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Cherry tomato productivity as influenced by liquid organic fertilizer under different growth conditions

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Abstract

This study was carried out to examine the impact of liquid organic fertilizer Ergonfill (LFE) on the yield and quality of cherry tomato (*Lycopersicon esculentum* Mill. Sakura F_1) under different growth conditions. The experiment was set up in a randomized blocks design with four variants in three replications. Experiment variants were as follows: drought-stressed seedlings with and without LFE treatment, and non-stressed seedlings with and without LFE treatment. Exposure of cherry tomato seedlings to controlled water stress conditions significantly increased fruit quality parameters (total soluble solids, titratable acidity, ascorbic acid, lycopene, total phenolic and flavonoids, total antioxidant capacity), but decreased yield. LFE treatment significantly increased by the all examined parameters under normal growth conditions as compared to untreated plants, and for total phenolic and flavonoids content, total antioxidant capacity and total soluble solids this increase was statistically significant. Positive impact of the LFE application on cherry tomato quality is result of fertilizer composition, as well as ability of cherry tomato plants to use bioactive substances in fertilizer for its growth and development.

Keywords: antioxidants, ascorbic acid, fruits, lycopene, quality, water stress, yield

Introduction

Crop cultivation takes place in an environment characterized by high risks and uncertainty, especially for its cultivation in arid and semi-arid areas where water supply to plants from rainfall is very unstable. Since the problem of water scarcity has been specially pronounced in last decades as result of global climate changes and

intensifying competition for water resources by industrial, agricultural and other users, optimal water management presents one of the most crucial challenges of the agriculture in 21st century. This primarily relates on the creating of optimal water management in the cultivation of crops sensitive to drought (Morison et al., 2007). Tomatoes are very sensitive to drought, particularly in the early vegetative growth (Nuruddin et al., 2003). Drought induces morphology, biochemistry and physiology changes in plant, leading to the reduction in photosynthesis and consequently on tomato growth and development. Also, drought can delay or inhibit flowering and fruiting of plants, resulting in a significant yield reduction (Atkinson and Urwin, 2012).

Tomato plants have developed a range of mechanisms to cope with drought stress: cell growth reduction, slower cell division, leaf area adjustment, osmotic adjustment, activation of efficient antioxidant systems and other mechanisms for plant physiological and morphological adaptions to stress conditions. However, if the plant response to stress is insufficient, the water stress can cause severe damage to plants, including wilt (Basu et al., 2016).

To overcome the problems arising from water scarcity, it is necessary devised approaches that help plant to improve its defense mechanisms against stress. One of the approaches in order to achieve this goal is application of liquid organic fertilizer with high content of physiologically active substances that can contribute to accumulation of osmotic active substances and antioxidants in plant cells, thus improving the adjustment of plant to stress conditions. Ergonfill (LFE) is relatively new liquid organic fertilizer obtained from the hydrolysis of proteins of animal origin and according literature data the effect of this fertilizer on growth and development of cherry tomato plants has not been studied. According to the product specification LFE contains 3.4% N, 10% C, 2% MgO, 0.2% Fe and 0.003% Mo, vitamin B_1, B_2 and nineteen essential amino acids, among them the highly presence in fertilizer have phenylalanine, tyrosine, tryptophan, glutamine, proline, leucine, lysine, asparagine and alanine. Since the LFE contains mineral elements: nitrogen, iron, magnesium and amino acids essential for plant metabolism, it is expected that LFE has a positive impact on the growth and development of the cherry tomato plants.

The objective of this research was to examine the effect of LFE application on the yield and quality of cherry tomato *Lycopersicon esculentum* Mill. Sakura F_1, grown under normal and water stress conditions.

