

EFFECT OF MICROBIOLOGICAL FERTILIZER FOR MITIGATING WATER STRESS IN CHERRY TOMATO

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Abstract

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This study was carried out to examine the effect of microbiological fertilizer ‘Slavol’ (MB) on selected physiological parameters for evaluating drought tolerance of seedlings (content of proline, leaf water potential, leaf area, content of photosynthetic pigments, total phenolic and flavonoids) and subsequently on the yield and fruit quality of cherry tomato (*Lycopersicon esculentum* Mill. var. *cerasiforme*). Cherry tomato seedlings treated by MB had a lower content of proline and higher leaf water potential compared to non-treated seedlings under water stress, which indicates that microorganisms present in fertilizers contributes to better adaptation of cherry tomato seedlings to stress. The research results also showed that application of MB contribute to increasing of phenolic compounds and consequently strengthening of cherry tomato antioxidant defense system. Fruit quality parameters (TSS, TA, TPC, TFC, FRAP, content of ascorbic acid and lycopene) were significantly higher in fruits of cherry tomato subjected to drought, regardless of MB treatment, suggesting that exposure of plant to controlled water stress conditions may represent a very promising approach to enhance the nutritional quality of cherry tomato.

Key words: drought; microorganisms; antioxidants; fruit quality; yield

Abbreviations: microbiological fertilizer ‘Slavol’ (MB), total soluble solids (TSS), titratable acidity (TA), total phenolic content (TPC), total flavonoid content (TFC), ferric reducing antioxidant power (FRAP)

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Introduction

Tomato (*Lycopersicon esculentum* Mill.) provide a wide variety of nutrients, vitamins, flavonoids, phenolic acids and carotenoids, important for nutrition and human health, and therefore the interest of producers and consumers for their cultivation constantly increased (Lim et al., 2014). This interest is particularly pronounced for growing of cherry tomato, since this type of tomato contains a significantly higher amount of ingredients relevant for human health, compared to commercial tomato cultivars. Also, the fruits of cherry tomato (*Lycopersicon esculentum* Mill. var. *cerasiforme*) have an excellent balance of flavor, color, and texture as well as a high content of vitamin C, all important components for the internal quality of tomato (Aguirre and Cabrera, 2012).

One of the major disadvantages in tomato cultivation is its susceptibility to drought. Drought can significantly reduce the yield of tomato, but also its survival is questionable, if the plants, especially seedlings, were subjected to water stress (Foolad et al., 2003). Any potential approach for improving defense system of plant against water stress is a welcome addition to an already existing agronomic practice in crop cultivation under stress conditions. The use of microbiological fertilizers is certainly one of the approaches that can help a crop to mitigate the negative effects of drought. Namely, the presence and activity of some microorganisms in soil-plant system can contribute to improve soil properties and increase uptake of nutrients by plants, thus indirectly improving the defense system of plant against stress (Bhattacharyya and Jha, 2012). MB contains nitrogen-fixing and phosphate solubilizing bacteria and plant growth hormones, so it can be assumed that their application has a positive effect on growth and development of cherry tomato under water stress conditions. This relatively new fertilizer is totally of natural origin which makes them also acceptable for organic agriculture.

The main objective of this study was to examine the effect of MB application on the selected physiological parameters for evaluating drought tolerance of cherry tomato (*Lycopersicon esculentum* Mill. var. *cerasiforme* 'Sakura F1'). An additional objective of this study was to examine its effect on the yield and quality of this cultivar of cherry tomato grown both under normal and water stress conditions.

Materials and Methods

The study was conducted under controlled conditions, in hothouse of public communal company 'Park' in Sarajevo. The experiment was set up in a randomized block design with four variants in three replications. Each of variants

was present with twenty plants. Experiment variants were as follows: (V₁) cherry tomato seedlings treated by MB and subjected to drought; (V₂) cherry tomato seedlings treated by MB and regularly watered; (V₃) non-treated cherry tomato seedlings subjected to drought; (V₄) non-treated cherry tomato seedlings regularly watered. Seedlings used in the experiment were produced at a certified nursery located near the hothouse and showed no significant difference in terms of size and appearance.

