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Response of cherry tomato seedlings to liquid fertiliser application under water stress

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Abstract

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The aim of this study was to examine the impact of different liquid fertilisers on selected physiological parameters in order to evaluate the drought tolerance of cherry tomato seedlings. The following physiological parameters were investigated: total phenolic and flavonoid content, total antioxidant capacity and proline content of leaf extracts. Total phenolic and flavonoid content were determined using the Folin-Ciocalteu and aluminium chloride colorimetric methods, respectively. The ferric-reducing/antioxidant power (FRAP assay) was used to measure the total antioxidant capacity, while proline content was evaluated according to the method of Bates. The contents of proline, total phenolics and flavonoids were significantly higher in the leaves of cherry tomato seedlings exposed to water stress, which suggests that the higher synthesis of these substances by plants represents an important defence mechanism of drought tolerance. The results also indicate that the application of all the used fertilisers in accordance with the manufacturer's instructions can significantly increase the content of phenol compounds and total antioxidant capacity of plants under normal growth conditions, thus improving survival under subsequent stress.

Keywords: phenolics; proline; drought; osmolytes; antioxidants

During the vegetation period, plants are exposed to the influence of various environmental factors which can cause stress for a plant. Water stress caused by drought during vegetative growth leads to considerable reductions in crop production (Wang et al. 2014).

Mechanisms of osmotic adjustment and adaptation of plants and antioxidant defence systems represent important defence mechanisms that enable plants to cope with water stress. Such mechanisms can be directly influenced by certain agro-technical measures. Osmotic adaptation is based on the accumulation of osmolytes such as proline, glycine betaine and sugar alcohols in plant cells (Saeidi et al. 2017). Hayat et al. (2012) reported that a stressful environment elicits a higher production of proline in plant cells, which in turn imparts stress tolerance primarily by maintaining cell turgor or osmotic balance. Furthermore, proline plays highly beneficial roles during stress, i.e., as a stabi-

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liser of sub-cellular structures and as a part of the antioxidant defence system of plants (Liang et al. 2013). Apart from the accumulation of proline and osmolytes in general, a stressful environment also evokes intensive synthesis of other substances that can help plants adapt or acclimatise to stress conditions (Cheynier et al. 2013). Phenolic compounds are plant secondary metabolites with significant effects on total antioxidant capacity, which is defined as the ability of the plant to cope with the toxic effects of free radicals. The antioxidant properties of phenolic compounds enable them to act as radical scavengers, hydrogen donors and metal chelators, abilities which mainly stem from their redox potential (Srivastava 2012).

Numerous products used in agricultural production as organic or bio-fertilisers contain both nutrients and physiologically active substances that can contribute to the accumulation of osmolytes and antioxidants in plants, enhancing protective mechanisms under stress conditions. Among them, particular emphasis is put on the relatively new liquid fertilisers Bio-algeen S-92, Ergonfill and Slavol. The main advantage of these fertilisers with respect to many others which are applied in agricultural production is that they are ecologically acceptable, which makes them potentially applicable in agriculture, particularly in organic agricultural production.

Bio-algeen S-92 is a liquid fertiliser derived from the seaweed *Ascophyllum nodosum* (L) Le Jolis, and contains numerous active natural substances including amino acids, vitamins and microelements that provide a large number of benefits for plant growth and development (Dobromilska et al. 2008; Arioli et al. 2015). According to the product specification, Bio-algeen S-92 contains 96% water, 0.02% N, 0.006% P, 0.096% K, 0.31% Ca, 6.3 mg/l Fe, 1 mg/l Zn and 0.6 mg/l Mn. Furthermore, this fertiliser contains different essential amino acids such as alanine, glycine, tryptophan, histidine, proline, glutamine and also vitamins B_1, B_3, B_6, B_9 and vitamin E. However, the chemical composition of Bioalgeen S-92 depends on many aspects, including environmental factors, time of harvest, water temperature and methods used to process the seaweed *Ascophyllum nodosum* (L.) Le Jolis (Lordan et al. 2011).

Ergonfill is a fertiliser produced by the hydrolytic degradation of animal proteins, and contains a high proportion of amino acids, minerals and other substances which stimulates physiological processes in plants. According to the product specification, Ergonfill contains 3.4% N, 10% C, 2% MgO, 0.2% Fe and 0.003% Mo. Also, Ergonfill contains vitamins B_1 and B_2 as well as 19 essential amino acids; glutamine, proline, leucine, lysine, asparagine and alanine are present in the highest concentrations in the fertiliser.