Materials and methods

Study area and experimental design

The study was conducted in 2015 under controlled conditions in a greenhouse at the nursery of public communal company 'Park' in Sarajevo. The experiment was laid out in a randomized complete block design with four variants in three replications. Each of variants was present with twenty plants. Experiment variants were as follows: (V_1) cherry tomato seedlings treated by LFE and subjected to drought, (V_2) cherry tomato seedlings treated by LFE and regularly watered, (V_3) non-treated cherry tomato seedlings subjected to drought, (V_4) non-treated cherry tomato seedlings regularly watered. Cherry tomato seedlings used in the experiment were produced in a same nursery and showed no significant difference in terms of size and appearance. The

first foliar treatment by LFE was carried out immediately after the transplanting of seedlings (8 April 2015), and the second treatment was performed fifteen days later. LFE was applied diluted with water at concentration of 0.1% in accordance with manufacturers' instructions. Five days after the second treatment, the cherry tomato seedlings in variant 1 and 3 were exposed to drought (non-watering), while the cherry tomato seedlings in variant 2 and 4 were not exposed to drought, that is, they were regularly watered. Exposure of seedlings to drought lasted until the appearance of first visual symptoms of drought on seedlings as leaves rolling and yellowing. Seedlings in all variants were then exposed to normal growth conditions (non-stressed conditions) until the time of technological maturity of fruits. This moment was represented the beginning of the determination of the yield and quality of cherry tomato fruits. The following parameters of fruit quality were determined: total soluble solids, titratable acidity, total phenolic and flavonoids content, prominent flavonoids content (naringenin and rutin), total antioxidant capacity, content of vitamin C and lycopene content.

Total soluble solids and titratable acidity

Total soluble solids (TSS) were determined for each sample fruit in three replications using an Atago PAL-1 digital refractometer and expressed as °Brix (ISO, 2003). Titratable acidity (TA) was obtained by titrating 10 ml of tomato juice with 0.1 mol*l^{-1} NaOH using phenolphtalein as indicator (AOAC, 2000). The results were expressed as grams of citric acid per 100 g of fresh tomato weight (%).

Determination of ascorbic acid

Ascorbic acid was determined by 2,6-dichlorophenolindophenol (DCPIP) titration method (AOAC, 2006) as follows: 25 g of fresh tomato fruit was weighed and homogenized with 20 ml of oxalic acid (1%) using mortar and pestle. The homogenate was filtered through coarse filter paper into 100 ml volumetric flask, which was followed by rinsing of pestle with another 20 ml of oxalic acid and at the end flask was filled to the mark with same acid. 10 ml of filtrate was pipetted into 250 ml conical flask and titrated with the DCPIP until a light rose pink persisted for 15 s. The amount of DCPIP used in the titration was recorded and this data was used for the calculation of vitamin C content, using formula prescribed by method.

Lycopene

Lycopene content was determined according to the method of Davis et al. (2003) as follows: approximately 0.5 g (determined to the nearest 0.01 g) of the homogenized samples of cherry tomato fruits were weighed in vial that contained 5 ml of 0.05% (w/v) butylated hydroxytoluene (BHT) in acetone, 5 ml of 95% ethanol and 10 ml of hexane. Samples were extracted on an orbital shaker at 180 revolutions per minute (RPM) for 15 min on ice, then 3 ml of deionized water were added to each vial and the vials were shaken for an additional 5 min on ice. The vials were then left 5 min at room temperature to allow phase separation. The absorbance of the hexane layer (upper layer) was read at 503 nm against a hexane blank. Lycopene content was

determined from the linear equation of a standard curve prepared with lycopene (0 - 3 mg*l^{-1}) and results were recalculated and expressed as μg per g of fresh weight (μg*g^{-1} FW).

Total phenolic content (TPC)

Total phenolic content was determined by the Folin-Ciocalteu method (Ough and Amerine, 1988) as follows: 1 g of dry fruit was mixed into 100 ml flask with ground glass stopper with 40 ml of an aqueous solution of ethanol (30%). The flask was boiled at 60 ^{0}C for 1 hour using a reflux condenser, then was filtered through coarse filter paper into 50 ml flask, and at the end flask was filled to the mark with 30% ethanol. The extract thus obtained was also used for the determination of the total flavonoid content and the total antioxidant capacity (FRAP). A volume of 0.25 ml of the above fruit extract was mixed into 25 ml flask with 15 ml of distilled water, and 1.25 ml of Folin-Ciocalteu's reagent (diluted by distilled water in the ratio 1:2). After 15 min 3.75 ml of Na_2CO_3 solution (7.5%, w/v) was added to the mixture. The volumetric flasks were then fill up to the mark with an aqueous solution of ethanol (30%) and then heated in water bath at 50 ºC, for 30 min with intermittent shaking for color development. After standing for 2 hours at room temperature, the absorbance of mixture was read at 765 nm. The total phenolic contents were determined from the linear equation of a standard curve prepared with gallic acid (0 - 500 mg*l^{-1}), and results were recalculated and expressed as mg of gallic acid equivalent per g dry weight (mg eq. GA*g^{-1} DW).

