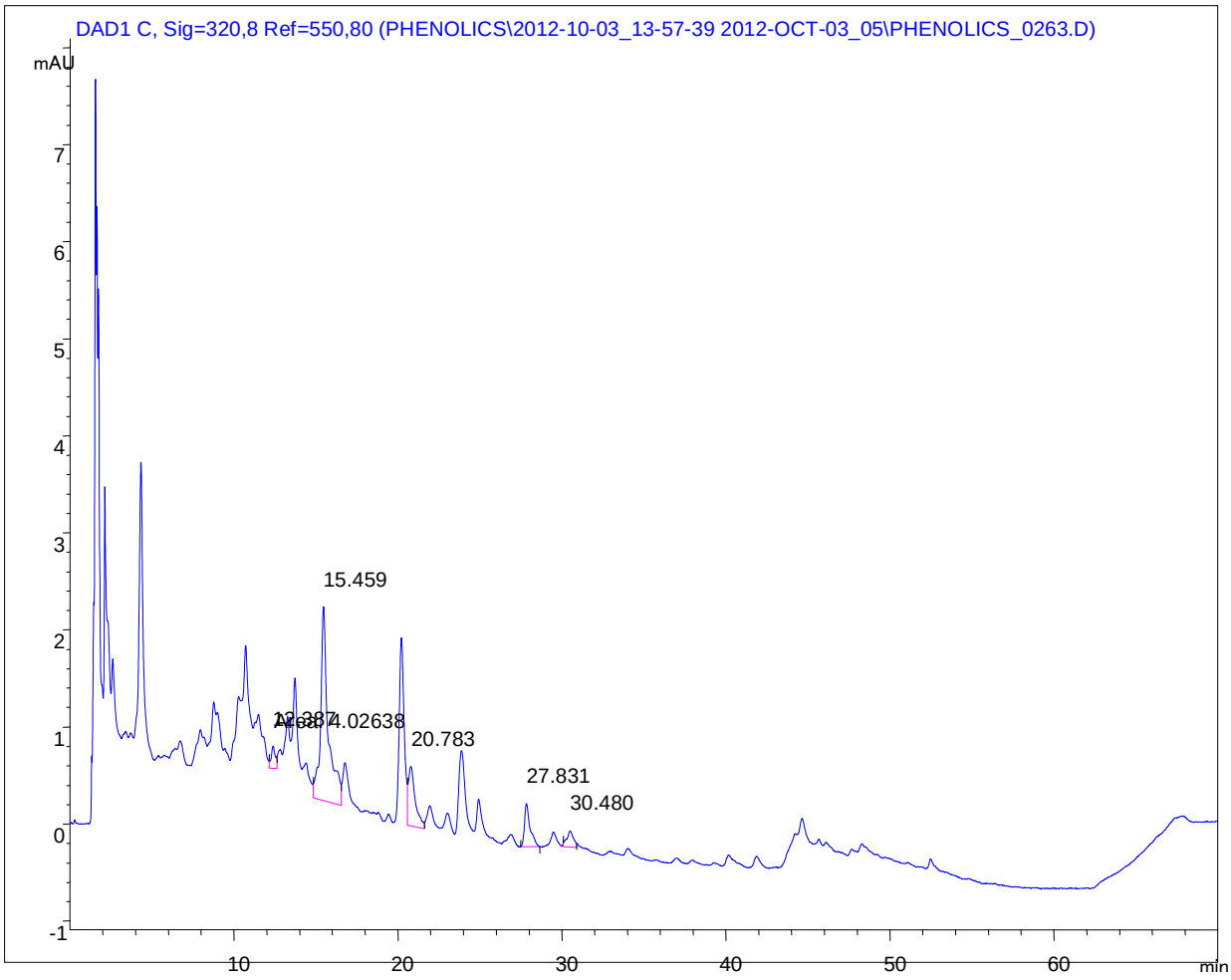

Figure 7. High performance liquid chromatography (HPLC) chromatogram for *Lycopersicon esculentum* - Moldoveanca (control), Ecologic.