Slavol is a liquid microbiological fertiliser which, apart from useful nitrogen-fixing and phosphate-solubilising bacteria, contains the plant hormone indole-3-acetic acid (IAA), which stimulates physiological processes, primarily plant growth.

Bearing in mind the features of the described fertilisers, it can be assumed that their application should have positive effects on plant defence mechanisms under water stress conditions.

The aim of this study was to examine the effects of the liquid fertilizers Bio-algeen S-92, Ergonfill and Slavol on selected physiological parameters in order to evaluate the drought tolerance of cherry tomato seedlings (*Lycopersicon esculentum* Mill. 'Sakura F_1') under water stress conditions. Cherry tomato was selected as the subject of this study, particularly because this species is commonly affected by a lack of moisture, and also because the plants features prominently in human diets.

MATERIAL AND METHODS

The experiment was carried out during 2015 under controlled conditions in the hothouse of the 'Park' public communal company in Sarajevo. The first phase of research included transplantation of the cherry tomato seedlings into pots (20 cm diameter × 13 cm height) containing the substrate Florahum-SP and was conducted on 8 April 2015. The substrate used in this study represented a fine mixture of white and black peat enriched with essential nutrients or macro- and microelements. The main characteristic of the substrate is its high capacity for water storage and ample supply of nutrients, which ensure proper plant vegetation for up to two months. The basic chemical properties of the Florahum-SP substrate were as follows: pH (H_2O) 5.5–6.5; EC 1.2–1.8 mS/cm; content of N 140–180 mg/l, content of P_2O_5 160–300 mg/l and content of K 180–400 mg/l.

The second phase consisted of setting up an experiment in which the cherry tomato seedlings

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were treated with different liquid fertilisers. The experimental trial was laid out in a randomized block design with four treatments in three replications. Each of treatments was applied to 40 plants, which means that a total number of 480 plants were included in this study.

Experimental fertilisation treatments were as follows: (1) Bio-algeen S92 0.2% (10 ml in 5 l of water); (2) Slavol 1% (50 ml in 5 l of water); (3) Ergonfill 0.1% (5 ml in 5 l of water); (4) untreated plants. Fertilisers were applied at the concentrations recommended by the manufacturers. The first application of fertiliser was carried out immediately after the transplanting of seedlings, and the second 15 days later. Five days after the second treatment, one half of the cherry tomato seedlings from each group (20 plants) was exposed to water stress conditions (non-watering), while the second half (also 20 plants) served as the controls which were not exposed to stress conditions, that is, they were regularly watered. Exposure to conditions of water stress lasted until the moment in which the first visible effects of drought in the form of falling leaves were observed; these symptoms appeared 72 h after the seedlings were exposed to drought. This moment was the beginning of the implementation of the third phase of the study, which included measurement of selected physiological parameters of defence mechanisms against drought stress, such as the content of proline, total phenolic and flavonoid contents and the antioxidant capacity of leaf extracts of cherry tomato seedlings. The third or fourth leaf from the growing tip of tomato seedlings was used for analysis and within each sampling only one leaf per plant was taken.

Determination of proline was carried out according to the method of Bates (1973) as follows: 1 g of fresh leaf sample was homogenised in 3% (w/v) aqueous sulfosalicylic acid and the homogenate was filtered through a glass-fibre filter into a plastic test tube. Then, 2 ml of filtrate were mixed with 2 ml of ninhydrin reagent and 2 ml of glacial acetic acid in a test tube and boiled for 1 h at 100°C in a water bath, after which the reaction was stopped by transferring the test tubes to ice (ninhydrin reagent was prepared by dissolving 2.5 g of ninhydrin in a mixture of 60 ml glacial acetic acid and 40 ml 6 M phosphoric acid). The reaction mixture in test tubes was extracted with 4 ml of toluene, and mixed vigorously with a vortex mixer for 15–20 sec. After the equilibration of test tubes to room temperature, the reddish layer was transferred to a cuvette using a micropipette and absorbance was read at 520 nm using toluene as blank. The proline concentration was determined from a standard curve (0–5 μg/ml) and then the values were recalculated on fresh weight (mg/g).