Total flavonoids content (TFC)

The total flavonoids content of the extract was determined by the Aluminium chloride colorimetric method (Zhishen et al., 1999) as follows: 1 ml of fruit extract was added to 10 ml volumetric flask containing 4 ml of distilled water. To the flask was added 0.3 ml 5% $NaNO_2$ and after 5 min 0.3 ml 10% $AlCl_3$. The mixture allowed to stand for 6 min, then 2 ml of 1 mol*l^{-1} NaOH was added and the total volume was made up to the mark with distilled water. After standing for 15 min at room temperature, absorbance was measured against the blank reactant at 510 nm. The total flavonoid contents were determined from the linear equation of a standard curve prepared with catechin (0 - 100 mg*l^{-1}) end results were recalculated and expressed as mg of catechin equivalent per g of dry weight (mg eq. C*g^{-1} DW).

Total antioxidant capacity (FRAP)

The total antioxidant capacity of the extract was determined by ferric reducing antioxidant power (FRAP) assay (Benzie and Strain, 1996) as follows: 80 μl of extract was mixed with 240 μl of distilled water and 2080 μl of FRAP reagent in glass tube and reaction mixture heated at 37 ºC for 5 min. FRAP reagent was prepared right away before analysis by mixing 0.3 mol*l^{-1} acetate buffer (pH = 3.6), 10 mmol*l^{-1} TPTZ (2,4,6-tripyridyl-s-triazine) in 40 mmol*l^{-1} HCl and 20 mmol*l^{-1} $FeCl_3$ x 6 H_2O in ratio 10:1:1). The absorbance of reaction mixture was read at 595 nm. The total antioxidant capacity was determined from the linear equation of a standard curve

prepared with $FeSO_4 \times 7H_2O$ (0 - 2,000 μmol*l^{-1} $FeSO_4 \times 7H_2O$) and results were recalculated and expressed as μmol Fe^{2+} per g of dry weight (μmol Fe^{2+}*g^{-1} DW).

Individual flavonoid compounds extraction and analysis

Extraction of flavonoid compounds from cherry tomato fruits was made according to Escarpa and Gonzales (2000) as follows: 5 g of homogenized tomato fruits was placed in the extraction vessel containing 10 ml of extracted solution (methanol + 3% formic acid + 1% m/v 2,6-di-tert-butyl-4-methyilphenol/BHT). Extraction vessel was left in an ultrasound bath for 60 min, and then the solvent was removed by centrifugation (for 7 min, at 10,000 x g, at 0 ^{0}C); Thermo Scientific SL16, Son Jose, USA). The obtained supernatant was filtered into vial through the Chromafil AO-45/25 polyamide filter (Macherey-Nagel Düren, Germany). This procedure was repeated for all fruit samples. A reversed-phase high-performance liquid chromatography method was performed for the detection and quantification of prominent flavonoids; rutin and naringenin (Marks et al., 2007). Analyses were performed on a Thermo Scientific liquid chromatograph model (Thermo Scientific, San Jose, CA, USA) controlled with a ChromQuest 4.0 chromatography workstation. Separation of flavonoid compounds was achieved by using Pursuit XRs 3 C-18 column (4.6 × 150 mm, 5 µm; Agilent Technologies, Santa Clara, CA, USA). The mobile phase consisted of 97% acetonitrile + 3% redistilled water + 0.1% formic acid (v/v; solvent A), and 97% redistilled water + 3% acetonitrile + 0.1% formic acid (v/v; solvent B). The flow rate through the column was 0.6 ml*min^{-1}; the total run time was 20 min; the column oven temperature was 25 ºC; the sample injection volume was 20 µl and absorbance detection wavelength was 280 and 350 nm. Naringenin and rutin were identified and quantified by comparing their retention times and their UV spectra with authentic standards. The results were expressed as mg per 100 g of fresh weight (mg naringenin or rutin*100 g^{-1} FW).

Statistical analysis

All experimental measurements were done in triplicates and the results were presented as mean ± standard deviation. Data analysis was performed using analysis of variance (ANOVA) with the SAS program package (statistical software), and the significant differences between the variants were determined using the least significant difference (LSD) test at 0.05 level of probability ($P<0.05$).