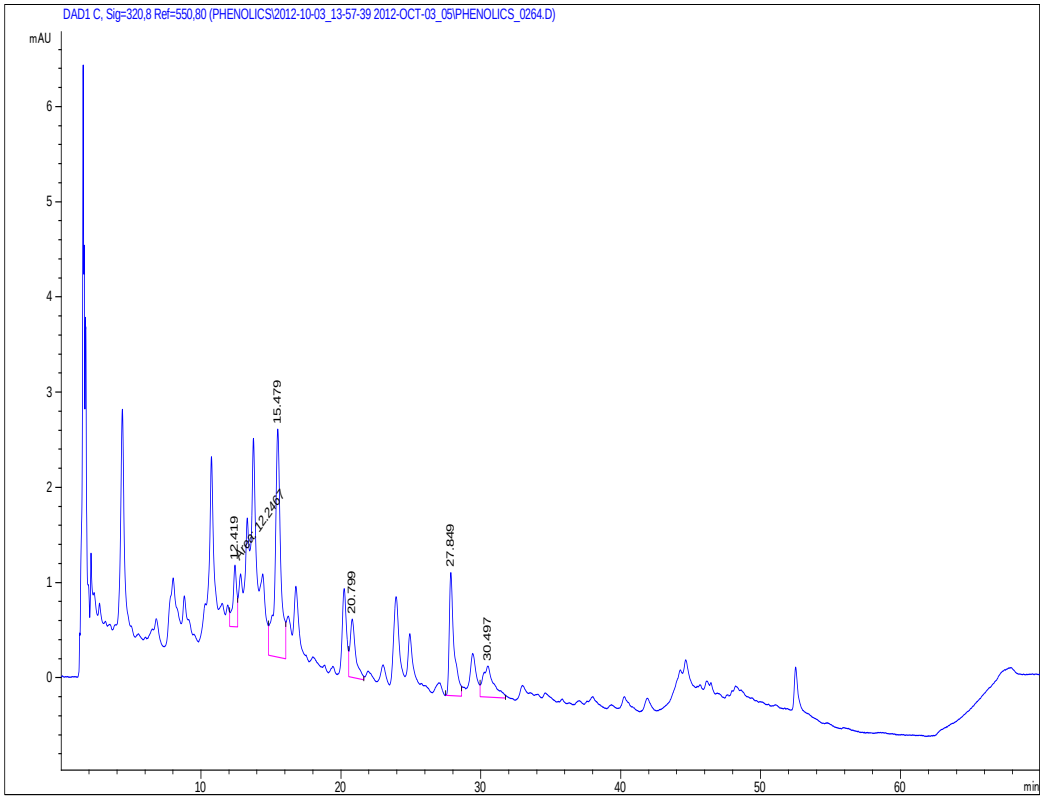

Figure 8. High performance liquid chromatography (HPLC) chromatogram for *Lycopersicon esculentum* Moldoveanca (control), Conventional.

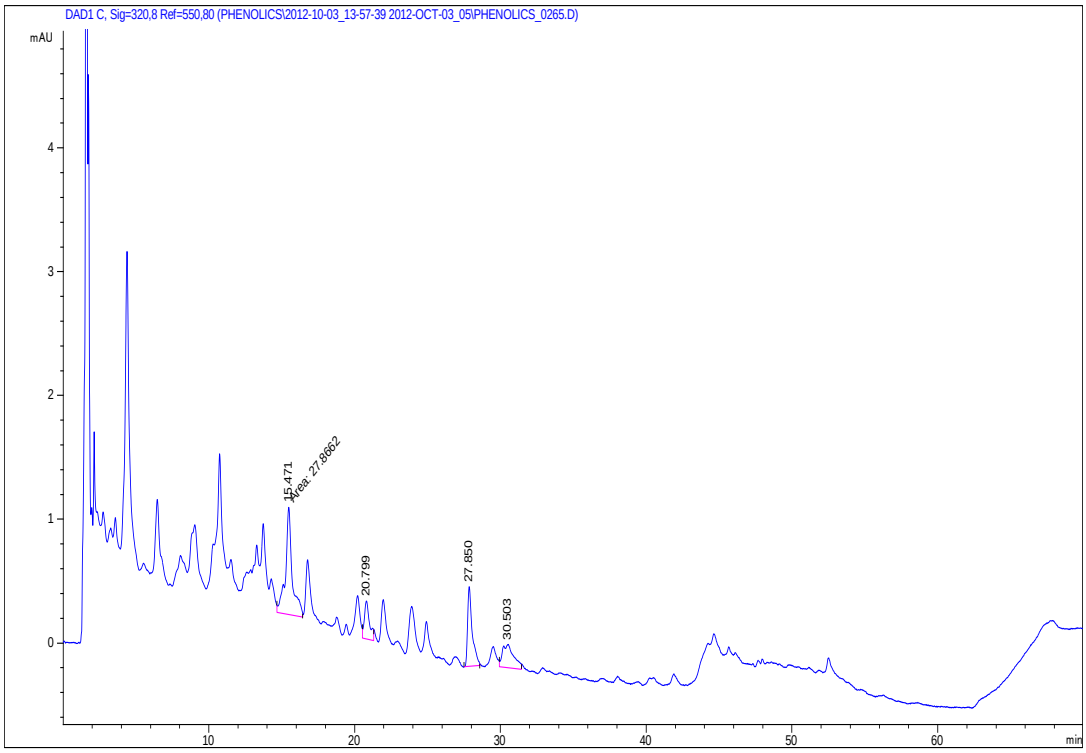

Figure 9. HPLC chromatogram for Lycopersicon esculentum - PL47 Ecologic.