The extraction of phenolics and flavonoids from the leaves of cherry tomato seedlings was carried out as follows: 1 g of dry mass of tomato leaves was added to a 100-ml Erlenmeyer flask containing 40 ml 30% ethanol, and the mixture was heated in a water bath at 60°C under reflux, for an hour. After the extraction, the homogenate was filtered through coarse filter paper into a 50-ml flask, and filled to the mark with 30% ethanol. Until the moment of analysis, the extracts were kept in a refrigerator at 4°C.

The total phenolic content of the extract was determined using the Folin-Ciocalteu method (OUGH and AMERINE 1998) as follows: 0.25 ml of extract, 15 ml of distilled water and 1.25 ml of Folin-Ciocalteu reagent (diluted using distilled water in a 1:2 ratio) were mixed in a 25-ml flask and the mixture was incubated at room temperature for 15 minutes. Then, 3.75 ml saturated sodium carbonate solution was added. The flask was filled to the mark with 30% ethanol and heated in a water bath at 50°C for 30 min. After cooling to room temperature, absorbance was measured at 765 nm. Gallic acid was used as a standard (0–500 mg/l). Total phenol values were recalculated and expressed as mg of gallic acid equivalents per 1 g of dry weight (mg eq. GA/g).

The total flavonoid content of the extract was determined using the aluminium chloride colorimetric method (Zhishen et al. 1999) as follows: 1 ml of extract was added to a 10-ml volumetric flask containing 4 ml of distilled water and 0.3 ml 5% $NaNO_2$. After 5 min, 0.3 ml 10% $AlCl_3$ was added and the mixture was allowed to stand for 6 min. Then, 2 ml of 1 M NaOH were added and the total volume was made up to 10 ml with distilled water. The solution was allowed to stand for 15 min and absorbance was measured at 510 nm. Determination of total flavonoid content was carried out in triplicate and calculated from the calibration curve obtained with catechin, which was used as a standard. The values obtained were recalculated end expressed as catechin equivalents in mg per g of dry weight (mg eq. C/g).

The total antioxidant capacity of the extract was determined using the ferric reducing/antioxidant

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power (FRAP) assay (Benzie, Strain 1996) as follows: 240 µL of distilled water, 80 µl of extract, 2,080 µl of FRAP reagent (reagent was obtained by mixing 0.3 M acetate buffer of pH 3.6, 10 mM TPTZ (2,4,6-tripyridyl-s-triazine) and 20 mM $FeCl_3 \times 6\ H_2O$ in a 10:1:1 ratio were added into a 10-ml Erlenmeyer flask and heated in a water bath at 37°C for 5 min. The absorbance of the samples was determined at 595 nm. Determination of total antioxidant capacity was carried out in triplicate and calculated from a calibration curve obtained with an aqueous solution of $FeSO_4 \times 7\ H_2O$, which was used as a standard (serial dilution of stock solution to 200, 400, 800, 1,200 and 1,600 µM). The values obtained were recalculated end expressed as µmol Fe^{2+} per 1 g of dry weight (µmol eq. Fe^{2+}/g).

All experimental measurements were performed in triplicate and the results were expressed as mean ± standard deviation. The data obtained were processed by applying standard analysis of variance (ANOVA) using the SAS computer software program, and significant differences between the groups were determined using the Tukey test at 0.01 probability level ($\alpha = 0.01$). Linear regression analysis and Pearson's correlation coefficient (ρ) were calculated using Microsoft Excel 2013 for Windows.

RESULTS AND DISCUSSION

High levels of proline accumulation were observed in the leaves of cherry tomato seedlings exposed to water stress in response to all fertiliser treatments (Table 1).

The data highlight the fact that plants accumulate large quantities of proline in response to water stress. This substance provides protection to plant cells by contributing to cellular osmotic adjustment, protection of membrane integrity and protein stabilisation (Sharma, Verslues 2010). Many scientific reports also showed a positive correlation between the accumulation of proline and plant stress (Lehmann et al. 2010; Szabados, Savouré 2010; Kavi Kishor, Sreenivasulu 2014). Accumulation of proline in the leaves of plants during water stress is caused both by activation of the synthesis of proline, which is mainly regulated at the transcriptional level, and by the inactivation of proline degradation (Yaish 2015).