Results

The yield of cherry tomato was significantly affected by growth conditions, regardless of LFE treatment (Table 1). The results showed that exposure of cherry tomato fruits to water stress conditions significantly reduced the yield.

Table 1. Yield, total soluble solids (TSS), titratable acidity (TA) and ascorbic acid content (AA) of cherry tomato fruits depending on the LFE treatment under different growth conditions

Experiment variant	Yield (kg*plant)	TSS (Brix)	TA (%)	AA (mg*100 g^{-1} FW)
V_1	1.6 ± 0.3^{c}	6.59 ± 0.02^{ab}	0.65 ± 0.02^{ab}	13.67 ± 1^{a}
V_2	2.19 ± 0.3^{a}	6.5 ± 0.07^{bc}	0.63 ± 0.02^{c}	13.22 ± 0.33^{c}
V_3	1.14 ± 0.5^{d}	6.65 ± 0.23^{a}	0.65 ± 0.02^{a}	13.66 ± 0.66^{ab}
V_4	2.07 ± 0.35^{ab}	6.36 ± 0.12^{d}	0.62 ± 0.01^{c}	13.11 ± 0.33^{c}
$LSD_{0.05}$	0.287	0.128	0.016	0.376

V_1 - LFE (stress), V_2 - LFE (non-stress), V_3 - non-treated (stress), V_4 - non-treated (non-stress); values expressed as main ± standard deviation; different letters in each column represent significant difference among variants ($P<0.05$); FW - fresh weight.

Data in Table 1 also showed that LFE application increased the yield of cherry tomato under both normal and water stress conditions as compared with non-treated plants. The increase of yield was not statistically significant under normal growth conditions (between V_2 and V_4) while under water stress was significant (between V_1 and V_3).

The quality parameters of cherry tomato fruits (total soluble solids, titratable acidity, ascorbic acid) were also affected by growth conditions (Table 1). In stressful growth conditions (V_1 and V_3) cherry tomato plants accumulate more total soluble solids, total acids, and ascorbic acid in fruits than in normal growth conditions (V_2 and V_4). Treatment with LFE positively influenced on the increase of the mentioned quality parameters in fruits as compared to non-treated seedlings under normal, non-stress growth conditions (between V_2 and V_4), but only for total soluble solids this increase was statistically justified.

The results of the present study also showed that in stressful growth conditions (V_1 and V_3) cherry tomato plants produce more lycopene, phenolic and flavonoids as compared by cherry tomato plants grown under non-stress growth conditions (V_2 and V_4), regardless of LFE treatment. The values of total antioxidant capacity were also higher in cherry tomato fruits subjected to controlled water stress conditions (Table 2).

Table 2. Lycopene, total phenolic (TPC) and flavonoids content (TFC) and total antioxidant capacity (FRAP) of cherry tomato fruits depending on the LFE treatment under different growth conditions

Treatment	Lycopene ($\mu g*g^{-1}$ FW)	TPC ($mg*g^{-1}$ DW)	TFC ($mg*g^{-1}$ DW)	FRAP ($\mu mol\ Fe^{2+}*g^{-1}$ DW)
V_1	90.33 ± 1.98^{a}	10.17 ± 0.32^{ab}	5.26 ± 0.24^{ab}	190.13 ± 10.53^{ab}
V_2	87.39 ± 1.84^{bc}	9.42 ± 1^{c}	4.62 ± 0.20^{c}	151.67 ± 6.49^{c}
V_3	89.31 ± 2.06^{ab}	10.24 ± 1.01^{a}	5.37 ± 0.31^{a}	194.98 ± 10.55^{a}
V_4	86.17 ± 3.11^{c}	8.49 ± 0.42^{d}	4.22 ± 0.12^{d}	141.55 ± 14.11^{d}
$LSD_{0.05}$	2.354	0.701	0.205	8.13

V_1 - LFE (stress), V_2 - LFE (non-stress), V_3 - non-treated (stress), V_4 - non-treated (non-stress); values expressed as main ± standard deviation; different letters in each column represent significant difference among variants ($P<0.05$); FW - fresh weight; DW - dry weight.