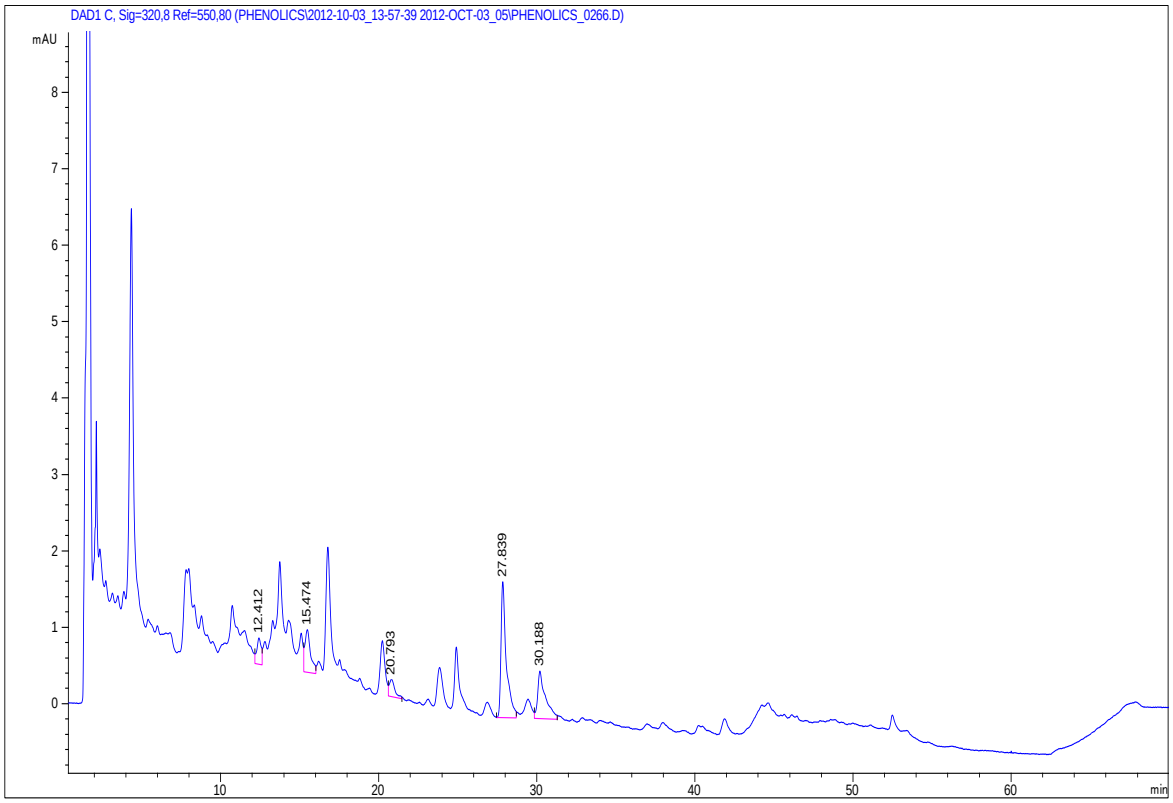

Figure 10. High performance liquid chromatography (HPLC) chromatogram for *Lycopersicon esculentum* - PL47 Conventional.

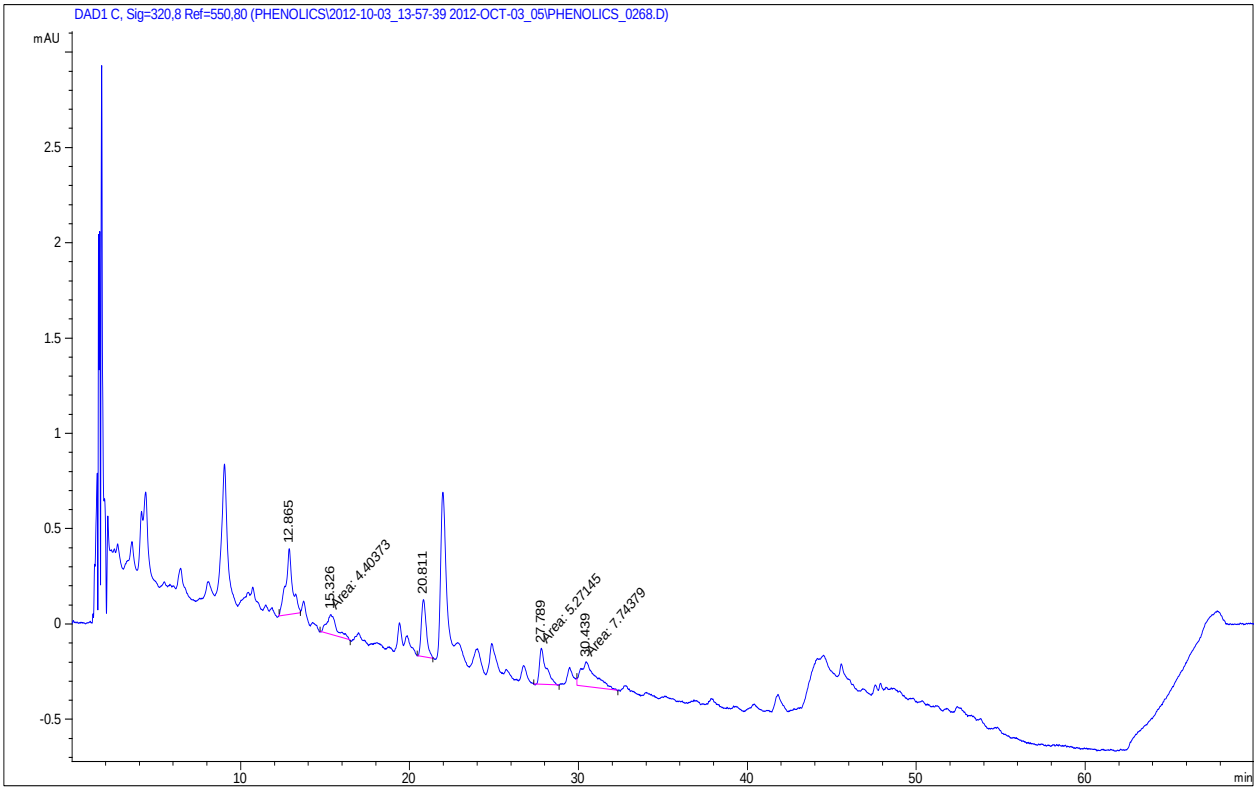

Figure 11. HPLC chromatogram for Lycopersicon esculentum – PL3 Ecologic.