Claussen (2005) reported that intensive synthesis of proline in the leaves of cherry tomato seedlings exposed to drought begins 16 hours after initiation of stress and that the substance accumulates over the following five days, which is comparable with the results obtained in the present study. The content of proline in plant leaves not exposed to water stress was low and did not change significantly in response to any of the applied fertilisers.

The fastest increase in proline content in the leaves of cherry tomato seedlings during water stress was found in untreated plants, as well as in cherry tomato seedlings treated with the fertiliser Slavol. Seedlings treated with Bio-algeen S-92 and Ergonfill exhibited a slower increase in proline content in leaves during water stress. In view of fact that a faster increase of proline in leaves indicates plant stress, the results support the hypothesis that application of Bio-algeen S-92 and Ergonfill mitigates the negative effects of water stress on cherry tomato seedlings.

Alongside osmotic adaptation, the ability of a plant to activate its defence mechanisms against

Table 1. Proline content in leaves of cherry tomato (mg/g fresh weight) during water stress

Treatment	Proline content (mg/g)				
	day 1	day 2	day 3	day 4	day 5
1. Bio-algeen S-92 (stress)	13.6 ± 0.5	84.4 ± 6.8	59.0 ± 5.6	69.5 ± 5.1	126.1 ± 8.1
1. Bio-algeen S-92 (non-stress)	7.0 ± 0.3	7.6 ± 0.4	8.9 ± 0.6	10.0 ± 0.7	8.0 ± 0.7
2. Slavol (stress)	10.2 ± 0.3	13.9 ± 0.3	63.1 ± 2.5	163.7 ± 9.8	314.2 ± 7.2
2. Slavol (non-stress)	8.4 ± 0.2	6.8 ± 0.5	14.4 ± 0.7	8.6 ± 0.6	6.7 ± 0.3
3. Ergonfill (stress)	13.2 ± 0.4	38.9 ± 1.1	29.6 ± 3.4	29.4 ± 4.1	44.2 ± 2.4
3. Ergonfill (non-stress)	7.2 ± 1.0	11.9 ± 0.7	7.4 ± 0.4	9.6 ± 1.0	9.4 ± 1.0
4. Non-treated (stress)	10.0 ± 0.6	28.0 ± 1.3	83.2 ± 7.2	172.8 ± 9.1	345.7 ± 8.0
4. Non-treated (non-stress)	6.8 ± 0.3	7.1 ± 0.4	7.3 ± 0.3	10.3 ± 1.2	9.8 ± 0.8

each value is a mean of three replicates ± standard deviation (SD), $n = 3$

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Table 2. Total phenolic and flavonoid contents (mg/g dry weight) and total antioxidant capacity (FRAP; μmol eq. Fe^{2+}/g dry weight) in leaves of cherry tomato seedlings at the beginning and end of drought stress

Treatment	Initial stress phase (day 1)		Final stress phase (day 5)		
	phenolic	flavonoids	phenolic	flavonoids	FRAP unit
1. Bio-algeen S-92 (stress)	3.38 ± 0.12^{a}	1.30 ± 0.05	9.06 ± 0.21^{a}	3.51 ± 0.15^{a}	118.59 ± 1.97^{a}
1. Bio-algeen S-92 (non-stress)	2.90 ± 0.08^{cdef}	1.23 ± 0.11	7.21 ± 0.37^{de}	2.47 ± 0.16^{de}	91.30 ± 2.70^{def}
2. Slavol (stress)	2.93 ± 0.10 ^{cde}	1.13 ± 0.06	8.51 ± 0.39^{bc}	3.02 ± 0.17^{c}	101.00 ± 2.03^{bc}
2. Slavol (non-stress)	3.24 ± 0.06^{b}	1.14 ± 0.08	6.48 ± 0.43^{g}	2.35 ± 0.09^{def}	86.65 ± 1.74^{defg}
3. Ergonfill (stress)	2.69 ± 0.14^{g}	1.08 ± 0.09	8.65 ± 0.24^{ab}	3.41 ± 0.14^{ab}	104.22 ± 2.11^{b}
3. Ergonfill (non-stress)	2.80 ± 0.08^{efg}	1.10 ± 0.06	7.28 ± 0.16^{d}	2.45 ± 0.19^{def}	91.61 ± 1.86^{de}
4. Non-treated (stress)	3.04 ± 0.11^{c}	1.12 ± 0.14	7.14 ± 0.29^{def}	2.53 ± 0.07^{d}	93.01 ± 4.20^{d}
4. Non-treated (non-stress)	2.97 ± 0.16^{cd}	1.07 ± 0.07	6.46 ± 0.23^{g}	2.14 ± 0.13^{f}	78.87 ± 2.30^{h}
Tukey test (α = 0.01)	0.165	–	0.468	0.351	6.833