The data in Table 2 showed that foliar application of LFE significantly increased phenolic and flavonoids content and total antioxidant capacity of cherry tomato fruits under normal growth conditions (between V_2 and V_4). LFE application also increased the lycopene content of cherry tomato under both normal and water stress conditions as compared with non-treated plants, but these increases were not statistically significant under the same growth conditions.

The results of the study have also demonstrated that dominant flavonoid in fruits of cherry tomato; naringenin and rutin accumulate more in cherry tomato fruits as response to water stress conditions (Table 3).

Data presented in Table 3 also showed that the content of naringenin and rutin were higher in fruits of cherry tomato treated by LFE under both stressful and non-stressful conditions.

Table 3. The content of dominant flavonoids (naringenin and rutin) of cherry tomato fruits depending on LFE treatment under different growth conditions

Treatment	Naringenin (mg*100 g^{-1} FW)	Rutin (mg*100 g^{-1} FW)
V_1	3.67 ± 0.08^{b}	5.41 ± 0.15^{ab}
V_2	3.14 ± 0.05^{c}	5.07 ± 0.02^{c}
V_3	3.81 ± 0.04^{a}	5.71 ± 0.53^{a}
V_4	2.91 ± 0.07^{d}	5.3 ± 0.14^{bc}
$LSD_{0.05}$	0.06	0.33

V_1 - LFE (stress), V_2 - LFE (non-stress), V_3 - non-treated (stress), V_4 - non-treated (non-stress); values expressed as main ± standard deviation; different letters in each column represent significant difference among variants ($P<0.05$); FW - fresh weight.

Discussion

Reduction in yield of cherry tomato due to exposure of the plants to water stress was expected since the drought limits productivity of crop plants by affecting photosynthetic processes, or by limiting cell division, shoot growth and fruit size (Farooq et al., 2009). Many studies have also noted the overall negative impact of water deficit on tomato yield (Birhanu and Tilahun, 2010; Nahar et al., 2011; Pék et al., 2014).

LFE application increased the yield of cherry tomato under both normal and water stress conditions as compared with non-treated plants. These results suggest that LFE application contributes to better adaptation of cherry tomato seedlings to drought conditions, thus improving their growth and development.

Quality of cherry tomato fruits is complex and strongly affected by genetic and environmental factors, especially by drought (Petrozza et al., 2014). Besides a decrease in photosynthesis and plant growth, drought also creates conditions that are conducive to photo-oxidative stress, i.e. the production of reactive oxygen species (ROS). Barbagallo et al. (2013) reported that the plant as a response to water stress stimulate the secondary metabolism, thereby potentially increasing the content of antioxidant substances involved in plant defense system against ROS, especially phenolic and flavonoids. Numerous studies have also found that the exposure of plant to controlled water stress conditions can significantly increase the content of antioxidants, and thus the quality and health benefits of tomato fruits (Atkinson et al., 2011; Murshed et al., 2013; Okunlola et al., 2015). There are many theoretical studies that tried to explain the influence of stress conditions on the synthesis of antioxidants. Caretto et al. (2015) noted that the water deficit slows growth more than photosynthesis at the beginning of water stress, and in these circumstances plants initiate the carbon fluxes from the primary to the secondary metabolic pathways, thus producing more carbon-based defensive substances such

as phenolic and flavonoids. Cramer et al. (2011) presented similar observations about this issue in their work. The results in present study also support the hypothesis that the plant increases secondary metabolism and consequently produces more phenolic compounds under stress conditions.

Considering the fact that phenolic and flavonoids are carriers of antioxidant capacity of plant (Vasco et al., 2008), it is quite realistic to expect higher values of antioxidant capacity in cherry tomato fruits subjected to controlled water stress conditions, and results of the present study confirm that fact.

Treatment with LFE positively influenced by the increase of total phenolic and flavonoids content in fruits of cherry tomato plants grown under normal growth conditions. These fertilizer effects could be attributed to its specific chemical composition. Namely, LFE contains some amount of amino acids: phenylalanine, tyrosine and tryptophan which serve as precursor in the formation of many phenolic compounds, so obtained data point out to the fact that application of LFE promote the synthesis of phenolic compounds in cherry tomato plants.