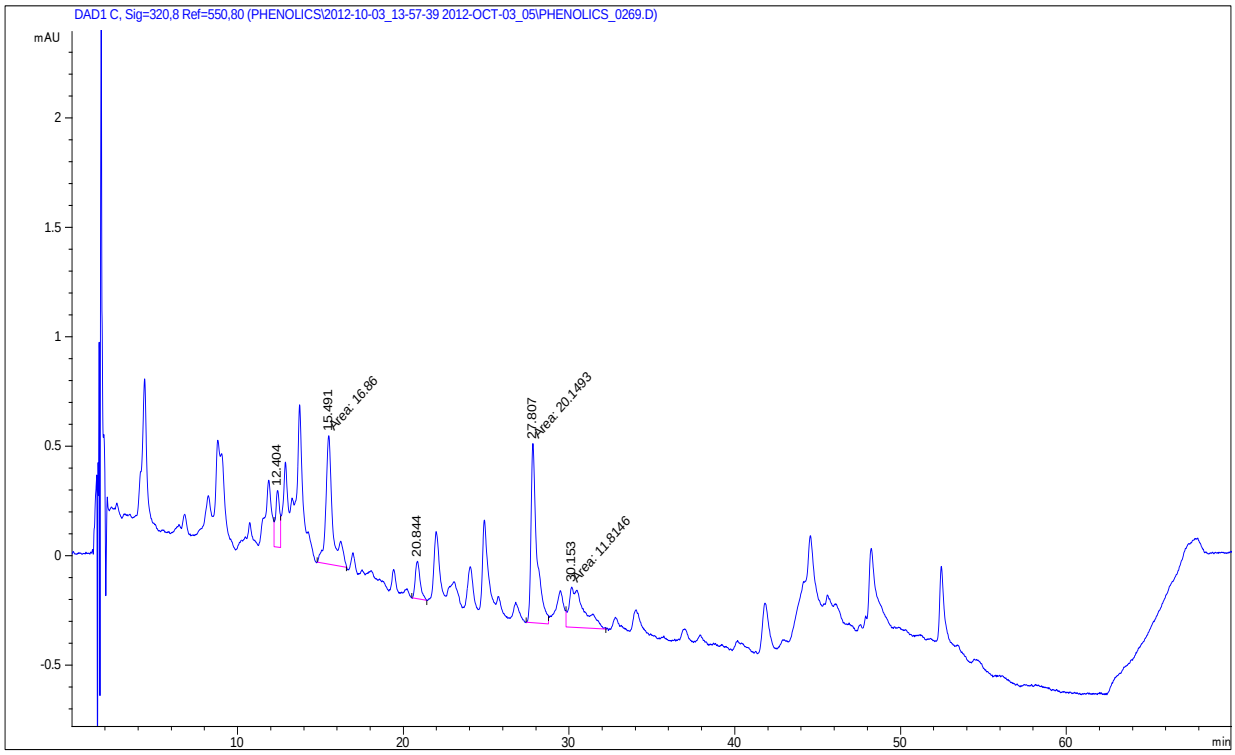

Figure 12. High performance liquid chromatography (HPLC) chromatogram for *Lycopersicon esculentum* – PL3, Conventional.