each value is a mean of three replicates ± standard deviation (SD), n = 3

stress is critically dependent on its ability to produce secondary metabolites, i.e., phenolic compounds, with strong antioxidant activity. The ability of phenolic compounds to bolster antioxidant defence mechanisms in plants has mainly been attributed to their ability to inhibit or quench free radical reactions and thus delay or inhibit cellular damage (Nimse, Pal 2015).

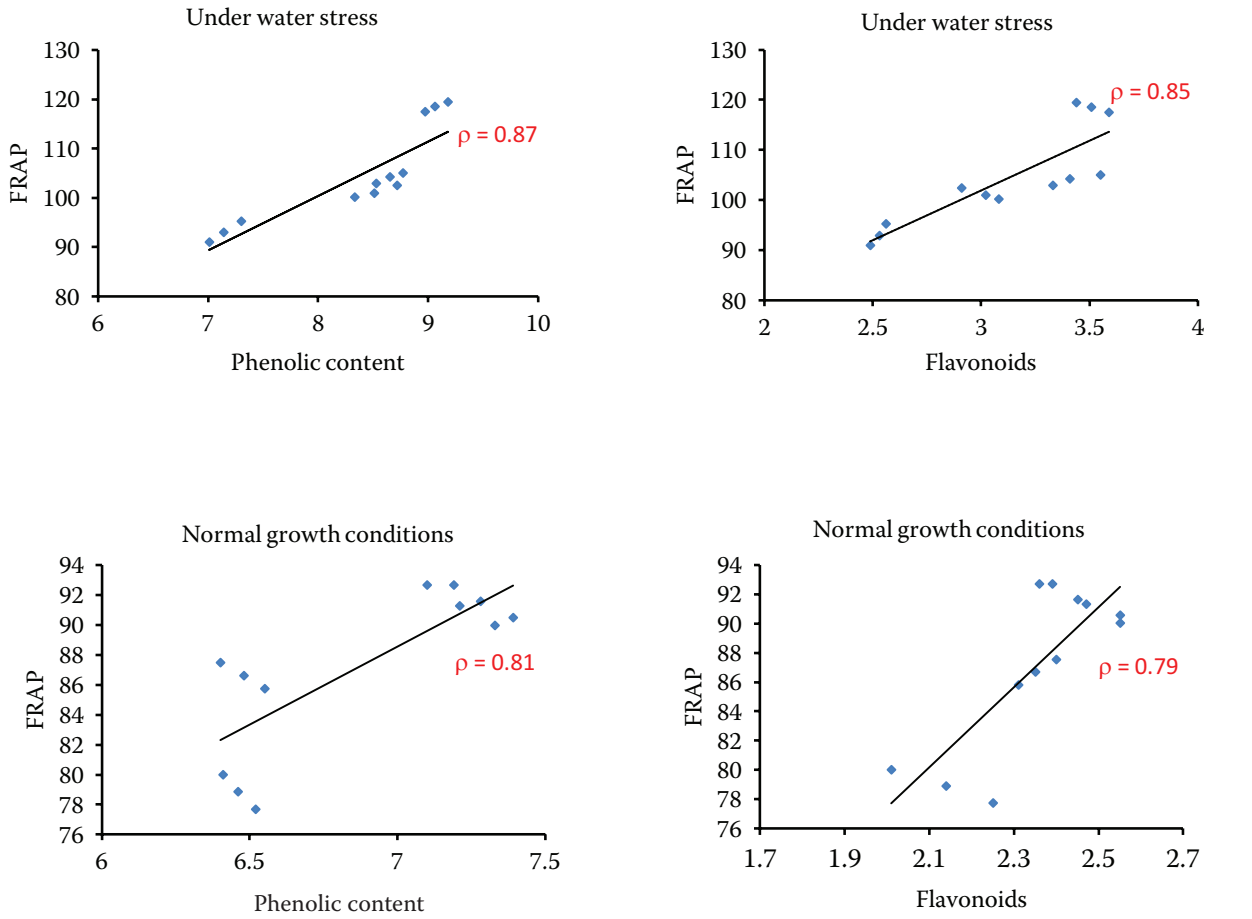

Fig. 1. Correlation between content of phenolic/flavonoids and total antioxidant capacity of leaves of cherry tomato seedlings under different growth conditions