In accordance with manufacturer's instructions, MB was applied through soil diluted with water at concentration of 1% (10 ml l⁻¹ water). The first application was performed immediately after the transplanting of seedlings, and the second two weeks later. Five days after the second application, one half of cherry tomato seedlings inside each variant (twenty plants) were exposed to drought, while the second half (also twenty plants) were regularly watered. Exposure of seedlings to drought lasted until the moment in which first visually observable effects of drought appeared on the seedlings as wilting leaves (three days after the seedlings were exposed to drought). This moment was represented the beginning of the measurement of the selected physiological parameters for evaluating drought tolerance of cherry tomato seedlings: content of proline, leaf water potential, leaf area, content of photosynthetic pigments, total phenolic and flavonoid content, and total antioxidant capacity of leaf extracts.

Leaf water potential was estimated by the dye method (Knippling, 1967), content of proline was measured by acid-ninhydrin method (Bates et al., 1973), photosynthetic pigments were extracted with 80% acetone (Wettstein, 1957) and the total amount of pigments were determined with equations recommended by Lichtenthaler and Weliburn (1983), leaf area was measured by millimeter graph paper method (Pandey and Singh, 2011), total phenolic content was estimated using Folin Ciocalteu method (Ough and Amerine, 1988), total flavonoids according to Aluminium chloride colorimetric assay (Zhishen et al., 1999), and the ferric reducing/antioxidant power (FRAP) assay was used to determine total antioxidant capacity (Benzie and Strain, 1996).

The next part of study involved the cultivation of cherry tomato under normal growth conditions until the time of technological maturity of fruits. The following parameters at this ripening stage of cherry tomato fruit were evaluated: yield, total soluble solids, titratable acidity, total phenolic and flavonoid content, total antioxidant capacity, and content of vitamin C and lycopene. Yield was determined by weighing and expressed as a kg per plant, total soluble solids by refractometric method (ISO, 2003), titratable acidity by titration with sodium hydroxide and phenolphthalein indicator (AOAC, 2000), vitamin C by 2,6-dichlorophenolindophenol

titration method (AOAC, 2006), and lycopene content by extraction with hexane and absorbance measurement at 503 nm (Davis et al., 2003).

All experimental measurements were done in triplicates and the results were presented as mean \pm standard deviation. SPSS 15.0 (statistical software) was used for statistical analysis of results. Least significance means were compared by LSD-test and significant differences were considered at $P < 0.05$.

Results

The results of the study suggest that cherry tomato seedlings treated by MB had a lower content of proline and higher leaf water potential (Ψ) compared to non-treated seedlings under water stress, as shown in Table 1. Since the high content of proline and low water potential indicates the stress in plants (Bhaskara et al., 2015), the presented data point out to the fact that microorganisms present in MB contributes to better adaptation of plants to stress.

The values of leaf area and content of chl *a*, chl *b* and carotenoids were higher in cherry tomato seedlings treated by MB, both in normal as well as stressful growth conditions (Table 2), indicating that application of MB enhances the photosynthetic capacity of cherry tomato seedlings.

Table 1
Content of proline and water potential (Ψ) in leaves of cherry tomato seedlings

Variant	Proline [mg g ⁻¹ FW]	Water potential [-MPa]
V ₁	62.42 \pm 13.93 ^b	-0.97 \pm 0.02 ^b
V ₂	9.96 \pm 7.56 ^c	-0.56 \pm 0.04 ^a
V ₃	81.32 \pm 18.46 ^a	-1.09 \pm 0.04 ^c
V ₄	8.24 \pm 3.24 ^c	-0.53 \pm 0.07 ^a
LSD _{0.05}	11.210	0.046

V₁ – MB stress; V₂ – MB watered; V₃ – non-treated stress; V₄ – non-treated watered; FW = fresh weight. Values are means \pm SD; the values marked with different letters in the same column indicate significantly differences ($p \leq 0.05$)