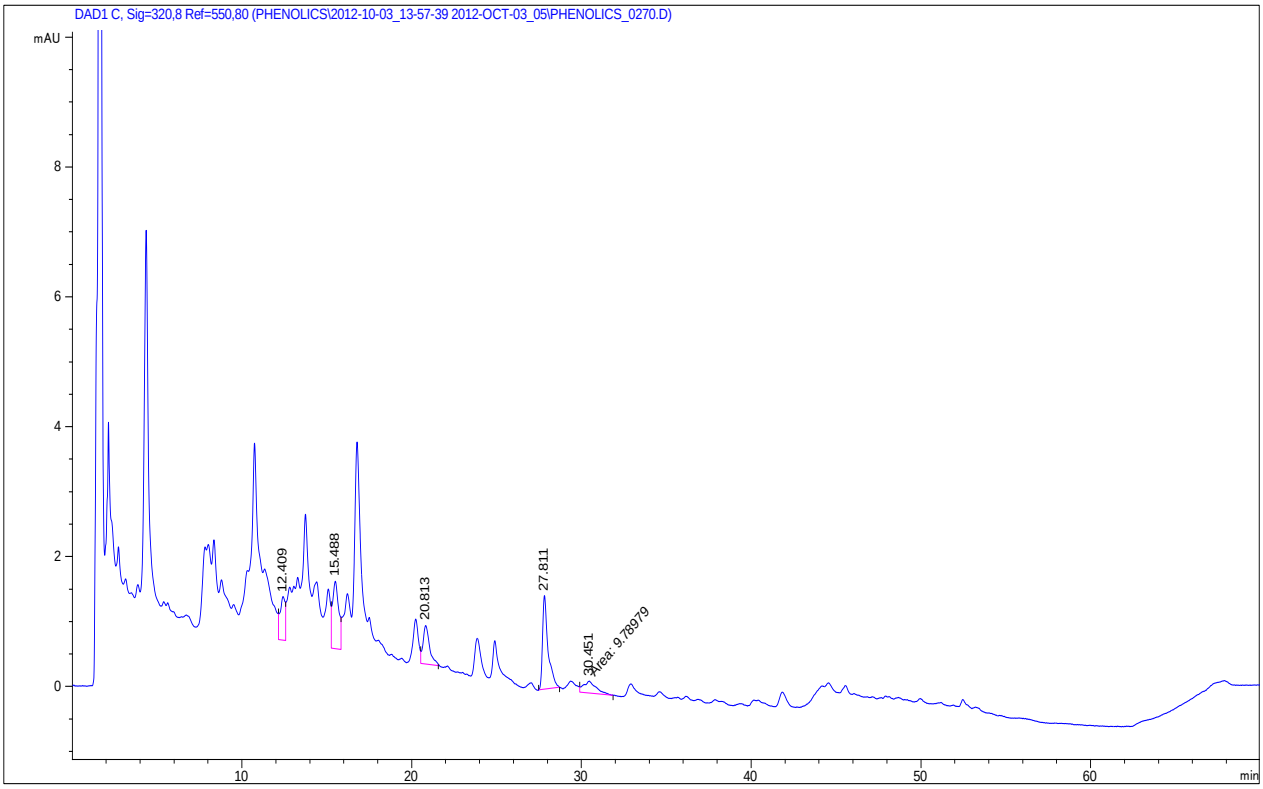

Figure 13. High performance liquid chromatography (HPLC) chromatogram for *Lycopersicon esculentum* - PL6, Ecologic.

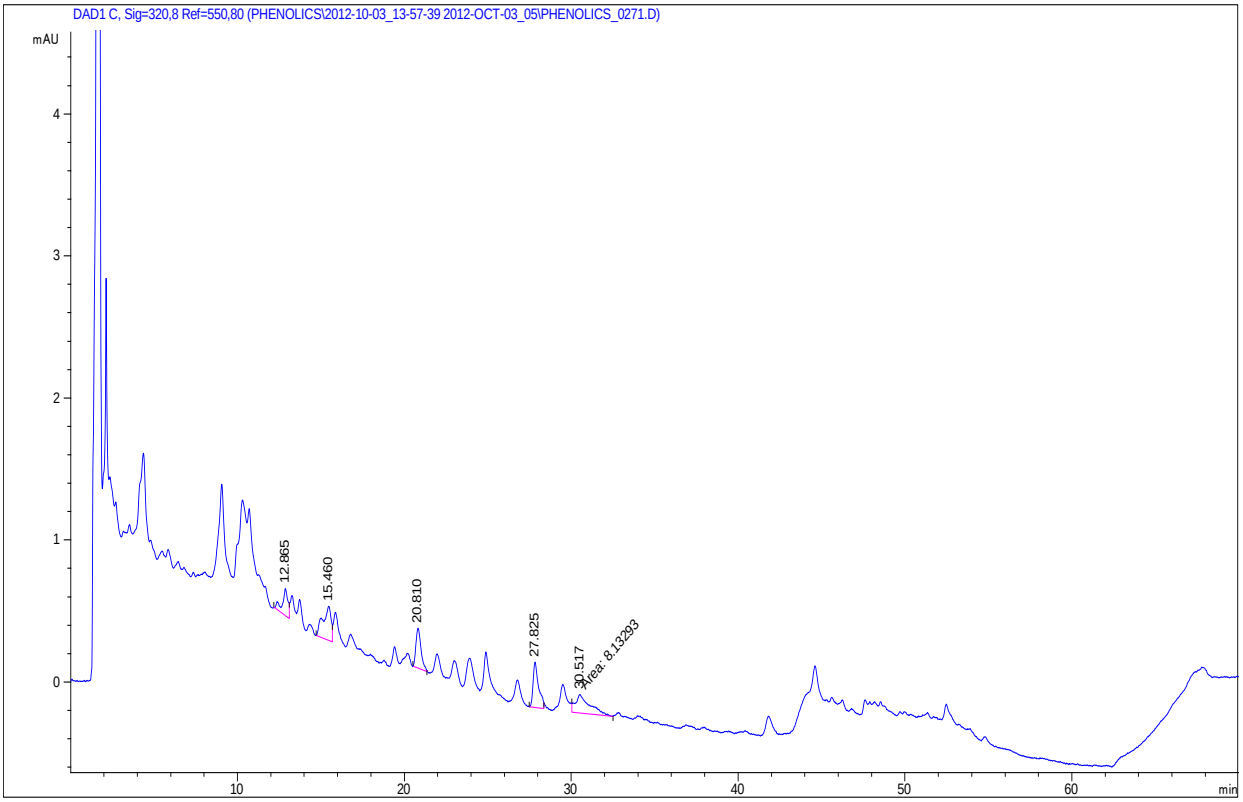

Figure 14. High performance liquid chromatography (HPLC) chromatogram for *Lycopersicon esculentum* – PL6.