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The results of this study show that the total antioxidant capacity and contents of phenolics and flavonoids were significantly higher in the leaves of cherry tomato seedlings exposed to water stress compared to non-stressed seedlings (Table 2), suggesting that the synthesis of phenolics, flavonoids and other substances with high antioxidant power is intensified under stress conditions (Nikolova et al. 2012; Sanchez-Rodriguez et al. 2013).

The data in Table 2 also show that the highest levels of phenolics and flavonoids in the leaves of cherry tomato seedlings were determined in stressed seedlings (day 5) treated with Bio-algeen S-92 and Ergonfill. The efficiency of these fertilisers in increasing phenolic content in plants has been mainly attributed to their chemical composition. Namely, these products contain the amino acids phenylalanine, tyrosine and tryptophan which serve as precursors for the synthesis of a wide range of phenolic compounds; thus, it can be assumed that the application of these fertilisers can contribute increasing the levels of phenolic compounds in the leaves of cherry tomato seedlings. The positive influence of Bio-algeen S-92 and seaweed liquid fertilisers in general on the levels of phenolics, flavonoids and other desirable substances in plants has been confirmed by the results of many studies (Craigie 2011; Calvo et al. 2014). Only a very small number of studies have described the application of the fertiliser Ergonfill in plant cultivation, so the results of this research on the physiological parameters of cherry tomato seedlings under water stress provides a better insight into the effects of this fertiliser. Slavol was also observed to positively influence the levels of phenolics and flavonoids in the leaves of tomato seedlings with respect to the non-treated group, especially when the seedlings were exposed to drought. The efficiency of Slavol is also a result of its chemical composition. This fertiliser contains the plant hormone IAA as well as nitrogen-fixing and phosphate-solubilising bacteria, which can contribute to a higher uptake of nutrients by plant roots and hence more successful plant metabolism. The results of this study support this idea.

Also, the application of Bio-algeen S-92, Slavol and Ergonfill resulted in statistically significant increases in the total antioxidant capacity of the leaves of cherry tomato seedlings with respect to non-treated plants, especially when the seedlings were exposed to drought. These data show that the application of fertilisers that can stimulate the plant to produce substances with powerful antioxidant effects is one ways to improve the plant antioxidant capacity.

A high correlation between the content of phenolics/flavonoids and total antioxidant capacity of the leaves of cherry tomato seedlings was found (Fig. 1). These results suggest that the antioxidant activities of plant can be mainly attributed to their phenolic compounds and this hypothesis has, in fact, been confirmed by many scientists (Gasemi et al. 2009; Hasna, Afidah 2009; Kaur, Mondal 2014).

The correlation between the content of phenolic/flavonoid compounds and total antioxidant capacity was a little higher in plants growing under water stress conditions. These data show that plants initiate an intensive synthesis of phenolic compounds under water stress, an observation confirmed by many other studies (Chandra, Ramalingam 2011; Petridis et al. 2012).

CONCLUSION

The content of proline, total phenolics and flavonoids were significantly higher in the leaves of cherry tomato seedlings exposed to water stress, which suggests that the increased synthesis of these substances by plants represents an important defence mechanism of drought tolerance.

The high correlation between phenolic/flavonoid content and total antioxidant capacity suggests that the antioxidant activities of the cherry tomato can be mainly attributed to their phenolic compounds.

The research presented here indicates that application of the fertilisers Bio-algeen S-92, Ergonfill and Slavol in accordance with the manufacturers' instructions can significantly increase the content of phenol compounds and total antioxidant capacity of the leaves of cherry tomato seedlings under non-stress growth conditions, thus improving survival under subsequent stress. The positive effects of the application of these fertilizers stem from their specific chemical compositions, as well as the ability of plants to maximally utilise the active substances in these products for growth and development.

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