The values of total soluble solids (TSS) and titratable acidity (TA) were also higher in fruits of cherry tomato exposed to water stress as compared to non-stressed plants, regardless of LFE treatment. These results correspond to results of other researchers who have examined this issue (Bertin et al., 2000; Giannakoula and Ilias, 2013; Gunawardena and De Silva, 2016). Guichard et al. (2001) reported that positive effects of water stress on TSS levels can be explained by a reduction in water accumulation in fruit without any significant change in the amount of the accumulated sugars or acids. In contrast, Beckles (2012) stated that higher accumulation of sugar in tomato fruits is primarily result of activity of sucrose-metabolizing enzymes in sugar import and metabolism under water stress conditions. These enzymes have been recognized as key metabolic enzymes involved in plant responses to drought (Ruan et al., 2010). However, the improvements of TSS levels and TA in fruits gained by water deficit are commonly accompanied by reduced yields, as confirmed by the results obtained in this research. Cherry tomato seedlings treated with LFE had a higher TSS and TA of fruits compared to non-treated seedlings under normal growth conditions. The efficiency of LFE application to increase these quality parameters has been mainly attributed to the presence of large number of nitrogenous compounds, microelements and other bioactive substances enhancing the plant metabolic pathways and consequently the increase of quality components in fruits.

In the present study, the content of ascorbic acid (AA) in fruits was also higher in experiment variants where cherry tomato seedlings were exposed to water stress (V_1 and V_3), regardless of LFE treatment. An increase of AA content in fruits of tomato plants grown under stress conditions has also been reported in many studies (Serio et al., 2004; Stevens et al., 2008; Gill and Tuteja, 2010). Khan et al. (2011) noted that tolerance of plants to stress depends largely on their capability to increase AA biosynthesis or the activity of AA - related enzymes when the plants are exposed to stress. Namely, AA acts as an antioxidant by removing different ROS or by reducing their damaging effects, so the higher presence of AA in plant cells is very favorable for plant defense mechanisms against stress. Considering the importance of ascorbic acid for the human health, crop producers use different agro-technical measures

such as exposure of plants to controlled stress conditions or application of bioactive fertilizers in order to achieve an increase of AA content and other antioxidant substances in edible parts of plants. Unfortunately, in the present study, the treatment of cherry tomato seedlings with LFE had not shown statistically significant influence on the increase of AA content in fruits as compared to untreated plants grown under the same conditions.

Cherry tomatoes contain high content of lycopene, which also exhibit a strong antioxidant capacity. Lycopene is major carotenoid pigment responsible for orange-red color of fruits. The consumption of tomato containing lycopene has been shown to be associated with decreased risk of many chronic diseases primarily due its antioxidant properties (Mynorsky, 2002). Enhancing the lycopene content of tomato fruits is therefore desirable from both an agricultural as well as a nutritional perspective. Results of this study showed that cherry tomato plants exposed to stress (V_1 and V_3) were characterized by a significantly higher content of lycopene as compared to non-stressed plants, regardless of LFE treatment. Many studies have also noted the similar results about the impact of exposure of plants to controlled stress conditions on the lycopene content in tomato fruits (Theobald et al., 2007; Favati et al., 2009). Understanding the impact of drought on lycopene production in fruits is still fragmentary. It is known that the lycopene accumulation in the fruits was accompanied by an increase of content of ABA which is a primary stress indicator for drought pathways in plants (Chaves et al., 2009). Theologis et al. (1993) reported that activity of plant hormone ethylene is also in high correlation with lycopene production what is expected, since the ethylene is hormone most associated with ripening, stage of fruit development where lycopene is mostly accumulated.

Conclusions

The results of present study lead to the conclusion that controlled exposure of cherry tomato seedlings to water stress significantly improved fruit quality, increased total soluble solids, titratable acidity, content of ascorbic acid and lycopene, total phenolic and flavonoids content, total antioxidant capacity but decreased yield.

LFE treatment significantly increased phenolic and flavonoids content of cherry tomato fruits under normal growth conditions, indicating that LFE promote the synthesis of phenolic compounds in cherry tomato plants. This treatment also positively influenced by the yield and quality parameters of cherry tomato (total soluble solids, titratable acidity, content of ascorbic acid and lycopene) under normal growth conditions as compared to untreated plants.

Positive influence of the LFE treatment on cherry tomato quality is result of fertilizer composition, as well as ability of cherry tomato plants to use bioactive substances in fertilizer for its growth and development.

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