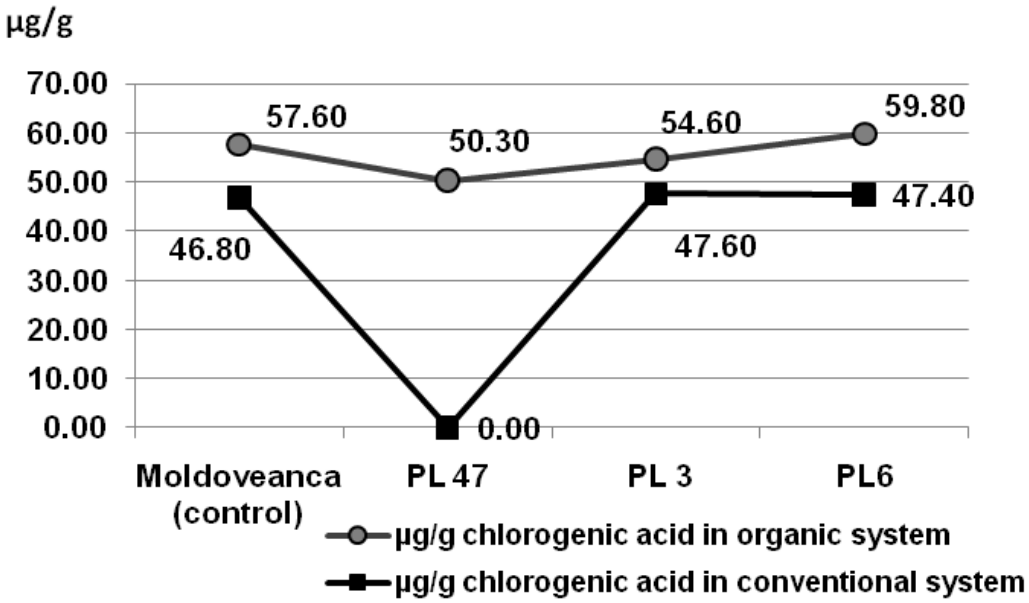

Figure 15. Variation of chlorogenic acid content in the two different culture systems for four studied genotypes.

environmental improvements and high quality outputs (González et al., 2002). Our study, developed at the Vegetable Research - Development Station Bacau, confirms that vegetable cultivation in organic farming conditions is biological feasible. We paid special attention to sanitation, and for pest-management purposes, we

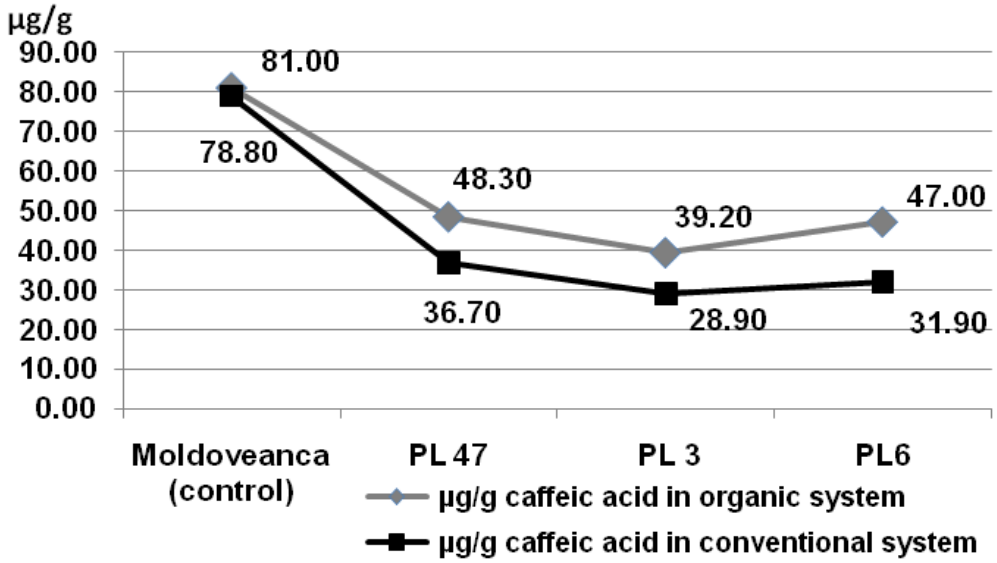

Figure 16. Variation of caffeic acid content in the two different culture systems for four studied genotypes.

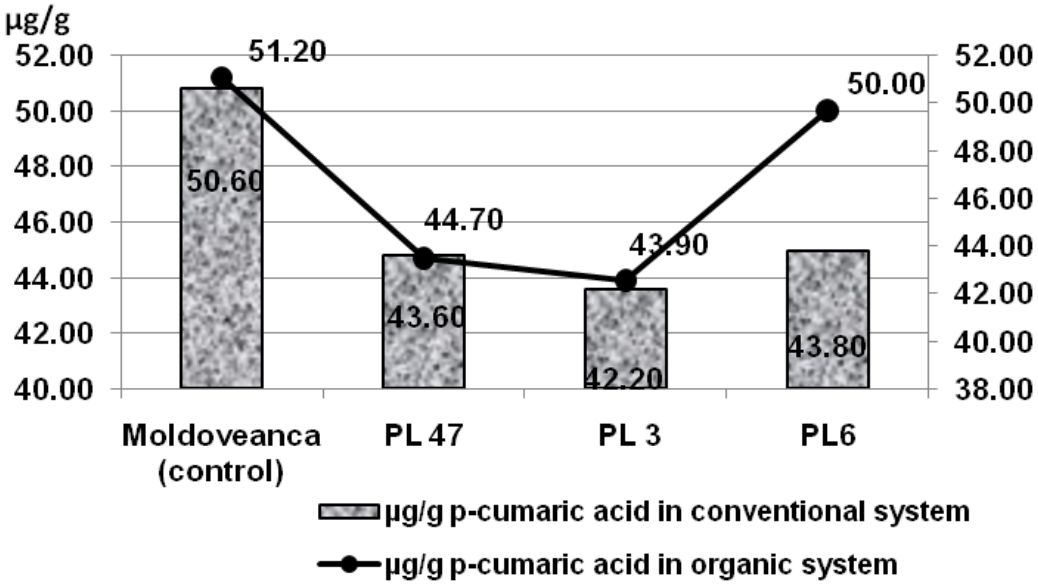

Figure 17. Variation of p-cumaric acid content in the two different culture systems for four studied genotypes.