Table 2
Leaf area and content of photosynthetic pigments in leaves of cherry tomato seedlings

Variant	Leaf area [cm ²]	Photosynthetic pigments [mg g ⁻¹ FW]		
		Chl <i>a</i>	Chl <i>b</i>	Carotenoids
V ₁	13.98 \pm 4.57 ^c	1.18 \pm 0.29 ^{bc}	0.50 \pm 0.03	0.49 \pm 0.03
V ₂	19.36 \pm 3.38 ^a	1.48 \pm 0.03 ^a	0.54 \pm 0.10	0.52 \pm 0.11
V ₃	13.35 \pm 5.17 ^c	1.04 \pm 0.09 ^d	0.45 \pm 0.06	0.43 \pm 0.04
V ₄	17.53 \pm 3.23 ^{ab}	1.29 \pm 0.20 ^b	0.50 \pm 0.06	0.44 \pm 0.08
LSD _{0.05}	2.167	0.138	-	-

V₁ – MB stress; V₂ – MB watered; V₃ – non-treated stress; V₄ – non-treated watered; FW = fresh weight; values are means \pm SD; the values marked with different letters in the same column indicate significantly differences ($p \leq 0.05$)

The results of analysis of total phenolic and flavonoid contents showed that the values of these parameters were significantly higher in leaves of cherry tomato seedlings exposed to water stress as compared to non-stressed seedlings, regardless of MB treatment, as shown in Table 3.

Table 3
Total phenolic content (TPC), total flavonoid content (TFC) and total antioxidant capacity (FRAP) in leaves of cherry tomato seedlings

Variant	TPC [mg g ⁻¹ DW]	TFC [mg g ⁻¹ DW]	FRAP [mmol Fe ²⁺ g ⁻¹ DW]
V ₁	8.51 \pm 0.39 ^a	3.02 \pm 0.17 ^a	101.00 \pm 2.03 ^a
V ₂	6.48 \pm 0.43 ^c	2.24 \pm 0.09 ^c	86.65 \pm 1.74 ^c
V ₃	7.14 \pm 0.29 ^b	2.53 \pm 0.07 ^b	93.01 \pm 4.20 ^b
V ₄	6.06 \pm 0.23 ^d	2.14 \pm 0.13 ^c	78.87 \pm 2.30 ^d
LSD _{0.05}	0.333	0.266	3.838

V₁ – MB stress; V₂ – MB watered; V₃ – non-treated stress; V₄ – non-treated watered; DW – dry weight; Values are means \pm SD; The values marked with different letters in the same column indicate significantly differences ($p \leq 0.05$)

The results of the analysis of yield and fruit quality parameters of cherry tomato, depending on MB treatment and growth conditions were presented in Table 4 and Table 5.

Data presented in Table 4 and Table 5 showed that all examined quality parameters were significantly higher in fruits of cherry tomato subjected to drought (experiment variant V₁ and V₃), regardless of MB treatment, indicating that exposure of plant to controlled water stress conditions can significantly increase fruit quality.

Discussion

As shown in Table 1 application of MB has contributed to better osmotic adjustments of plants to stress conditions. Numerous studies have also found that the application of ni-

Table 4
Yield, total soluble solids (TSS), titratable acidity (TA) and lycopene content of cherry tomato fruits

Variant	Yield [kg per plant]	TSS [Brix]	TA [%]	Lycopene [mg g ⁻¹ FW]
V ₁	1.29 ± 0.35 ^c	6.66 ± 0.13 ^{ab}	0.65 ± 0.03 ^b	91.63 ± 5.57 ^a
V ₂	2.20 ± 0.40 ^a	6.43 ± 0.20 ^c	0.62 ± 0.01 ^c	85.30 ± 9.57 ^{bc}
V ₃	1.14 ± 0.50 ^c	6.67 ± 0.17 ^a	0.67 ± 0.01 ^a	89.63 ± 5.43 ^{ab}
V ₄	2.07 ± 0.35 ^{ab}	6.41 ± 0.25 ^c	0.63 ± 0.01 ^c	79.92 ± 5.86 ^c
LSD _{0.05}	0.287	0.202	0.016	6.322