applied only products approved by actual legislation. Regarding the influence of factor A (culture system) on factor B (genotype), the data presented in Table 4 reveals that all genotypes achieved production increases of between 6.2 - 13.2 t / ha in the conventional system compared to the organic system. The biggest difference was observed in the case of genotype PL6. This genotype registered 55.8 t/ha in the conventional system, 13.2 t/ha more than in the organic system. Significant differences in yield between the conventional and organic systems were registered for PL3 (12.9 t/ha) and PL47 (12.8 t/ha). The smallest difference observed was in the case of Moldoveanca, where the conventional variant obtained only 6.2 more t/ha than the organic, meaning that this cultivar behaves similarly in both culture systems. The conventional system resulted in increased yield due to the application of fertilizer and other treatments against pests and disease. In our experimental field, the mean yield in the conventional system was higher by 11.2 t/ha than in the organic system. Even

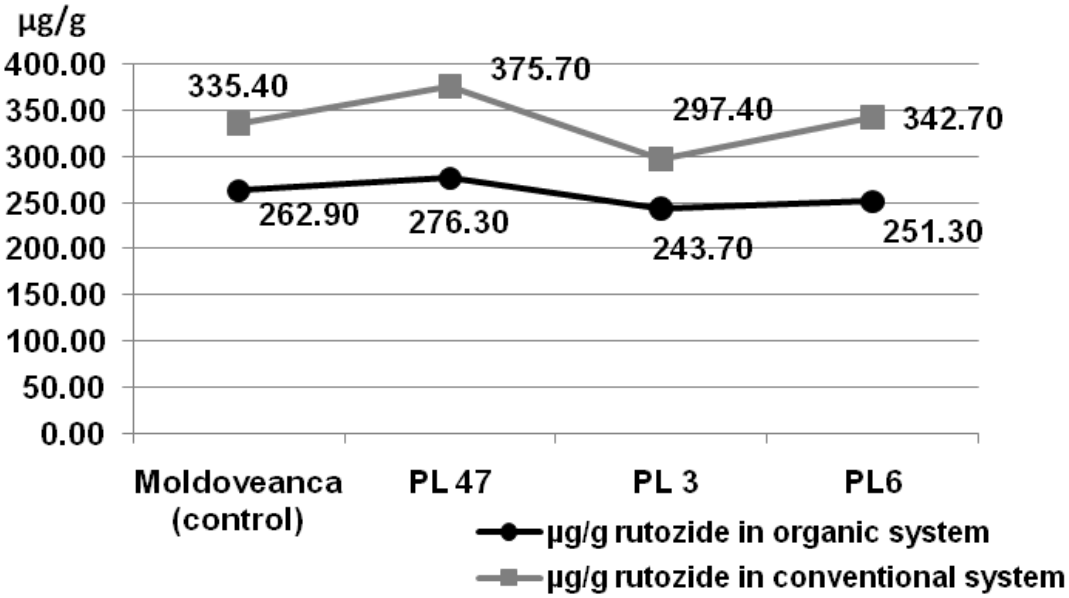

Figure 18. Variation of rutozide content in the two different culture systems for four studied genotypes.

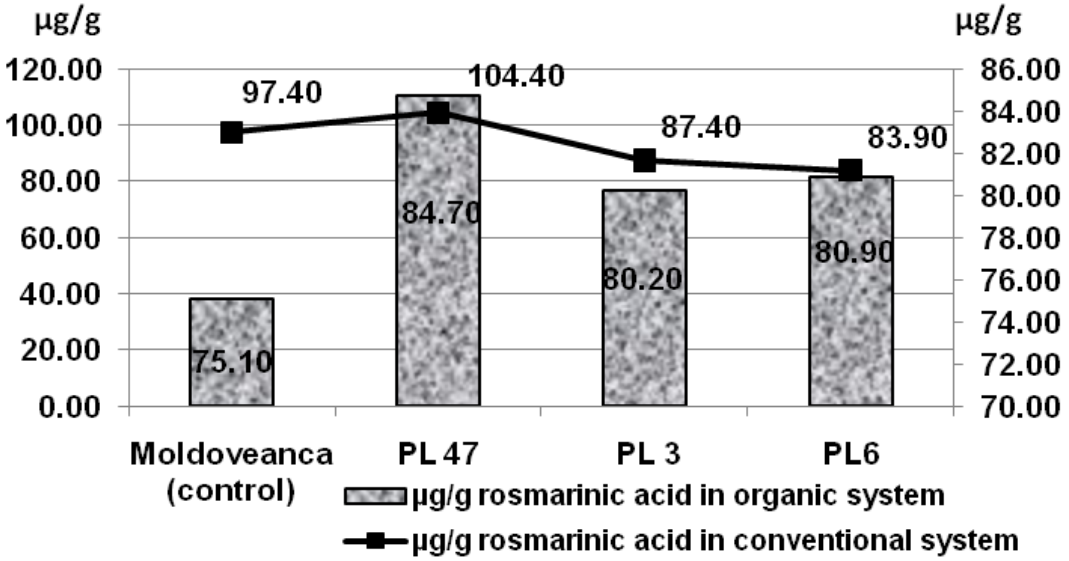

Figure 19. Variation of rosmarinic acid content in the two different culture systems for four studied genotypes.

though yields obtained in the conventional system were higher, the market value of the organic produce was significantly higher.

In the case of local organic markets, it is important for small growers to explore niche markets, such as selling the produce directly, even before placing the culture. "Biological quality" is an intrinsic quality, assessed on criteria other than just physical or aesthetic characteristics (size, uniformity, color, among others). Both the genotype and the culture system influence the yield quantity and quality. Obtained yield was higher in the conventional system than in the organic system, which can be explained by small losses due to pathogen attacks in the conventional system, as opposed to the organic system. The best results were obtained by all cultivars at a density of 30,000 plants per hectare, followed by cultivars planted at 40,000 plants per hectare, in both culture systems. The lowest yield was achieved at a density of 20,000 plants per hectare in both culture systems and is not recommended. The cultivar with the highest yield potential was PL6. The results obtained in the organic system can be explained through the biological

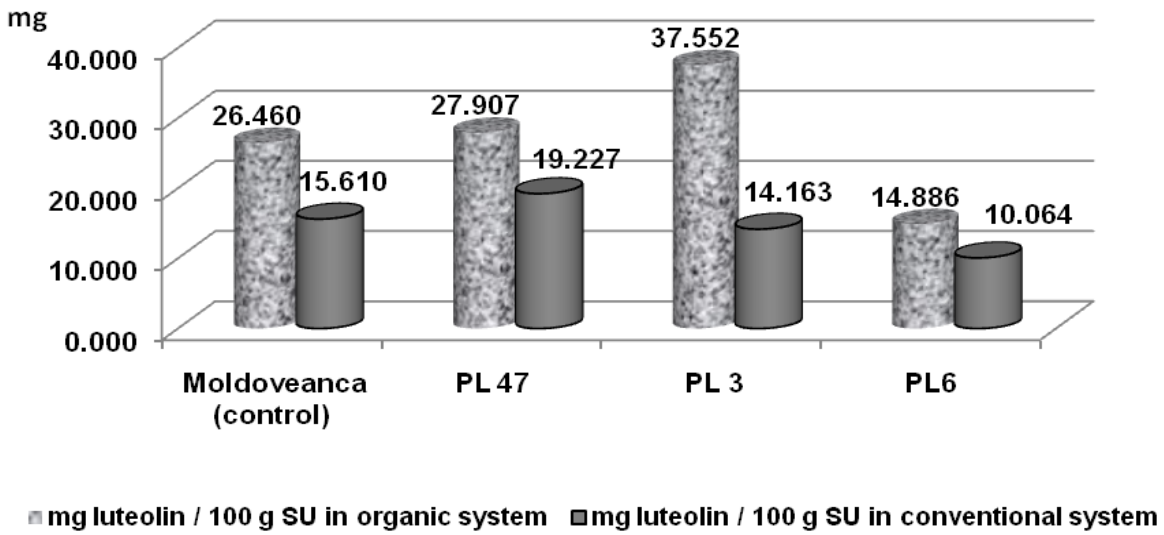

Figure 20. Variation of luteoline content in the two different culture systems for four studied genotypes.

fertilization (one of the fundamental principles) that was applied in the present study and represents a key to distinguishing the organic system from the conventional one.

Contemporary literature illustrates an apparent trend toward higher levels of phenolic antioxidants, ascorbic acid, and soluble solids in organic foods. (For example, A.E. Mitchell and A.W. Chassy, University of California, Davis, USA, personal communication). Phenolic compounds are organic molecules found in many vegetables with proven human health benefits. Consumption of fruits and vegetables has been associated with good health and prevention of numerous chronic diseases, including cancer, cardio and cerebro-vascular diseases, and ocular and neurological diseases (Barber and Barber, 2002). Our findings indicate significantly greater concentrations of antioxidant compounds in tomato fruits cultivated organically. These results indicate that the phenolic content of tomato fruits is significantly affected by the different treatments applied in the two culture systems. Since phenolic compounds play an important role as antioxidants in human nutrition, subtle differences in phenolic content between the two cultivation systems as a result of different applied treatments may have considerable importance from a nutritional standpoint.

More research examining the relationship between agricultural production and the synthesis of phytochemicals in specific crops is needed. Future studies should emphasize the potential for agricultural manipulations to alter levels of both beneficial and potentially toxic phytochemicals in foods. The ability to manage and control levels of beneficial phenolic antioxidants in plants through cultivation has the potential to enhance the nutritive quality of foods (A.E. Mitchell and A.W. Chassy, University of California, Davis, USA, personal communication).

The results of this study are summarized below:

1. The organic culture system represents an alternative to the conventional system, and the organic system can provide high quality products and preserve the environment at the same time;
2. The yield quantity and quality were influenced by both genotype and system culture;
3. The best yield results were obtained by all cultivars at a density of 30,000 plants per hectare;
PL6 had the highest yield production in both systems;
4. The registered values are quantitatively superior in fruit grown in the organic system compared to those grown in the conventional system;
5. In the organic system, chlorogenic acid, caffeic acid, p-coumaric acid, and flavones registered higher values than in the conventional system.

We conclude, therefore, that these four tomato varieties studied have great antioxidant potential and are suitable for cultivation under organic conditions.

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