V₁ – MB stress; V₂ – MB watered; V₃ – non-treated stress; V₄ – non-treated watered; FW – fresh weight; values are means ± SD; the values marked with different letters in the same column indicate significant differences (p ≤ 0.05)

Table 5
Vitamin C, total phenolic content (TPC), total flavonoid content (TFC) and total antioxidant capacity (FRAP) of cherry tomato fruits

Variant	Vitamin C (mg 100g ⁻¹ FW)	TPC (mg g ⁻¹ DW)	TFC (mg g ⁻¹ DW)	FRAP (mol Fe ²⁺ g ⁻¹ DW)
V ₁	13.66 ± 0.34 ^a	11.28 ± 0.49 ^a	5.60 ± 0.49 ^a	201.20 ± 9.36 ^a
V ₂	13.11 ± 0.66 ^{bc}	9.60 ± 0.78 ^c	4.54 ± 0.22 ^c	150.01 ± 6.66 ^c
V ₃	13.33 ± 0.33 ^{ab}	10.75 ± 0.64 ^{ab}	5.52 ± 0.35 ^{ab}	197.98 ± 10.55 ^{ab}
V ₄	12.77 ± 0.67 ^c	9.30 ± 0.42 ^c	4.43 ± 0.12 ^c	145.50 ± 14.11 ^c
LSD _{0.05}	0.489	0.702	0.304	10.096

V₁ – MB stress; V₂ – MB watered; V₃ – non-treated stress; V₄ – non-treated watered; FW – fresh weight; DW – dry weight; Values are means ± SD; the values marked with different letters in the same column indicate significant differences (p ≤ 0.05)

trogen-fixing bacteria (the bacterial genera *Rhizobium*) and phosphate solubilizing bacteria (the bacterial genera *Bacillus*) contained in applied microbiological fertilizer greatly improved the plant adaptation to drought (Chauhan et al., 2015; Rfaki et al., 2015). Namely, the nitrogen-fixing bacteria convert the nitrogen gas in the atmosphere into a biologically useful form, while phosphate solubilizing bacteria transform organic compounds of phosphorus into available forms which directly provide the better supply of nitrogen and phosphorus by plant and thus help improve plant functionality, especially under water stress conditions (Chen et al., 2006). Studer et al. (2007) reported that the maintained turgor of roots under water stress obtained with an optimal nutrient supply, primarily with nitrogen and phosphorus which plants need in larger quantities, results in better root growth, suggesting that osmotic adjustment is an adaptation not only for surviving stress, but also for growth under such conditions.

Data presented in Table 2 showed that the content of photosynthetic pigments and leaf area were higher in leaves of cherry tomato seedlings treated by MB under both stressful and non-stress conditions. However, the efficiency of MB to increase leaf area and content of chlorophyll *a* in leaves of cherry tomato seedlings was lower under stressful conditions, suggesting that drought can highly reduce the activity of nitrogen-fixing bacteria and phosphate solubilizing bacte-

ria in soil. The decreasing of nitrogen-fixing bacteria activity by drought is usually attributed to a reduction in respiration of the root nodules (Zahran, 1999), while the decreasing of phosphate solubilizing bacteria activity in drought-affected soils is primarily result of slow decomposition of organic phosphorus compounds under water stress conditions (Sharma et al., 2013).

Maréchaux et al. (2015) reported that drought decreases leaf area owing to reductions in leaf water potential, rate of cell division, and cell elongation due to loss of turgor. A reduction in leaf area allows plants to adapt under conditions of less water availability, as small leaf area implies less transpiration area (Silva et al., 2010).

Besides reducing the leaf area and osmotic adjustments to maintain homeostasis in plant cells, plants possess a series of other mechanisms at the physiological, biochemical and cellular levels to overcome water stress. One of these mechanisms is related to maintain balance between the production and scavenging of reactive oxygen species (ROS). Since the ROS production is enhanced under water stress conditions, plants activated its defense systems to establish the previously disturbed balance, including enzymatic and non-enzymatic systems. The enzymatic antioxidant system includes primarily superoxide dismutase, catalase, and peroxidase enzymes, while the non-enzymatic antioxidant system includes phenolic compounds, flavonoids and many

other antioxidant substances. A plant that has a higher ability to synthesize of those antioxidants has also a higher ability to reestablish a balance between production and scavenging of ROS and thus eliminating the negative effects of oxidants on the cell structure and functionality (Nimse and Pal, 2015).

As shown in Table 3 content of total phenolic and flavonoid were significantly higher in leaves of cherry tomato seedlings exposed to water stress as compared to non-stressed seedlings, regardless of MB treatment. Obtained data point out to the fact that plants, as a response to water stress, intensive produce phenolic and flavonoids compounds and this fact has been confirmed by many scientists (Sanchez-Rodriguez et al., 2011; Al Hassan et al., 2015). Scheible et al. (2004) reported that other stress factors such as nutrient deficiency may also initiate intensive synthesis of phenolic compounds. There are many hypotheses that attempt to explain impact of environmental stress on the synthesis of phenolic compounds and one of the most widely accepted hypotheses is carbon-nutrient balance hypothesis. This hypothesis suggests that in situations when growth is more limited than photosynthesis, plants are predicted to use more carbon to produce carbon-based defensive substances such as phenolic and flavonoids (Hamilton et al., 2001). However, phenolic compounds play a significant role in protecting plant cells against oxidative species and it is preferable that plant produces more phenolic compounds (Parvaiz and Satyawati, 2008).

The values of the total antioxidant capacity were also higher in leaves of cherry tomato seedlings exposed to stress. Taking into consideration the fact that leaves of these seedlings have a higher phenolic content, it is quite clear that phenolic compounds are carriers of antioxidant capacity of cherry tomato (Vasco et al., 2008). These results also lead to the conclusion that MB application has a positive impact on the strengthening of plant defense system against ROS-induced oxidative damage.

Data presented in Table 4 and Table 5 showed that all examined quality parameters were significantly higher in fruits of cherry tomato subjected to drought (experiment variant V₁ and V₃), regardless of MB treatment. Many studies have also indicated that exposure of plant to controlled water stress conditions can significantly increase fruit quality (Agbemafle et al., 2015; Alaoui et al., 2015).

Ripoll et al. (2014) reported that the stress conditions stimulates the secondary metabolism, thereby potentially increasing the content of ascorbic acid, phenolic compounds, lycopene and other antioxidant substances involved in plant defense system and health benefits. These data lead to the conclusion that exposure of plant to controlled water stress conditions may represent a very promising approach to enhance the nutritional quality of cherry tomato. Drawback of

exposure of plant to water stress is a very significant reduction in cherry tomato yield (Okunlola et al., 2015), and results of this study confirm this hypothesis.

Table 4 and Table 5 data also showed that the application of MB not improved the yield and quality of cherry tomato within the same growth conditions. Since it was previously found the positive effect of MB on growth and development of cherry tomato seedlings, obtained results were quite unexpected. One of the probably reasons for lower efficiency of MB on quality parameters of tomato fruits was the time of applying fertilizers. Namely, the microbiological fertilizer in the present study was applied before fruit set, and thus its impact on the development of the fruit was reduced. Furthermore, survival and distribution of microorganisms in soil depend on many factors such as plant-microbe interactions, soil properties, organic matter and other factors that can also influence their efficiency (Menge and Chazdon, 2016; Hungria et al., 2013).

Conclusion

MB application in cherry tomato cultivation contributes to better adaptation of seedlings to drought conditions. This treatment can also be used as a good alternative or a supplement to chemical fertilization. If we want to achieve maximum benefits of applying microorganisms in terms of fertilizer savings, it is necessary to harmonize the application of microorganisms with appropriate levels of fertilization, crop requirements for nutrients and soil properties.

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